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Original Article

Delayed Treatment and Adverse Effects of Drug Resistance Tuberculosis Impact on Outcome, Survival and Quality of Life

Linda Soebroto, Reviono, Yusup Subagio Sutanto, Farih Raharjo, Jatu Aphridasari

Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Sebelas Maret, RSUD dr. Moewardi, Surakarta, Indonesia

Abstract

Background: Global TB Report 2020 states that 78% of tuberculosis patients experience drug resistancy with a global treatment success rate of 57%. Drug resistance tuberculosis (DR TB) patients whom experience side effects such as arthralgia and hyperuricemia with inadequate treatment will affect the treatment result.

Methods: Retrospective cohort study to DR TB patients who underwent treatment from January 2015 to August 2020 at RSUD dr. Moewardi Surakarta. Survival analysis using Kaplan Meier method and Cox regression test for the effect of risk factors on the safety and survival of TB RO patients. Quality of life analysis using Mann Whitney test.

Results: From 372 patients, delayed treatment factor (OR=2.906; 95% CI=1.890-4.469; *P*≤0.001) and arthralgia factor (OR=1.775; 95% CI=1.148-2.744; *P*=0.010) were variables that had a significant effect on recovery of DR TB patients. Delayed treatment and arthralgia have risk (2,906 times and 1.775 times) for non-recovered DR TB patients. Delayed treatment factor with HR=14.772 (95% CI=13.381-16.163), arthralgia with HR=15.170 (95% CI=13.960-16.379), and anemia with HR=15.304 (95% CI=14.074-16.535; *P*=0.002) affect on the survival of DR TB patients. Anemia affect on the quality of life DR TB patients.

Conclusion: Delayed treatment for more than 14 days and arthralgia that is not treated adequately can affect the recovery of DR TB patients. The survival and quality of life of DR TB patients can be increased by monitoring the time of taking medication, monitoring side effects of drugs such as arthralgia, and adequate nutritional intake so that anemia does not occur.

Keywords: anemia, arthralgia, delayed treatment, drug resistant TB, hyperuricemia, quality of life, recovery, survival

Corresponding Author:
Linda Soebroto | Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Sebelas Maret Surakarta, RSUD dr. Moewardi, Surakarta, Indonesia | soebrotolinda@hotmail.com

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INTRODUCTION

Global TB report 2020 states that there are 465,000 cases of *rifampicin resistant* (RR) tuberculosis which 78% of them are drug-resistant TB (DR TB). The number of patients receiving DR TB treatment was 177,099 in 2019, this number increased by 10% from the previous year. The death rate due to tuberculosis is still a problem in Indonesia, especially when *Mtb. bacilli* strain appeared that are resistant to several types of drugs. The decline in health services and the increase in drug resistance will exacerbate TB deaths.[1–5]

Individuals who are susceptible to physical, biological, psychological, and socio-economic stressors can exacerbate TB mortality. Depression, anxiety, drug use, stigma, discrimination, and psychological distress are considered to be the most commonly reported psychological and social problems among TB patients. Azam et al in 2020 did the research about the correlation between depression and gender. Almost 40% women and 16.7% man had depression (OR=7.6; 95% CI=1.79-32.21). Drug side effects also higher in subject with depression due to perception and stigma from community.[1–5]

Delay in diagnosis and delay treatment can increase the severity of the disease, increased transmission time, and poor treatment outcomes up to death. Asres et al's study (2018) in Ethiopia assessed the effects of treatment delay on TB outcome. Treatment success in 735 TB patients differed between patients starting treatment within 30 days and after 30 days of diagnosis. TB patients starting treatment in 30 days after diagnosis had 94.2% treatment success and the mortality rate is 1.9%. TB patients who started treatment more than

30 days after diagnosis had 88.4% treatment success with a mortality rate of 4.8%. Delay in starting treatment will increase the risk of transmission, morbidity, and mortality high in the patient. Arthralgia or joint pain is a side effect that often occurs after gastrointestinal disorders. Research by Reviono et al in 2014 in Surakarta stated that the third most common side effect in the treatment of DR TB was arthralgia in 90 (78.9%) patients.[6,7]

The World Health Organization (WHO) recommends nutritional assessment to be an integral aspect of TB care. Anemia in pulmonary TB patients can be caused by several causes including: the result of chronic disease, increased blood loss from hemoptysis, decreased red blood cell production, and anemia due to malnutrition. The 2019 Holden, et al study in Denmark examined 1188 TB patients of which 640 patients (53.87%) were anemic at the time of diagnosis. Unfavorable outcomes associated with male gender, alcohol abuse, a history of mental disorders, and anemia. Anemia is associated with slowed sputum conversion, worsening of the disease and death. Research by Bhargava, et al in 2020 in India found that anemia occurs in 75% of TB patients and can cause morbidity and mortality in as much as 5% due to TB.[1,8–13]

Treatment of DR TB in Indonesia are a constraint both from the patient and the health care system. There is no comprehensive report on the timing of delay in treatment initiation and its predictors in Indonesia. The results of this study are expected to evaluate the side effects of treatment and delay in treatment so as to increase the resilience and quality of life of DR TB patients. This research is expected to help the national program to control drug-resistant tuberculosis to take appropriate action to achieve zero TB.

METHODS

A retrospective cohort study was conducted at the drug-resistant TB outpatient clinic at RSUD Dr. Moewardi Surakarta from June–August 2021. This study used primary data and medical record data by taking 372 DR TB patients who had received treatment from January 2015 to August 2020 and declared completed at RSUD Dr. Moewardi Surakarta. This study used a total sampling method (consecutive sampling). Inclusion criteria were DR TB patients, who received treatment from January 2015 to August 2020, who had finished receiving DR treatment, age >18 years, complete medical records related to the data needed in this study. Exclusion criteria for DR TB patients with blood disorders, patients in pregnancy who had not finished treatment, prior history of anemia and arthralgia, incomplete medical record data related to this study, patients who refused to be interviewed and or fill out a questionnaire.

The basic data were processed descriptively. Statistical analysis has been done with resistance and quality life of DR TB patient as the dependent variable. Delay on treatment, arthralgia, hyperuricemia, and anemia were independent variables. Estimation of the magnitude of the association of two variables with relative risk. Survival analysis to analyze data with time until the occurrence of a certain event as a response variable. Survival rate and median survival using survival analysis and Log Rank test to know the difference between the two survival curves (Kaplan Meier). Quality of life scores were analyzed using the Mann Whitney Test. The relationship between the independent variable and the dependent variable used the Chi square test followed by Cox regression analysis (multivariate). The statistical test used 95% confidence limit value (95% *Confidence Interval*) or a significance limit value of $P<0.05$. Data analysis using computer program SPSS 21 for windows.

RESULTS

Baseline characteristic data was obtained at the beginning of the TB treatment, while the data about arthralgia, anemia, and hyperuricemia was taken during the TB treatment. The primary data taken is the data on the quality of life of DR TB patients. The purpose of this study was to determine the effect of delay in treatment, arthralgia,

hyperuricemia, and anemia on healing, survival, and quality of life of drug-resistant TB patients.

The results of recording the medical records of drug-resistant TB patients obtained as many as 397 drug-resistant TB patients, but those used in this study were 372 patients, because as many as 25 patients did not have complete data. The results of the characteristics of research subject data characteristics based on the results of research on 372 DR TB patients can be seen in Table 1 as follows.

Table 1. Basic Characteristics of Research Subjects (N=372)

Variable	Frequency	%
Gender		
Male	232	62.3
Female	140	37.7
Age		
18–40 years	154	41.3
41–60 years	179	48.1
>60 years	39	10.6
Healed		
Cured	215	57.8%
Not cured	157	42.2%
Died		
Alive	282	75.8%
Died	90	24.2%
Late treatment		
No	205	55.1%
Yes	167	44.9%
Hyperuricemia		
No	76	20.4%
Yes	296	79.6%
Arthralgia		
No	165	44.4%
Yes	207	55.6%
Anemia		
No	177	47.6%
Yes	195	52.4%

Among 327 patients who are selected as the subjects of this study, most of the patients were male (62.3%) and aged between 41–60 years (48.1%). Baseline characteristic data showed that 57.8% of patients were cured at the end of TB DR treatment and 75.8% of patients were still alive.

The data about delay in DR TB treatment among the patients was obtained. Among 327 patient, 44.9% patients were failing to receive a TB DR treatment before 14 days after they diagnosed with TB DR. Based on the data, 79.6% patients had hyperuricemia, 55.6% had arthralgia, and 52.4% had anemia.

Table 2. Multivariate analysis of variables that affect the recovery of DR TB patients

Variable	OR (95%CI)	*P*
Treatment delay	2.906 (1.890-4,469)	<0.001*
Arthralgia	1.775 (1.148-2.744)	0.010*
Treatment delay	2.906 (1.890-4,469)	<0.001*

Based on logistic regression analysis, it was found that treatment delay (OR=2.906; 95% CI=1.890-4.469; *P*≤0.001) and arthralgia (OR=1.775, 95% CI=1.148-2.744; *P*= 0.010) is a variable that has a significant effect on the recovery of DR TB patients. Delay in treatment has a higher risk (2.906 times) of non-healing DR TB patients compared to arthralgia (1.775 times).

Because there are 3 variables that have a significant effect on the *outcome of* death, namely delay in treatment (OR=2.385; 95% CI=1.467–3.879); *P*≤0.001), arthralgia (OR=2.563; 95% CI=1.529–4.299; *P*≤0.001) and anemia (OR=2.178; 95% CI=1.325–3.579; *P*=0.002) then followed by multivariate regression analysis logistics as follows.

Table 3. Multivariate analysis of variables that affect the *outcome* of DR TB patients

Variable	OR (95%CI)	*P*
Delay in Treatment	2.309 (1.402–3.802)	0.001*
Arthralgia	2.302 (1.349–3.928)	0.002*
Anemia	1.817 (1.084–3.047)	0.024 *

Note: Logistic regression test = *Significant at *P*≤0.05

The effect of delay in treatment, arthralgia, hyperuricemia, and anemia on the quality of life of DR TB patients involved 103 recovered patients. The effect of delay in treatment, arthralgia, hyperuricemia, and anemia on the quality of life of DR TB patients in this study used a different independent t-test if the data met the assumption of normality.

If the data does not meet the assumption of normality, then use the Mann-Whitney test, this is because the quality of life data (SF-12 score) is in the form of numerical data. The results of statistical analysis of the effect of delay in treatment, hyperuricemia, arthralgia and anemia on the quality of life of DR TB patients can be seen in Table 4 as follows.

Table 4. Effect of delay in treatment, arthralgia, hyperuricemia and anemia on quality of life of cured DR TB patients (N=103)

Variable	N	Score SF-12 (Quality of Life)		
		Mean±SD	Mean Difference	*P*
Late Treatment				
No	68	40.04±5.57	0.66	0.525
Yes	35	39.82±4.50		
Hyperuricemia				
No	22	37.82±6.38	2.74	0.082
Yes	81	40.56±4.72		
Arthralgia				
No	52	40.60±5.29	-1.26	0.131
Yes	51	39.33±5.10		
Anemia				
No	55	41.00±4.93	-2.21	0.021*
Yes	48	38.79±5.32		

Note: Whitney-Mann test = *Significant at *P*≤0.05

Based on table 16 it is known that treatment delay (diff mean = -0.66; *P*=0.525), arthralgia (diff mean = -1.26; *P*=0.131) and hyperuricemia (diff mean = 2.74; *P*=0.082) did not show a significant effect on the SF-12.

Anemia (diff mean = -2.21; *P*=0.021) showed a significant effect on the SF-12 score (Quality of Life). Where DR TB patients who do not have anemia (41.00±4.93) have a better quality of life compared to DR TB patients with anemia (38.79±5.32).

Survival analysis has been done in this study to see the effect of delay in treatment, arthralgia, hyperuricemia, and anemia on DR TB patient survival. The overall survival rate of 90 died patients among 372 subjects who are selected in this study is 75.8%, and the average survival is 17.364 (95% CI=16.510–18.218) months.

Table 5 shows the overall survival analysis of DR TB patient who were delayed in treatment and had certain medical condition such as hyperuricemia, arthralgia, and anemia. Among 205 patients who were not delayed in the DR TB treatment, 35 patients died. The survival rate is 82.9% and the average survival is 18.887 (95% CI=17.919–19.855) months.

The analysis also has been done based on the prior history of arthralgia, hyperuricemia, and anemia. Approximately 65 patient died among 207 patients without arthralgia, with survival rate 68.6% and average survival is 15.170 (95% CI=13.960–16.379) months. The survival rate of 75 deceased patients with hyperuricemia is 74.7%, with average survival 16.362 (95% CI=15.426–17.298) months. Sixty deceased patients with anemia has survival rate approximately 69.2%, and the average of survival is 15.304 (95% CI=14.074–16.535) months.

Table 5. Effect of delay in treatment, arthralgia, hyperuricemia and anemia on DR TB patients' survival

Variable	Total N	N of events	Mean	95% CI
Late Treatment				
No	205	35	18.887	17.919–19.855
Yes	167	55	14.772	13.381–16.163
Hyperuricemia				
No	76	15	18.422	16.746–20.098
Yes	296	75	16.362	15.426–17.298
Arthralgia				
No	165	25	19.230	18.195–20.266
Yes	207	65	15.170	13.960–16.379
Anemia				
No	177	30	18.916	17.867–19.964
Yes	195	60	15.304	14.074–16.535
Overall	372	90	17.364	16.510–18.218

Note: Log rank = *Significant at *P*≤0.001

Log rank analysis has been done to know the significancy of patient's survival in each group. It comes to the conclusion that there is a significant difference of survival rate between DR TB patient who were had the treatment on time and the patient who were delayed in treatment (Log Rank = 16.582; *P*≤0.001). The same result also found in two other variables. There is significant difference of survival rate between two groups of each medical condition, Arthralgia (Log Rank = 14.390; *P*≤0.001) and Anemia (Log Rank = 9.297; *P*=0.002). Based on the result of the analysis, there is no significant difference between survival rate of DR TB patient with and without Hyperuricemia (Log Rank = 0.951; *P*=0.329).

DISCUSSION

The characteristics of drug resistant tuberculosis patients in this study during the period January 2015 – August 2020 as many as 372 patients, after exclusion in as many as 25 patients Gender DR TB patients in this study are predominantly male (62.3%). In this study 48.1% patients were aged 41–60 years. The male gender tends to be at higher risk of being infected with DR TB because many men work outside the home and interact with the community, while not all of the

female gender work outside, many also work as housewives. In productive age, humans tend to have more mobility so that they have a higher possibility of exposure to Mtb germs.

In this study, delays in treatment with *outcomes* did not heal as much as (59.9%). Treatment delay (OR=2.902; 95% CI=1.895–4.444; *P*≤0.001) in this study was a risk factor for the non-healing of DR TB patients. Most of the DR TB patients are patients with secondary resistance. Patients with a history of drug-sensitive TB who have already undergone TB treatment with all the side effects that arise, when diagnosed with DR TB patients may feel discouraged and not eager to seek treatment. Moral support from the psychological side can play a role since the patient is diagnosed with DR TB and before starting treatment to be able to accompany DR TB treatment.

The delay in treatment (OR=2.385; 95% CI=1.467–3.879; *P*≤0.001) in this study is a risk factor for the death outcome of DR TB patients. Patients with delay in treatment are at risk of dying 2.385 times greater than patients who do not experience delays in treatment. Statistical tests showed that there was a significant effect between delay in treatment and the outcome of dying of DR TB patients. One of contributing factor to delay in treatment is the never-ending bureaucracy and administration due to tiered healthcare insurance system. It is possible that the patient's lack of education and knowledge about drug-resistant tuberculosis contributed to the delay in treatment. Delay in treatment can cause DR TB patients tend to experience clinical deterioration with opportunistic infections to severe complications and cause death based on the Adiwinata's research in 2018, the national health insurance rules for tuberculosis treatment had to step by step from primary healthcare facilities. This rules also take the important step of delay in treatment and diagnosis.[6,12,14]

The survival of 207 patients who experienced arthralgia, there were 65 patients died with the survival rate of 68.6%. DR TB patients who died with arthralgia had an average survival of 15.170 months. The Log Rank test showed that there was a significant difference in patient survival between those without arthralgia and patients with arthralgia (Log Rank = 14.390; *P*≤0.001). Arthralgia has a significant effect on the survival of DR TB patients. Patients with arthralgia have lower survival because there are disturbances in daily activities that affect the psychology of DR TB patients. Arthralgia in this study was not the main cause of death. Arthralgia is a factor that can aggravate the patient's condition, resulting in clinical deterioration or death. There are many factors associated with a direct effect on the survival of DR TB patients.[8,13]

Hyperuricemia (OR=1.324; 95% CI=0.788–2.227; *P*=0.289) in this study was not a risk factor for non-healing DR TB patients. There was no significant effect between hyperuricemia and recovery in DR TB patients (*P*>0.05). In the classification of antitubercular medications of DR TB for individual regimens, they are divided into 3 groups, group A which is considered the main drug with the best efficacy, group B and group C. The fluoroquinolones (levofloxacin, moxifloxacin) are included in group A which is in the treatment of individual TB therapy. The DR must consist of at least 5 drugs with the division of 3 drugs from group A and 2 drugs from group B. Group C is additional if there is one class A or B drug that cannot be given.[1,3,7,8]

In group C treatment, Ethambutol is the first choice while Pyrazinamide is the third choice, thus increasing the possibility of hyperuricemia caused by antitubercular medications. In the short-term therapy regimen according to WHO 2020 guidelines, treatment is given for 9–11 months where levofloxacin, pyrazinamide and ethambutol are continued during treatment. If hyperuricemia is controlled and does not cause complaints, it is not necessary to change the treatment regimen. Hyperuricemia can cause disturbing joint pain symptoms, which is one indication of changing the regimen to an individual regimen. The patient's recovery had no effect on increased uric acid levels.[1,3,7,8]

Based on the incidence of hyperuricemia (OR=1.380; 95% CI=0.740–2.572; *P*=0.309) in this

study was not a risk factor for the death outcome of DR TB patients. There was no significant effect between hyperuricemia and the outcome of DR TB patients (*P*>0.05). Hyperuricemia is associated with high blood pressure, atherosclerosis, kidney failure, and diabetes mellitus so that the outcome of death is not directly caused by hyperuricemia. In cardiac disorders, hyperuricemia is a risk factor that causes vascular endothelial damage through the mechanism of ROS formation.

The survival rate of DR TB patients who did not experience hyperuricemia was 80.3% with an average survival of 18.422 (95% CI=16.746–20.098) months. The average survival was 16.362 (95% CI=15.426–17.298) months, or it can be said that DR TB patients who died and experienced hyperuricemia had an average survival of 16.362 months. The Log Rank test showed that there was no significant difference between the survival of DR TB patients between patients who did not have hyperuricemia and had hyperuricemia (Log Rank = 0.951; *P*= 0.329). Hyperuricemia has no significant effect on the survival of DR TB patients.

The use of Pyrazinamide has been shown to increase the rate of sputum conversion. The success and cure of therapy depends on the patient's tolerance and medication adherence. Factors that cannot be controlled by researchers are the consumption of foods or diets that are high in purines, which can affect uric acid levels. The research subjects in this study were included in the criteria for hyperuricemia by looking at the increase in uric acid levels during baseline blood tests and when taking antitubercular medications. Hyperuricemia can be divided into a*symptomatic hyperuricemia* and *symptomatic hyperuricemia*. Hyperuricemia that causes symptoms can affect *activities of daily living and* affect survival. In this study, researchers did not distinguish between symptomatic and asymptomatic hyperuricemia. There is no significance of hyperuricemia on survival.[7,8]

According to the Guideline: *Nutritional care and support for patients with tuberculosis* from WHO, malnutrition in TB patients can be caused, among others: polypharmacy, decreased appetite, decreased oral intake, nausea, vomiting, abdominal pain, diarrhea and vomiting. catabolic reaction. TB infection and malnutrition are related. Patients with malnutrition or undernutrition will reduce their immunity so that they are easily infected with TB disease or reactivation of TB infection.[1,3]

Anemia (OR=2.178, 95% CI=1.325–3.579; *P*=0.002) in this study is a risk factor for the death outcome in DR TB patients. Patients with anemia risk of dying 2.178 times greater than patients without anemia. Anemia in this study was associated with chronic inflammatory disease and inadequate nutritional intake. This is in line with the research by Fantaw et al, in 2018 which stated that DR TB patients who had a lower initial body weight had 56% higher risk of dying. Multivariate Cox-regression analysis in this study assessed anemia as a significant predictor of mortality among drug-resistant TB patients. Malnutrition that triggers anemia is associated with drug toxicity which can contribute to default and eventually lead to death.[8–11,15]

Log Rank test in this study showed that there was a significant difference in the survival of DR TB patients between those who were not anemic and anemic (Log Rank = 9.297; *P*=0.002). Anemia has a significant effect on the survival of DR TB patients. Patients with anemia has lower survival. Economic support and nutritional intervention can reduce the mortality of DR TB patients.

Tuberculosis is a disease that can reduce the patient's quality of life. Long-term treatment, polypharmacy therapy, toxic reactions and drug side effects, medication adherence, social support, social and family acceptance, lifestyle changes, marital status, level of access to health care services, socioeconomic status, knowledge of patients and families about the disease, treatment, as well as complications of the disease mutually influence the quality of life of DR TB patients. According to WHO there are four aspects of quality of life, namely: 1) physical health which includes daily activities; 2) psychological well-being which includes self-image and appearance, spirituality, thinking, learning, memory and concentration; 3) social relationships

which include personal relationships and support social; 4) relationship with the environment which includes financial resources, freedom, physical security and health insurance. In this study, anemia (diff mean = -2.21; *P*=0.021) showed a significant effect on the SF-12 score. DR TB patients who did not have anemia (41.00±4.93) had a better quality of life than DR TB patients who had anemia (38.79±5.32).[8,16,17]

Some of the patients that we contacted and some of the patients who came to the DR TB polyclinic still felt tired quickly and were unable to carry out strenuous activities even though they had been declared cured, thus affecting the patient's daily activities and the quality of life of the patient. The social aspect has not changed much because DR TB patients who have recovered have received a certificate of completion of treatment and are declared cured. Drug side effects due to hyperuricemia are not felt by many patients after recovering. Arthralgia side effects some of the patients still feel especially in some patients suffering from TB with diabetes. A side effect that is quite disturbing until after recovery is hearing loss which we did not investigate further in this study.[8,18]

LIMITATION

Limitations in this study are secondary data regarding the date time for treatment, complaints of joint pain, uric acid levels, hemoglobin levels that do not complete data collection can be difficult. A lot of phone number data which has changed and the latest telephone number data is not included so that the researcher was unable to contact to ask for a quality of life questionnaire. When this research took place, it coincided with a spike in the number of the incidence of covid and restrictions on community activities were carried out so that some research subjects could not come to the workshop.

CONCLUSION

Delay in treatment, arthralgia, affects the recovery of TB RO patients. Treatment delay, arthralgia, and anemia affect the survival of RO TB patients. Anemia affects the quality of life of TB RO patients.

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CONFLICT OF INTEREST

None.

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Original Article

Change in Exhaled Volatile Organic Compounds (VOC) Profile and Interleukin-17 Serum in Lung Cancer Patient

Agil Dananjaya,[1] Ungky Agus Setyawan,[1] Susanthy Djajalaksana,[1] Arinto Yudi Ponco Wardoyo[2]

[1]Departement of Pulmonology and Respiratory Medicine, Faculty of Medicine Universitas Brawijaya, RSUD Saiful Anwar, Malang, Indonesia
[2]Departement of Physical Science, Faculty of Medicine Universitas Brawijaya, Malang, Indonesia

Abstract

Background: In recent years, there have been studies regarding biomarkers for early detection of lung cancer. The expansion of tumor is accompanied by distinct metabolic process product, which results in identifiable changes in the volatile organic compounds (VOC) emission profile. The content of such molecules differs between healthy and lung cancer patients. Furthermore, the expression of Interleukin-17 (IL-17) was linked to the clinical and pathological aspects of lung cancer patients. The aim of this study is to profile the exhaled VOC and the level of IL-17 in the serum of lung cancer patient.

Methods: Fourty patients with confirmed lung cancer and 42 healthy subjects as control were gathered for this study. VOC was measured using breath analyzer and sensor array, while IL-17 was measured by ELISA. Statistical analysis was conducted using Kruskal-Wallis test and Spearman correlation test with $P<0.05$ considered significant.

Results: We examined 15 VOCs and found that ethanol (C_2H_5OH), formaldehyde (CH_2O), toluene (C_7H_8) and ammonia (NH_3) in lung cancer patient were increased significantly compared to control ($P<0.05$; $P<0.05$; $P<0.05$ and $P=0.001$ respectively). However, the level of IL-17 in control subjects was higher ($P=0.299$) than patients with lung cancer.

Conclusion: Ethanol, formaldehyde, toluene and ammonia can potentially be used as biomarkers for lung cancer. However, the role of IL-17 in lung cancer screening still needs further investigations.

Keywords: interleukin-17, lung cancer, VOC

Corresponding Author:
Agil Dananjaya | Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Brawijaya, RSUD Saiful Anwar Malang, Indonesia | akkun18@student.ub.ac.id

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INTRODUCTION

In recent years, lung cancer has become one of the main causes of cancer deaths globally. Data from GLOBOCAN 2018 stated that there is an estimation of 2.09 million new cases and 1.76 million deaths from lung cancer.[1] Lung cancer is predicted to be the main cause of cancer death in men and women in the next 20 years as well.[2] All lung malignancies, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) combined, have a 5-year relative survival rate of 19%, and NSCLC has higher 5-year survival rate (23%) than SCLC (6%).[3] One of numerous factors that contributes to the poor outcome in lung cancer is that the disease is frequently only diagnosed at an advanced stage after the patient has developed symptoms. This highlights the importance of early detection of lung cancer in order to improve the patients' survival.[4]

The one method that's currently approved for lung cancer screening is low-dose computerized tomography scan (LDCT). Studies show that this method lowers lung cancer mortality by 20% and is indicated for high-risk individuals. However, there is a risk of radiation exposure, high expense and high likelihood of false positives when employing LDCT as a screening approach.[4,5]

Therefore, several other methods have been developed as screening tools, namely the analysis of volatile organic compounds (VOCs) or specific genomic approaches. VOCs referred to volatile organic compounds that can be found in the human body. In patients with lung cancer, VOCs are metabolized differently and its profile is changed. However, there are currently no consistent VOC biomarkers for lung cancer and the VOC set used in investigations also varies. Therefore, breath analysis is still in an early stage of clinical application.[6]

T-helper 17 (Th17) cells are the principal producers of interleukin-17 (IL-17), a proinflammatory cytokine. IL-17 and IL-17-expressing cells have lately been investigated in a variety of cancers, including NSCLC. In NSCLC patients, high level of IL-17 in serum was found to be associated with late stage of the disease, overall survival (OS) and disease-free survival (DFS). IL-17 expression was also significantly elevated in human NSCLC tissues and associated with clinical and pathological characteristics of patients, such as TNM staging, OS and DFS. Furthermore, in NSCLC patients, the frequency of IL-17-producing T cells have been observed to be dysregulated.[7]

In spite of both VOCs and IL-17 increase in lung cancer, the clear explanation for the correlation remains unclear. In this study, we aim to profile exhaled VOC and serum IL-17 in lung cancer patients.

METHODS

This study was conducted in RSUD dr. Saiful Anwar Malang, East Java, Java, Indonesia from October 2021 to January 2022. Patients with lung cancer found in outpatient clinics and wards were enrolled in the study. The inclusion criteria were patients with primary lung cancer who were in stable condition and consented to take part in the study. Subjects with secondary lung cancer and in acute or unstable condition were excluded. The control group consisted of healthy subjects without lung cancer based on anamnesis, physical examination and radiological finding. Minimal number of samples from each group is 31. Samples were obtained by consecutive sampling. Eighty-two subjects who met inclusion and exclusion criteria were measured for their exhaled VOC and IL-17 serum.

Exhaled VOCs were measured using a breath analyzer. The breath analyzer was developed by Universitas Brawijaya, Malang (Ubreath) which does analysis and measurement using sensor array. Samples of VOCs were collected using breath apparatus and connected to Ubreath. Data was automatically entered and collected in the computer. Serum was obtained by phlebotomy and the samples were sent to biomolecular laboratory to have the IL-17 level measured by ELISA.

Data were logarithm-transformed as necessary to meet normality and homoscedasticity criteria. Kruskal-Wallis one-way analysis of variance (ANOVA) was used to find any significant changes in VOC profile and IL-17 levels between lung cancer and control group. To examine the difference in VOC profiles in lung cancer patients depending on the cancer type, stage and management, researchers used repeated ANOVA. The Spearman correlation test was used to find the association between VOC profile and IL-17 in the two groups. With value of $P<0.05$, differences were measured using IBM SPSS software version 25.0.

RESULTS

Among the 82 subjects, 40 were lung cancer patients and 42 healthy controls (Table 1). The median age for lung cancer group was 56.6 years old, which is older than the control group. There were more male than female subjects, with percentage of 60% vs 40% in lung cancer group and 57.14% vs 42.86% in control group. Most subjects in lung cancer group were smokers (55% vs 45%), while the opposite was found in control group in which 97.7% were non-smokers.

Table 1. Demography of Study Subject

Characteristic	Lung Cancer (n=40)	Control (n=42)
Age, range (mean)	40-72 (56.6)	25-38 (30.8)
Sex		
Male	24 (60.00%)	24 (57.14%)
Female	16 (40.00%)	18 (42.86%)
Smoking		
Smoker	22 (55.00%)	1 (2.30%)
Non-smoker	18 (45.00%)	41 (97.70%)
Histological type		
Adenocarcinoma	27 (67.50%)	-
Adenosquamous cell carcinoma	4 (10.00%)	-
Squamous cell carcinoma	5 (12.50%)	-
Small cell lung cancer	4 (10.00%)	-
Stage		
IIIB	2 (5.00%)	-
IVA	15 (37.50%)	-
IVB	23 (57.50%)	-
Chemotherapy		
Chemotherapy	34 (85.00%)	-
Targeted therapy	6 (15.00%)	-

In lung cancer group (n=40), the most prevalent type of lung cancer was adenocarcinoma (67.50%), followed by squamous cell carcinoma (12.50%), adenosquamous cell carcinoma (10.00%) and small cell carcinoma (10.00%). All patients were found at late stage of the disease, with 57.50% in stage IVB, 37.50% IVA and 5.00% IIIB. All lung cancer patients had received chemotherapy (85.00%) or targeted therapy by tyrosine kinase inhibitor (15.00%).

We evaluated the concentrations of 15 distinct VOCs in exhaled air between lung cancer patients and healthy controls, using value of *P*<0.05 to account for multiple comparisons (Table 2). Ethanol, toluene, formaldehyde and ammonia concentrations in the exhaled air of lung cancer patients were considerably higher (*P*<0.05; *P*<0.05; *P*<0.05 and *P*=0.001 respectively) than in the subject control group. Thus, the researcher focused on those four compounds in this study.

Table 2. Profile of Volatile Organic Compound Subject

VOCs	Lung Cancer (Mean) ppm	Control (Mean) ppm	*P*
Oxygen (O_2)	21.22254	20.7988	0.282
Ozone (O_3)	58.14644	110.8965	0.0001
Carbon Dioxide (CO_2) (1)	1470.21055	701.1388	0.188
Carbon Dioxide (CO_2) (2)	1496.00533	714.5339	0.204
Ethanol (C_2H_5OH)	1.24580	0.8148	0.0001
Formaldehyde (CH_2O)	0.51899	0.0453	0.0001
Toluene (C_7H_8)	0.61858	0.0167	0.0001
Acetone (C_3H_6O)	0.08536	0.2279	0.0001
Ammonium (NH_4)	0.44946	0.9996	0.0001
Hexane (C_6H_{14})	0.41880	0.4589	0.0001
Nitrogen (NO_2)	0.98333	1.5615	0.001
Carbon Monoxide (CO)	0.00009	0.0000	0.306
Ammonia (NH_3)	0.90681	0.6637	0.001
Methane (CH_4)	0.47846	0.5175	0.0001
Sulphur Dioxide (SO_2)	2.59492	2.5316	0.133

Furthermore, we investigated the differences between histological types of lung cancer, stage and therapy with the VOCs (Table 3). The concentration of ethanol, toluene, formaldehyde and ammonia in the exhaled air of lung cancer patients did not differ significantly between each histological type of lung cancer (*P*=0.404; *P*=0.978; *P*=0.967 and *P*=0.535 respectively), neither did they with the cancer stages (*P*=0.298; *P*=0.086; *P*=0.086 and *P*=0.107 respectively) nor the therapy (*P*=0.570; *P*=0.081; *P*=0.081; *P*=0.130 respectively).

Table 3. The differences between the histological types, stage and therapy of lung cancer with VOCs and IL-17

VOCs	Ethanol (*P*)	Formaldehyde (*P*)	Toluene (*P*)	Ammonia (*P*)	IL-17 (*P*)
Histological type	0.404	0.967	0.978	0.535	0.751
Stage	0.298	0.084	0.086	0.107	0.342
Therapy	0.570	0.081	0.081	0.130	0.363

While comparing the IL-17 level between both groups, we found the level of IL-17 in control group was higher (*P*=0.299) than lung cancer group (Figure 1) with no significant difference between those two groups.

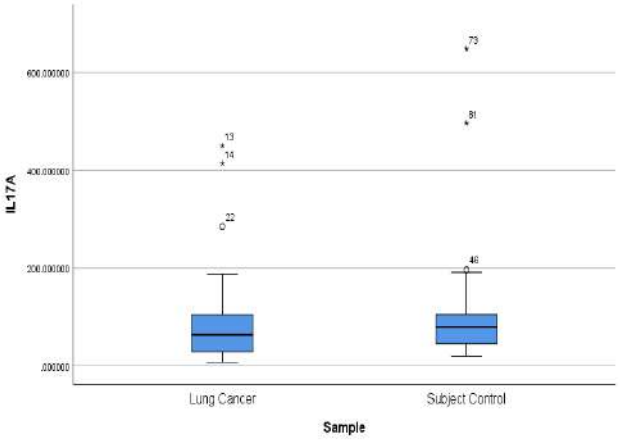

Figure 1. IL-17 comparison between lung cancer and control groups

We also examined the level of IL-17 on patient with lung cancer, according to the lung cancer type, stage and therapy. No significant differences were found with *P*=0.751; *P*=0.342 and *P*=0.363 respectively (Table 3). There wasn't any correlation either between the concentration of those four VOCs and the level of IL-17 statistically (*P*=0.277; *P*=0.477; *P*=0.412 and *P*=0.269 respectively) (Table 4).

Table 4. Association between VOC and IL-17

VOCs	IL-17 (*P*)
Ethanol	0.277
Formaldehyde	0.477
Toluene	0.412
Ammonia	0.269

DISCUSSION

The goal of this study is to determine whether VOCs and IL-17 may be used for early detection of

lung cancer. Lung cancer is a disease that's mostly asymptomatic in the early stages but fatal in an advanced stage. Radiological imaging can be used for lung cancer screening while a definitive diagnosis is determined by histopathology examination. VOCs from exhaled air may reflect metabolic changes caused by the disease and play a role as biomarkers for lung cancer.[8]

We identified ethanol, toluene, formaldehyde and ammonia as possible biomarkers for lung cancer, especially in advanced stages. Several studies have been conducted to identify components of VOC compounds. One study conducted by Oguma et al. concluded that ethanol and toluene concentration had shown an increase in lung cancer compared to the control group.[9]

Differences in VOC concentrations caught on the sensor devices are influenced by metabolic activity in cancer cells. Cancer cells directly undergo changes in their metabolic products since they need large amount of energy to support its uncontrolled proliferation. The Warburg effect is a cancer metabolism process in which the activation of aerobic glycolysis occurs as the main pathway for obtaining energy. Changes in cellular metabolism result in metabolic changes that accelerate the growth of cancer cells and also change the profile of respiratory VOCs.[10] The increased ethanol concentrations in the study were most likely caused by the Warburg effect on the cancer patient group.

The concentration of formaldehyde was also found to elevate in this study. Endogenous formaldehyde may increase in malignancy. In studies involving patients with breast and prostate cancer, it has been reported that there's an increase of the endogenous formaldehyde concentration in the urine. In vivo studies also show abnormally elevated formaldehyde inside cancer cell tissue. Endogenous formaldehyde is produced through a number of biochemical pathways in cells through the enzymatic reaction process of oxidative demethylation. Other factors such as cigarette smoke, electronic cigarettes and aspartame sweeteners can also cause increase in the amount of formaldehyde.[11]

The increase of ammonia concentration found in this study can be explained by cancer cells' need to absorb and process high amount of glutamine as a supplement for nucleotide biosynthesis. Glutamine is a non-essential amino acid that can be synthesized by cells through glutamine synthetase and is present in the blood in the form of free amino acids. Ammonia is formed as a result of the breakdown of glutamine into glutamate.[12] This is similar to Spinelli's research (2018) which stated that ammonia is a cellular metabolism product that is mainly excreted by proliferating cells, for example cancer cells. Ammonia accumulates in tumor microenvironment 10 times higher compared to healthy tissue.[13]

Toluene has been researched as biomarker for detecting lung cancer. Toluene is a metabolite product of cancer cells, hence its concentration tends to elevate in lung cancer. However, the mechanism remains unclear.[14]

The global incidence of lung cancer shows that NSCLC accounts for 80-85 percent of all lung cancer. According to Chen et al, the cytokine IL-17 is vital in the process of microangiogenesis in the tumor microenvironment, stimulates cell proliferation and has a role in the metastatic process. Wu et al. also found that IL-17 promotes tumor angiogenesis and cell proliferation while also inhibits apoptosis via inflammatory activation pathways.[15,16]

Several studies have been conducted to investigate the expression of IL-17 cytokines in the peripheral blood of NSCLC patients. Dutkowska et al. revealed that IL-17 is a proinflammatory cytokine that plays a role in chronic inflammation, autoimmunity and malignancies associated with inflammation. They further claim that IL-17 plays a direct or indirect function in lung cancer spread and progression, boosting tumor angiogenesis and cell proliferation while blocking apoptosis. Hence, higher level of IL-17 expression was linked to earlier stages of cancer.[17]

In our study, the average levels of IL-17 in lung cancer were smaller than those of healthy subjects (87.77 and 101.03 pg/mL) with a $p > 0.05$. This result is not in line with the study conducted by Chen et al which found that IL-17 expression in lung cancer was

higher compared to the control group. Since Chen et al's study used subjects with untreated lung cancer, we assumed that these differing results happened because our patients have been treated by chemotherapy that might affect the concentration of IL-17. This is consistent with the study by Xiang et al which revealed that there was a decrease in serum IL-17 levels in breast cancer patients who received chemotherapy and radiotherapy compared to the control group, though the underlying mechanism remains unclear.[16,18]

Another study by Wang et al explored the association between lung cancer prognosis and IL-17 level, which shows that the increase in IL-17 expression was closely related to poor clinical output in lung cancer patients. Our study didn't find any significant differences between IL-17 levels to lung cancer stadium and types.[19]

LIMITATION

Our research has a number of limitations. Firstly, our study used patients who were receiving chemotherapy at the time of enrollment, which might have influenced the concentration of inhaled VOCs and IL-17. Secondly, due to the small number of early-stage patients, the current investigation was underpowered to discover exhaled VOCs relevant for early diagnosis of lung cancer. To continue and complete the VOC and IL-17 profile that is useful in evaluating patients with suspected lung cancer, a prospective study is required.

CONCLUSION

Some exhaled VOCs such as ethanol (C_2H_5OH), formaldehyde (CH_2O), toluene (C_7H_8) and ammonia (NH_3) in lung cancer patients were higher compared to control group, hence their potential as biomarkers of lung cancer. However, the level of IL-17 in this study was higher in control group instead. IL-17 has weak correlation with VOCs.

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CONFLICT OF INTEREST

None.

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Original Article

The Degree of Inflammation and Length of Hospital Stay in Acute Exacerbation of COPD Patients After Secretome Administration

Hendrastutik Apriningsih,[1,4] Yusup Subagio Sutanto,[1,3] Reviono,[1,4] Artrien Adhiputri,[1,3] Windu Prasetya,[4] Fahlevie,[1] Fitrotin Aziza[2]

[1]Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia
[2]Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia
[3]Moewardi Regional General Hospital, Surakarta, Indonesia
[4]Sebelas Maret University Hospital, Sukoharjo, Indonesia

Abstract

Background: Acute exacerbation of COPD (AECOPD) is a decrease in respiratory conditions compared with regular conditions that require additional treatment, increase risk of hospitalization or demand intensive care unit. The neutrophil-to-lymphocyte ratio (NLR) describes the balance between the severity of inflammation and the immune system and is considered as an important systemic inflammatory marker. Length of hospital stay (LHS) is important in predicting the severity of AECOPD, in which longer LHS indicates greater severity of AECOPD. Secretome has been shown to have the ability to exert immunomodulatory effects, reduce lung injury and inflammation in several models of lung inflammation and immune-mediated lung disease. This study aimed to assess the differences between NLR and LHS in AECOPD patients who received a secretome and those who did not.

Methods: This study involved 30 AECOPD patients whom assigned into two groups. Secretome and standard therapy were administered in the treatment group, whilst the control group only received the standard therapy. Statistical analysis used different test, the unpaired group difference test using Mann Whitney and the independent test, the paired group difference test using Wilcoxon rank test and Pair test. Result is significant if the *P*<0.05.

Results: NLR value in the treatment group experienced a lower increase than the control group, yet statistically insignificant (*P*=0,187). Secretome decreased the LHS in AECOPD patients, and statistically significant with (*P*=0,028).

Conclusion: Administration of secretome led to a lower increase in NLR value and decreased LHS AECOPD patients.

Keywords: acute exacerbation of COPD, secretome, NLR, LHS

Corresponding Author:
Hendrastutik Apriningsih Department of Pulmonology and Respiration Medicine, Faculty of Medicine, Sebelas Maret University, Universitas Sebelas Maret Hospital, Sukoharjo, Indonesia | hendrasapriningsih@staff.uns.ac.id

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INTRODUCTION

The GOLD definition of COPD is a widespread, preventable, and treatable condition, defined by persistent respiratory symptoms and airflow limitation resulting from airway and/or alveolar abnormalities.[1] 10% of people around the world have COPD, which lowers patients' quality of life and life expectancy.[2] The prevalence of COPD increased from 11% in 1990 to 44% in 2015.[3] If COPD is not effectively managed, it is predicted to be the third most common cause of death in the world by 2030.[4]

The annual expense of treating COPD rises in direct proportion to the frequency of COPD exacerbations. Hoogendoorn et al claimed that incidence of exacerbation in 2017 in Netherlands occurred in >50% of COPD patients. COPD patients may experience several exacerbations each year.[5] According to Hurst et al, in the UK in 2021, 23% of COPD patients reported having one or more moderately severe exacerbations per year, and 14% of patients had at least three such episodes.[6] One of the four most severe non-communicable diseases, COPD is responsible for 60% of deaths in Indonesia and will continue to add to the burden of disease in developing countries by 2030.[7,8] AECOPD raises hospitalizations as well as the cost of care and therapy.[9,10]

Systemic inflammation in COPD is characterized by elevated levels of neutrophils, IL-8, IL-6, TNF-α, and decreased levels of lymphocytes. Those play a role in the development of disease and

comorbidities, causing an increased risk of morbidity and mortality.[11,12] Current standard therapy for COPD has not been able to slow the decline in lung function and mortality, so it is necessary to develop additional therapies for COPD.[8,13]

Stem cells have the ability to self-renew repeatedly and produce a single type of highly differentiated cell lineage. Maintaining cell regeneration is one of the important stem cell functions. Stem cells are present in most body tissues from early embryogenesis throughout adult life and are thought to play a role in tissue maintenance and repair.[13] One of the most studied stem cell is mesenchymal stem cells (MSC) and has a broad therapeutic effect in various pre-clinical models of lung disorders. Secretome is one of the MSCs that provides various beneficial effects. MSC-derived molecules have the capacity to modify inflammatory and regenerative activity in paracrine ways because they contain a variety of bioactive molecules, including cytokines, chemokines, growth factors, angiogenic factors, and extracellular vesicles. MSC-based therapies are safe and well-tolerated in clinical research.[14] Research by Weiss et al in 2021 reported the results of a placebo-controlled trial of MSC in 62 patients with moderate to severe AECOPD showing no serious side effects, no increase in the level of exacerbations, and no deterioration of the disease.[15]

There are no studies that address the effect of *secretome* on the degree of inflammation in patients with AECOPD. Therefore, this study was designed to explain the potential of *secretome* as adjuvant therapy for acute exacerbations of COPD in decreasing inflamation and LHS in acute exacerbating COPD patients treated at Moewardi General Hospital Surakarta, Universitas Sebelas Maret General Hospital Sukoharjo, and Soehadi Prijonegoro General Hospital Sragen. This study was approved by the medical research Ethics Committee No: 421/IV/HREC/2022, issued by the Moewardi General Hospital Surakarta.

METHODS

The study design was an experimental randomized controlled trial using pre-test and post-test control group design. This study was conducted at three separate hospitals, including Moewardi Regional General Hospital, Sebelas Maret University Hospital, and Soehadi Prijonegoro Regional General Hospital in Central Java, Indonesia between July-October 2022. Our study had received approval from the Research Ethics Committee of the Moewardi Regional General Hospital Surakarta.

This study involved 30 AECOPD patients whom assigned into two groups, with 15 patients each. Patients included in this study were COPD patients who experienced exacerbations, namely worsening of acute respiratory symptoms requiring additional therapy and hospitalization. The criteria for acute exacerbations taken were patients with moderate or severe clinical degree (at least two of three symptoms, including increased shortness of breath, increase in sputum production, and sputum purulence) who did not require intensive care unit.

All groups received standard care and therapy according to the current clinical guidelines. Treatment group received intramuscular injection of secretome 1cc/12 hours for three days. Secretome was collected from umbilical cord mesenchymal stem cell (UC-MSC) cultured. The UC-MSC cultures in passage 3 were cultured to a confluence of about 80% using complete growth media. Culture media was then obtained and centrifuged 500xg for 5 minutes to remove debris. The results were then filtered and stored at -80°C. Before the product is used, conditioned medium (CM) is then thawed and transferred into a sterile vial and sent to the hospital where the study was carried out in a cooler box at 2-8°C. UC-MSC preparation is carried out in the laboratory. Secretome contains DMEM supplemented with human platelet lysate that processed with pharmaceutical heparin. Secretome contains IL-10 which acts as an anti-inflammatory agent. BDNF, SDF-1, VEGF, PDGF, EGF, NGF, and FGF are detected in secretome.

The inclusion criteria were AECOPD requiring hospitalization, age ≥40 years. The exclusion criteria were patients without spirometry data, asthma, bronchiectasis, pulmonary tuberculosis or post TB

with fibrotic, pneumoconiosis, interstitial lung diseases (ILDs), immunosuppressive status like HIV, systemic steroid use within last 2 weeks.

The diagnosis of COPD by pulmonologist is based on several criteria, namely a history of exposure to harmful gas/particles, risk elements, clinical symptoms, and spirometry results (FEV_1/FVC% <0.7 post bronchodilator test). AECOPD is the addition of acute respiratory symptoms beyond the normal daily variation that requires additional therapy.[1] The neutrophil-to-lymphocyte ratio is the number of neutrophils divided by the lymphocytes in the blood. The average LHS in AECOPD patients according to Muslin's study was 6-7 days.[16] Crisafulli et al classified LHS in AECOPD patients into normal (≤7 days) and prolonged (>7 days).[17]

Demographic data, NLR, and LHS were recorded and collected at the day of admission. 1cc/12hours of secretome was injected in treatment group on the first until the third day. Blood samples for neutrophil and lymphocyte examination were collected both in the treatment and control group at the fourth day of hospitalization. Neutrophil and lymphocytes value was screened by a hematologic analyzer. Spirometry test was carried out to establish COPD diagnosis after patient's condition was stable or had previous spirometry with the result of FEV_1/FVC value <0,7 post bronchodilator test. The spirometry test used spirometer by COSMED.

Data analysis in this study used SPSS software version 22.0. Authors used frequency distribution (%) to describe categorical data, means of SD to describe numerical data, and the unpaired group difference test for categorical data with chi square or fisher exact test. Analysis of differences in pre, post, and post-pre NLR value differences in treatment and control groups used different test. The unpaired group differences test on abnormal distribution data used Mann Whitney, while data with normal distribution used independent t-test. The paired group differences test on abnormal distribution data used Wilcoxon rank test, while data with normal distribution used Paired t-test. Result is significant if value of P<0.05.

RESULTS

The gender of patients in both groups share similar proportion, in which the treatment group had 11 male patients (73.3%) and 12 male patients (80.0%) in the control group.

Table 1. Patient's demographic data

Baseline characteristic	Group		*P*
	Treatment	Control	
Sex[a]			
Male	11 (73.3%)	12 (80.0%)	1.000
Female	4 (26.7%)	3 (20.0%)	
Age[b]	62.40 ±12.89	64.20 ±9.58	0.668
Severity of COPD Exacerbation[c]			
Mild	1 (6.7%)	2 (13.3%)	
Moderate	6 (40.0%)	8 (53.3%)	0.261
Severe	8 (53.3%)	5 (33.3%)	
Baseline NLR	7.06 ±3.70	7.05 ±7.10	0.330

Note=[a]Difference test with chi square/fhiser exact test; [b]Difference test with independent t-test; [c]Difference test with Mann-Whitney.

The mean age of the patients in treatment group was 62.40±12.89 years while in the control group was 64.20±9.58 years. The exacerbation severity of patients in the treatment group was mostly severe in 8 patients (53.3%), while the control group were mostly moderate in 8 patients (53.3%). The statistical test results obtained P=0.261 which indicates that there was no significant difference in the characteristics of the study subjects based on the degree of exacerbation between the treatment and control groups. Table 1 shows demographic data.

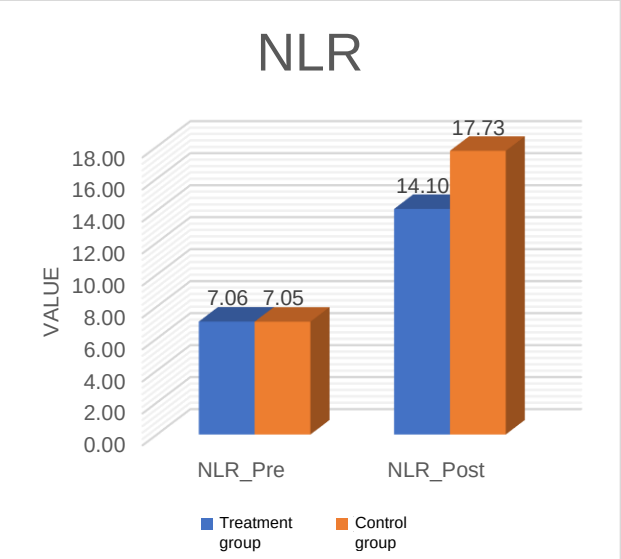

Fig 1. Bar chart of the NLR value of both groups

In the treatment group, the mean baseline/pre-test NLR value of patients was 7.06±3.70, and

14.10±9.02 for the post test. The mean difference in the post-pre-treatment group's NLR changes was found to have increased by 7.05±9.41. The increase of NLR was statistically significant with *P*=0.012. In the control group, the mean pre-test NLR value of patients was 7.05±7.10, and 17.73±12.13 for the post-test (Figure 1).

Table 2. Difference test of NLR between treatment and control group.

Group	NLR		NLR differences	*P*
	Pre	Post		
Treatment	7.06±3.70	14.10±9.02	7.05±9.41	0.012[d*]
Control	7.05±7.10	17.73±12.13	10.68±9.05	0.001[c*]
P	0.330[a]	0.361[b]	0.187 [a]	

Note=[a]difference test using Mann Whitney; [b]difference test using independent t-test; [c]difference test using Wilcoxon rank test; [d]difference test using Pair test; *significant if *P*<0.05

The mean difference in post-pre control group's NLR changes was found to have increased by 10.68±9.05, and the increase was statistically significant with *P*=0.001. The results indicate that the control group had a higher increase in NLR than the treatment group, but the comparison of changes in NLR increase was not statistically significant, with *P*=0.187 (Table 2).

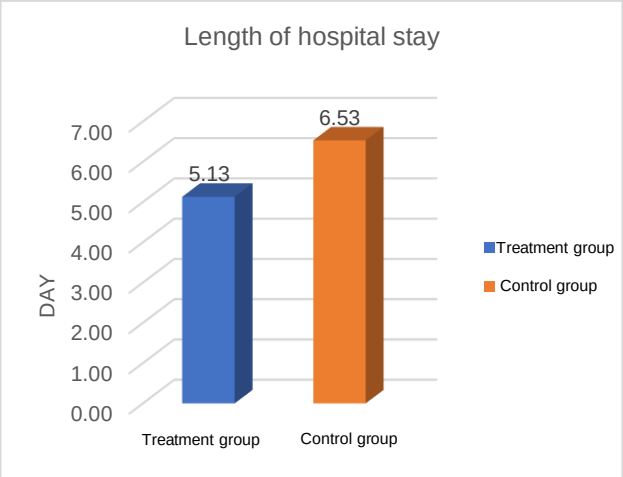

Fig 2. Difference in LHS between treatment and control group

The mean LHS of patients in the treatment group was 5.13±1.30 days while in the control group was 6.53±1.92 days. LHS of patients in the treatment group tend to be shorter than in the control group. The result was statistically significant with *P*=0.028, implying that there was a difference in the LHS between treatment group and control group. Figure 2 show LHS of patients of both groups and Table 3 show the differences test of LHS between two groups.

Table 3. Differences test of LHS between treatment and control group

Variable	Group		*P*
	Treatment	Control	
LHS	5.13 ±1.30	6.53 ±1.92	0.028*

Note=Difference test using independent t-test; *significant if *P*<0.05

DISCUSSION

Even though existing COPD prevalence data were varied, gender and age were still used to estimate the prevalence of COPD. Male and those who are over 40 years old still have a higher prevalence of COPD compared to female and those who are less than 40 years old, respectively.[1] Our demographic data showed similar characteristics as well. The number of male COPD participants in our study was higher than female. The mean age of participants in our study was more than 60 years old.

AECOPD are complicated conditions that are frequently accompanied by increased mucus production, increased airway inflammation, and disguised gas trapping. These issues exacerbate dyspnea, a defining sign of an exacerbation with detrimental effects on health, hospitalization and readmission rates, and disease progression.[1] Exacerbations lead to increased airway and systemic inflammation. The inflammatory cascade releases inflammatory mediators such as cytokine and chemokines which attract and activate immune cells. This cascade contributes to the local structural damage, development of COPD, and systemic inflammation.[18]

The major reason of application of MSC in clinical research is due to its extensive anti-inflammatory and regenerative activities. MSC participates in the healing of lung tissue and has the ability to develop into type I and/or type II alveolar epithelial cells. By activating macrophages, neutrophils, and lymphocytes in the lungs and causing the release of inflammatory cytokines, cigarette smoke contributes to the development of COPD. MSC have shown the ability to reduce COPD progression through the mechanism of reducing the inflammatory response by releasing classically attenuated macrophage cytokines IL-6, IL-1β, and

TNF-α. Systemic administration of allogenic bone marrow (BM)-MSCs in COPD patients can decrease CRP levels 1 to 3 month after infusion.[13]

Armitage et al study was a phase I clinical trial in Australia, observing in vivo stem cells distribution and systemic inflammatory response after systemic administration. Nine patients received two infusions of allogenic BM-MSCs of $2x10^6$ cells/kg one a week for 2 weeks. BM-MSCs were detected in the lung within 30 min and still detectable after 24 hours, then distributed mainly in the liver. There was a trend of decreasing inflammatory mediators such as IL-6 in 1 to 7 days following the treatment.[19]

NLR serum has been shown to be a good valuable predictor of inflammatory conditions. This index is a rapid, easy and cost-effective method in clinical daily practice. AECOPD patients are reported to have higher NLR values than stable patients, and are associated with severity.[20] In the submucosa of the airway in COPD, neutrophils were found in greater numbers and lymphocytes in lower numbers. Significant inflammation and lowered immunity were indicated by high NLR values. In AECOPD patients who are hospitalized, an increase in NLR value is related to worse prognosis. NLR is shown to possess a significant sensitivity and specificity in assessing the probability of in-hospital mortality in AECOPD patients, according to the findings of the study by Karauda et al In hospitalized AECOPD patients, the neutrophil-to-lymphocyte ratio offers potential indicators of LHS and prognosis.[21]

As described in our study, NLR were increased in all subjects at the beginning of diagnosis. The increase in NLR persisted until the fourth day of the disease course although the treatment group had received secretome administration for three days as adjuvant therapy. However, the increase of NLR in the control group was higher than in the treatment group. It means secretome has the ability to withstand the high surge of inflammatory in AECOPD, though not statistically significant. The result of this study supported by Weiss et al 2021, which state that there was a decrease in CRP levels within 1 month after systemic administration of allogenic BM-MSCs in COPD patients.[15] From the statement, it can be concluded that the reduction of inflammatory cells takes at least 1 month.

AECOPD patients requiring hospitalization experienced lower quality of life, higher hospital expenses, and mortality. LHS becomes a significant predictor of hospital costs and the use of medical resources by COPD patients. Increased LHS was a significant risk factor for 30 and 90-day all-cause readmission, according to a meta-analysis.[21] Age and smoking history are the only two factors that may affect LHS. According to a retrospective study by Li M et al, 2021, Macao had a higher mean of LHS for AECOPD patients than China (9.38 days), North West England (8.7 days), the United States (5.9 days), and European nations (12.28 days) (8.7 day).[22]

The severity of AECOPD can be predicted by LHS. The average LHS of AECOPD patients was 6 - 7 days in the study by Muslin et al Longer LHS is associated with an increased risk of hypercapnic respiratory failure.[16] LHS greater than or equal to 7 days was independently linked with a modified Medical Research Council (mMRC) score of 2 and increased the likelihood of developing acute respiratory acidosis in Crisafulli et al's study of AECOPD patients.[17] Study results from Armitage et al found that systemic administration of BM-MSCs reduced the risk of COPD hospitalization.[19] Their study shared similar result as ours. In our study, LHS for AECOPD in the treatment group was shorter than the control group. This result implied that secretome were able to reduce the degree of inflammation and accelerate the patient's clinical improvement.

LIMITATION

The study has several limitations, including small sample size and short period of NLR re-examination (3 days) where it was possible that secretome might need more time to reduce the degree of inflammation.

CONCLUSION

The neutrophil to lymphocyte ratio can be a promising predictor of LHS and severity, related to

the prognosis of hospitalized AECOPD patients. The longer COPD patients are hospitalized, the lower the quality of life, the higher the hospital costs, and the higher the mortality rate. Administration of secretome can provide a lower effect on increasing the high NLR value and decreasing the length of stay in AECOPD patients. Further studies of secretome administration in AECOPD patients with a longer follow-up period should be carried out.

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CONFLICT OF INTEREST

This study has no conflict of interest.

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Original Article

Risk Factors Associated with 28-Day Mortality of COVID-19 Patients at RSUP Dr. M. Djamil Padang

Lusi Agustini Arda,[1] Oea Khairsyaf,[1] Russilawati,[1] Tuti Handayani[2]

[1]Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Andalas, RSUP Dr. M. Djamil, Padang, Indonesia
[2]Department of Radiology, Faculty of Medicine, Universitas Andalas – RSUP Dr. M. Djamil, Padang, Indonesia

Abstract

Background: The COVID-19 mortality rate varies widely around the world. The COVID-19 death rate in Indonesia is currently relatively higher than the world average and is the highest in Southeast Asia. Data regarding risk factors of COVID-19 mortality in Indonesia, particularly West Sumatra, are still scarce. This study aims to determine the risk factors associated with the 28-day mortality of COVID-19 patients at RSUP Dr. M. Djamil Padang.

Methods: This was an observational analytic study with a retrospective cohort approach on confirmed COVID-19 inpatients who were treated at RSUP Dr. M. Djamil Padang between January 1 and March 31, 2021. A bivariate analysis using Chi square was calculated to see the correlation between clinical severity, and routine blood values, markers of inflammation, liver function, kidney function, blood gas analysis, the RALE score and comorbidities with a 28-day mortality outcome. To assess the dominant risk factors, multivariate analysis was performed using logistic regression.

Results: From 245 samples, patients aged >50 years and women were the most treated group of patients. Bivariate analysis obtained the following critical clinical grade factors: Hb <10 g/dl, leukocyte level >10.0x10^3/mm^3, monocyte level 8.0%, procalcitonin level >0.5 ng/ml, interleukin-6 level >7 pg/ml, ferritin >159/ml, D-Dimer level >500 ng/dl, SGOT level >38 µ/l, urea >50 mg/dl, creatinine >1.3 mg/dl, PO_2 <80 mmHg, SO_2 ≤90%, PO_2/FiO_2 ≤300 mmHg, high RALE score, comorbid of chronic renal failure, hypertension, type II DM; and comorbidities >1 were associated with 28 days of death. Multivariate analysis identified critical clinical severity as the dominant risk factor (OR=8.47; 95% CI=2.55–28.14; *P*<0.001).

Conclusion: Critical clinical severity was the dominant risk factor associated with the 28-day mortality of COVID-19 patients at RSUP Dr. M. Djamil.

Keywords: COVID-19, risk factors, 28-day mortality, clinical severity

Corresponding Author: *Lusi Agustini* Arda | Department Pulmonology and Respiratory Medicine, Facuty of Medicine, Universitas Andalas, RSUP Dr. M. Djamil, Padang, Indonesia | lusi.arda@gmail.com

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INTRODUCTION

Coronavirus Disease-19 (COVID-19) is caused by the new Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2). The World Health Organization (WHO) announced COVID-19 as a global pandemic on March 11, 2020.[1] Data from the Ministry of Health of the Republic of Indonesia as of October 5, 2021, recorded 4.2 million confirmed cases of COVID-19 with 142,338 deaths.[2]

The COVID-19 mortality rate varies widely around the world, from 0.5% to 10%. The mortality rate is 4.9% in Wuhan, and 2.1% overall in China. The mortality rate is higher in Europe than in Asia or America, which is more than 10%.[2] The current COVID-19 death rate in Indonesia is 2.7%, relatively higher than the world average mortality rate set by the WHO, which is 2%, so that is the highest in Southeast Asia based on data from the Center for Strategic and International Studies (CSIS).[3] Therefore, it is important to understand the risk factors associated with COVID-19 deaths in Indonesia.

Several risk factors have been studied and accepted by the scientific community as increasing the risk of death in COVID-19.[4] Izcovich et al in their study concluded that the prognostic factors for mortality doubled in the elderly (age increase per 10 years), and in men. In the same study, it was also concluded that comorbidities increased the risk of death from COVID-19 three times compared to those without comorbidities.[5] Vivas et al stated that factors which predict death within 30 days of patients with COVID-19 were age, Asian ethnicity,

immunodeficiency conditions, and increased body mass index (BMI).[6]

In addition, signs and symptoms indicating respiratory failure or organ damage assessed from laboratory markers or radiological features are also considered to be potential risk factors for death in COVID-19. Currently, data related to risk factors for 28-day mortality of COVID- 19 in Indonesia and West Sumatra in particular are still very limited. Therefore, the authors were interested in examining the risk factors for 28-day mortality of COVID-19 patients at Dr. RSUP. M. Djamil Padang, a type A referral hospital in West Sumatra.

METHODS

This was a retrospective cohort observational study of confirmed COVID-19 inpatients at Dr. M. Djamil Hospital Padang between January 1 and March 31, 2021. The sample was, population that met the inclusion criteria: confirmed COVID-19 from the results of RT PCR of SARS-CoV-2, aged >18 years, including the clinical severity of moderate, severe and critical illness. Exclusion criteria were patients with incomplete medical record data. The independent variables in this study were age, gender, clinical degree, laboratory values (complete blood count/CBC, markers of inflammation, liver function, kidney function, blood gas analysis), chest X-ray (CXR) RALE score and comorbidity. While the dependent variable was the outcome of 28-day mortality for COVID-19 patients.

The data were processed descriptively and analytically. A bivariate analysis with Chi square test was calculated to see the relationship between clinical severity factors, CBC, inflammation markers, liver function, kidney function, blood gas analysis, RALE scores and comorbidities with a 28-day mortality outcome. Multivariate analysis with logistic regression was calculated to assess the dominant risk factor.

RESULTS

Table 1 provides a description of the characteristics of COVID- 19 inpatients at Dr. M. Djamil Hospital Padang for the period January 1 to March 31, 2021. The COVID-19 patients being treated at Dr. M. Djamil Hospital Padang were mostly in the age group above 50 years for as much as 62.86% and the female patients (55.10%) were found to be more than the male. Moderate clinical severity accounted for the highest proportion of hospitalized patients (58.77%), followed by critical (37.14%) and severe (4.10%).

Table 1. Characteristics of COVID-19 Inpatients at Dr. M. Djamil Hospital (N=245)

Characteristics	N	%
Age		
<50 years	91	37.14
50–59 years	63	25.71
60–69 years	54	22.04
≥70 years	37	15.10
Gender		
Female	135	55.10
Clinical severity		
Moderate	144	58.77
Severe	10	4.10
Critical	91	37.14

For laboratory parameters, about 82.44% of patients were treated with Hb >10 g/dl, 66.93% with leukopenia, 65.71% with neutrophilia, 67.34% with monocytopenia, 95.51% with lymphopenia, and 86.53% with normal platelets count. On the inflammatory markers, almost 77.14% had procalcitonin <0.5 ng/ml, 86.53% had an increase in interleukin-6 level of >7 pg/ml, 69.38% had an increase in ferritin value >159 ng/ml, and 84.08% had an increase in D-Dimer of >500 ng/ml. According to the liver function profile, 58.36% of the patients had normal SGOT values (<38 µ/l) and 74.28% had normal SGPT values (<41 µ/l). On the renal function, about 74.69% had normal ureum level and 83.67% had normal creatinine level. For oxygenation, 66.5% of patients had normal PO_2 (>80 mmHg), 75.10% had SO_2% above 90% and 52.65% had PO_2/FiO_2 >300 mmHg.

The parameters of the CXR showed that the median RALE score was 2, with a minimum score of 0 and the highest score of 48. A total of 66.11% of treated COVID-19 patients had comorbidities, with hypertension and diabetes mellitus (DM) ranked as the most common, at 7.75% and 7.34%, respectively.

Table 2. Laboratory parameters of COVID-19 Inpatients at Dr. M. Djamil Hospital (N=245)

Parameters	N	%
Complete Blood Count		
Hemoglobin		
Hb <10 g/dl	43	17.55
Hb ≥10 g/dl	202	82.44
Leukocytes		
≤5.0–10.0 x 10^3/mm^3	164	66.93
>10.0 x 10^3/mm^3	81	33.06
Neutrophil		
≤50.0–70.0%	84	34.28
>70.0%	161	65.71
Monocyte		
≤2.0–8.0%	165	67.34
>8.0%	80	32.65
Lymphocytes		
≤20.0–40.0%	234	95.51
>40.0%	11	4.48
Thrombocytes		
<150 x 10^3/mm^3	33	13.46
>150 x 10^3/mm^3	212	86.53
Inflammation marker		
Procalcitonin		
≤0.5 ng/ml	189	77.14
>0.5 ng/ml	56	22.85
Interleukin-6		
≤7 pg/ml	33	13.46
>7 pg/ml	212	86.53
Ferritin		
≤9.3–159 ng/ml	75	30.61
>159/ml	170	69.38
D-Dimer		
≤500 ng/ml	39	15.91
>500 ng/dl	206	84.08
Liver function		
SGOT		
≤38 u/l	143	58.36
>38 u/l	102	41.63
SGPT		
≤41 u/l	182	74.28
>41 u/l	63	25.71
Kidney function		
Urea		
≤10–50 mg/dl	183	74.69
50 mg/dl	62	25.30
Creatinine		
≤0.8–1.3 mg/dl	205	83.67
1.3 mg/dl	40	16.32
Blood Gas Analysis		
PO_2		
<80 mmHg	82	33.46
80 mmHg	163	66.53
SO_2%		
≤90%	60	24.48
>90%	184	75.10
PO_2/FiO_2		
≤300 mmHg	129	52.65
300 mmHg	116	47.34

Based on the number of comorbidities, 34.28% of patients had >1 comorbidity A total of 31.83% of patients treated at Dr. M. Djamil Hospital Padang for the period January – March 2021 died, and another 68.16% recovered.

Table 3. Chest X-Ray parameters of COVID-19 Inpatients at Dr. M. Djamil Hospital (N=245)

Parameters	N	%
Chest X-Ray [Median (Min – Max)]	2.00 (0–48)	
RALE SCORE		
Comorbidities		
Cerebrovascular disease	1	0.40
Hypertension	19	7.75
Cardiovascular disease	8	3.26
Chronic lung disease	4	1.63
Chronic liver disease	1	0.40
Chronic kidney disease	8	3.32
Diabetes mellitus	18	7.34
Malignancy	7	2.85
Immunodeficiency (HIV)	1	0.40
Obesity	12	4.89
No comorbid	83	33.87
Number of comorbidities		
No. comorbid	83	33.87
1 comorbid	78	31.83
>1 comorbid	84	34.28
Outcome		
Survived	167	68.16
Death	78	31.83

The correlation of demographic factors, clinical degrees, laboratory values, chest radiographs and comorbidities with the 28-day mortality of COVID-19 patients at Dr. M. Djamil Hospital can be seen in Table 4. There was a relationship between age and 28-day mortality of COVID-19 patients (P<0.05), where, based on the greatest risk opportunity, it is known that age 70 years was associated with OR=4.44 (95% CI=1.92–10.29). It was also found that there was a relationship between clinical severity and 28-day mortality in COVID-19 patients (P<0.05), and the probability of 28-day mortality in COVID-19 patients was found in patients with critical clinical severity with OR= 31.92 (95% CI=14.81–68.81).

Based on the parameters of the laboratory description, it was observed that there was a correlation with 28-day mortality of COVID-19 patients at hemoglobin <10 g/dl (P<0.05) with OR=2.43 (95% CI=1.24–4.75).

Table 4. Correlation of Demographic Factors, Clinical Severity, Laboratory Values, Chest X-Rays and Comorbidities with 28-Day Mortality of COVID-19 Patients at Dr. M. Djamil Hospital

Variables	Survived (n = 167)	Death (n = 780	*P*	OR (95% CI)
Age, f (%)				
<50 years	75 (82.4)	16 (17.6)		Reff.
50–59 years	40 (63.4)	23 (36.5)	0.002*[a]	2.69 (1.28–5.68)*
60–69 years	33 (61.1)	21 (38.9)		2.98 (1.38–6.43)*
≥70 year				
Gender, f (%)				
Male	71 (64.5)	39 (35.5)	0.337	1.35 (0.79–2.32)
Female	96 (68.2)	39 (28.9)		0.74 (0.43–1.27)
Clinical severity, f (%)				
Moderate	133 (92.4)	11 (7.6)		Reff.
Severe	9 (90.0)	1 (10.0)	<0.001*[a]	1.34 (0,16–11.60)
Chritical	25 (27.5)	66 (72.5)		31.92 (14,81–68.81)*
Laboratory finding, f (%)				
Hemoglobin				
Hb <10 g/dl	22 (51.2)	21 (48.8)	0.014*[a]	2.43 (1.24–4.75)*
Hb ≥10 g/dl	145 (71.8)	57 (28.2)		Reff.
Leukocytes				
≤5.0–10.0 x 103/mm^3	129 (78.7)	35 (21.3)	<0.001*[a]	n/a
>10.0 x 103/mm^3	38 (46.9)	43 (53.1)		
Neutrophil				
≤50.0–70.0%	80 (95.2)	4 (4.8)	<0.001*[a]	n/a
>70.0%	87 (54.0)	74 (46.0)		
Monocytes				
≤2.0–8.0%	99 (60.0)	66 (40.0)	<0.001*[a]	n/a
>8.0%	68 (85.0)	12 (15.0)		
Lymphocytes				
≤20.0–40.0%	156 (66.7)	78 (33.3)	n/a	n/a
>40.0%	11 (100.0)	0 (0.0)		
Thrombocytes				
≤150 x 103/mm^3	21 (63.6)	12 (36.4)	0.690	n/a
>150 x 103/mm^3	146 (68.9)	66 (31.1)		
Inflammation markers				
Procalcitonin, f (%)				
≤0.5 ng/ml	152 (80.4)	37 (19.6)	<0.001*[a]	n/a
>0.5 ng/ml	15 (26.8)	41 (73.2)		
Interleukin-6				
≤7 pg/ml	31 (93.9)	2 (6.1)	0.001*[a]	n/a
>7 pg/ml	136 (64.2)	76 (35.8)		
Ferritin				
≤9.3–159 ng/ml	67 (89.3)	8 (10.7)	<0.001*[a]	n/a
>159 ng/ml	100 (58.8)	70 (41.2)		
D-Dimer				
≤500 ng/ml	37 (94.9)	2 (5.1)	<0.001*[a]	n/a
>500 ng/ml	130 (63.1)	76 (36.9)		
Liver function, f (%)				
SGOT				
≤38 u/l	110 (76.9)	33 (23.1)	0.001*[a]	n/a
>38 u/l	57 (55.9)	45 (44.1)		
SGPT				
≤41 u/l	130 (71.4)	52 (28.6)	0.088[a]	n/a
>41 u/l	37 (58.7)	26 (41.3)		

Table 4. Correlation of Demographic Factors, Clinical Severity, Laboratory Values, Chest X-Rays and Comorbidities with 28-Day Mortality of COVID-19 Patients at Dr. M. Djamil Hospital (Cont.)

Variables	Survived (n = 167)	Death (n = 780	*P*	OR (95% CI)
Kidney function, f (%)				
Urea				
≤10–50 mg/dl	147 (80.3)	36 (19.7)	<0.001*[a]	n/a
>50	152 (80.4)	42 (67.7)		
Creatinine				
<0.8–1.3 mg/dl	151 (74.0)	53 (25.9)	<0.001*[a]	n/a
>1.3 mg/dl	16 (39.0)	25 (62.5)		
Blood gas analyses, f (%)				
PO_2				
<80 mmHg	31 (37,8)	51 (62.2)	<0.001*[a]	8.29 (4.51–15.22)*
≥80 mmHg	136 (83,4)	27 (16.6)		Reff.
SO_2%				
≤90%	22 (36,1)	39 (63.9)	<0.001*[a]	6.59 (3.51–12.39)*
>90%	145 (78,8)	39 (21.2)		Reff.
PO_2/FiO_2				
≤300 mmHg	58 (45,0)	71 (55.0)	<0.001*[a]	19.06 (8.24–44.12)*
>300 mmHg	109 (94.0)	7 (6.0)		Reff.
Chest X-ray, Median (min-max) RALE SCORE	0 (0–48)	16.0 (0–48)	<0.001*[a]	1.09 (1.06–1.12)*
Comorbidities				
Cerebrovascular disease	2 (40.0)	3 (60.0)	0.330	3.30 (0.54–20.16)
Hypertension	45 (58.4)	32 (41.6)	0.039*[a]	1.89 (1.07–3.32)*
Cardiovascular disease	24 (63.2)	14 (36.8)	0.595	1.30 (0.63–2.68)
Chronic lung disease	6 (66.7)	3 (33.3)	1.000	1.07 (0.26–4.41)
Chronic heart disease	4 (80.0)	1 (20.0)	1.000	0.57 (0.06–4.82)
Chronic kidney disease	10 (35.7)	18 (64.3)	<0.001*[a]	4.71 (2.06–10.78)*
Diabetes mellitus	33 (49.3)	34 (50.7)	<0.001*[a]	3.14 (1.74–5.65)*
Malignancy	11 (68.8)	5 (31.3)	1.000	0.97 (0.33–2.89)
Immunodeficiency (HIV)	1 (50.0)	1 (50.0)	0.536	2.16 (0.13–34.92)
Obesity	17 (63.0)	10 (37.0)	0.692	1.29 (0.57–2.98)
No comorbidity	70 (84.3)	13 (15.7)	<0.001*[a]	0.28 (0.14–0.54)*
Number of comorbidities				
No comorbidity	70 (84.3)	13 (15.7)	<0.001*[a]	Reff.
1 comorbidity	51 (65.4)	27 (34.6)		2.74 (1.29–5.82)*
>1 comorbidity	46 (54.8)	38 (45.2)		4.65 (2.23–9.69)*

Also, there was leukocytes >10.0x10^3/mm^3 with OR=4.17 (95% CI=2.35–7.41), neutrophils >70.0% with OR=17.01 (95% CI=5.95–48.66), monocytes 8.0% with OR=3.78 (95% CI=1.89–7.52), procalcitonin >0.5 ng/ml with OR=11.23 (95% CI=5.62–22.43), interleukin-6 >7 pg/ml with OR=8.66 (95% CI=2.02–37.19), ferritin >159/ml with OR=5.86 (95% CI=2.65–12.97), D-Dimer >500 ng/dl with OR=10.82 (95% CI=2.54–46.14), SGOT >38 u/l with OR=2.63 (95% CI=1.52–4.57), ureum >50 mg/dl with OR=8.58 (95% CI=4.49–16.35), and creatinine >1.3 mg/dl with OR=4.45 (95% CI=2.21–8.96).

There was a relationship between 28-day mortality of COVID-19 patients and each of blood gas analysis parameters, namely PO_2 levels <80 mmHg with OR=8.29 (95% CI=4.51-15.22), SO_2 90% with OR=6.59 (95% CI=3.51–12.39), and PO_2/FiO_2 300 mmHg with OR=19.06 (95% CI=8.24–44.12). Based on the CXR description with RALE scores, there was a correlation between RALE scores and 28-day mortality of COVID-19 patients (*P*<0.05) with OR=1.09 (95% CI=1.06–1.12).

Based on comorbidities, it was known that both chronic kidney disease and DM were associated with 28-day mortality of COVID-19 patients (*P*<0.05), and the OR=4.71 (95% CI=2.06–10.78) and OR=3.14 (95% CI=1.74–5.65), respectively. Furthermore, hypertension was also associated with 28-day mortality in COVID-19 patients (*P*<0.05), and the OR=1.89 (95% CI=1.07–3.32).

Table 5. Dominant Risk Factor for 28-Day Mortality of COVID-19 Patients at Dr. M. Djamil Hospital

Variables	B	SE	*P*	OR (95% CI)
Age				
50–59 years	0.956	0.731	0.191	2.60 (0.62–10.89)
60–69 years	0.458	0.681	0.501	1.58 (0.42–6.01)
≥70 years	0.665	0.779	0.393	1.95 (0.42–8.95)
Clinical Severity				
Severe	-0.771	1.317	0.558	0.46 (0.04–6.12)
Critical	2.136	0.613	<0.001*	8.47 (2.55–28.14)*
Hb ≥10 g/dl	0.999	0.679	0.141	2.72 (0.72–10.27)
Leukocytes 10.0 x 10^3/mm^3	0.619	0.507	0.222	1.86 (0.69–5.02)
Neutrophil >70.0%	1.621	0.933	0.082	5.06 (0.81–31.49)
Monocytes ≤2.0–8.0%	-0.415	0.705	0.557	0.66 (0.17–2.63)
Procalcitonin >0.5 ng/ml	0.829	0.539	0.124	2.29 (0.79–6.59)
Interleukin-6 >7 pg/ml	-0.332	1.002	0.740	0.72 (0.10–5.12)
Ferritin >159/ml	0.539	0.776	0.487	1.71 (0.38–7.84)
D-Dimer >500 ng/dl	-0.818	1.152	0.478	0.44 (0.05–4.22)
SGOT >38 u/l	0.361	0.536	0.500	1.44 (0.50–4.11)
SGPT >41 u/l	0.161	0.602	0.789	1.18 (0.36–3.82)
Urea >50 mg/dl	1.119	0.697	0.109	3.06 (0.78–12.02)
Creatinine >1.3 mg/dl	-0.798	0.886	0.368	0.45 (0.08–2.56)
PO_2 <80 mmHg	0.799	0.624	0.200	2.22 (0.65–7.55)

Patients without comorbidities had protective factors to prevent adverse outcomes in COVID-19 patients with an OR=0.28 (95% CI=0.14–0.54). The number of comorbidities was associated with 28-day mortality in COVID-19 patients (*P*<0.05). Patients with more than one comorbidity had the highest risk factor for 28-day mortality with an OR=4.65 (95% CI=2.23–9.69), followed by patients with only 1 comorbidity with an OR=2.74 (95% CI=1.29–5.82).

However, there was no relationship between gender, platelet count, SGPT, cerebrovascular disease, cardiovascular disease, chronic lung disease, chronic liver disease, malignancy, immunodeficiency and obesity with 28-day mortality of COVID-19 patients (*P*>0.05).

The analysis was continued with a multivariate analysis by first selecting candidate variables based on the previous bivariate analysis. Based on bivariate analysis, the variables that passed the selection were those with *P*<0.25. These variables were age, clinical grade, hemoglobin level, leukocyte count, neutrophil count, monocyte count, procalcitonin, interleukin-6, ferritin level, D-Dimer, SGOT, SGPT, ureum, creatinine, PO_2, SO_2, PO_2/FiO_2, Rale score, comorbidities of hypertension, chronic kidney disease, DM, without comorbidity, and the number of comorbidities. The most dominant factor in the 28-day mortality of COVID-19 patients was critical clinical degree with an OR=8.47 (95% CI=2.55–28.14), as can be seen in Table 5.

DISCUSSION

The results showed that COVID-19 patients who were treated at Dr. M. Djamil Hospital Padang were mostly in the age group less than 50 years (37.1%), followed by the age group 50–59 years (25.7%) and the age group 60–69 years (22%). This result was in line with the study by Islam, which had the highest age range in the 25–39 years group (39.4%) followed by 40–59 years group (34.3%).[7] This age range is a productive age group that is active in outdoor activities, thereby increasing the risk of exposure to the SARS-CoV-2 virus.

Based on the gender of the hospitalized patients, women were found to be more than men (55.1% compared to 44.9%). Similar findings were obtained in study from Mardewi in Bali, where women with COVID-19 outnumbered men (53.9% vs 46.1%).[8] The higher number of women treated compared to men in this study could be influenced by

the data on the distribution of COVID-19 in West Sumatra, where the number of COVID-19 female patients was higher than that of male patients (54.9% vs. 45.1%).[9] An earlier study in China also found that in East Asian women, ACE2 expression was higher, so they were more likely to get COVID-19.[10] This factor could be one of the factors causing the high number of female patients compared to men in this study.

Moderate clinical severity accounted for the highest proportion of hospitalized patients (58.8%), followed by critical clinical severity (37.1%) and severe clinical severity (4.1%). This result was higher than in Wuhan (17.7%) and Hubei (10.4%).[11] The majority of clinical cases had been treated at Dr. M. Djamil Hospital due to comorbidities or accidents that required specialized management.

These specialties cannot be carried out in regional hospitals, such as hemodialysis, sectio caesarea or other surgical procedures, so even though the clinical severity was moderate, they still required treatment at a referral hospital.

The CBC revealed that 17.6% of patients had Hb below 10 g/dl, 66.9% had leukopenia, 65.7% had neutrophilia, 67.3% had monocytopenia, 95.5% had lymphopenia, and 13.5% had thrombocytopenia. Zhou et al in Wuhan also concluded that lymphopenia, leukopenia, and thrombocytopenia were the most common laboratory abnormalities found in COVID-19.[12] Cytotoxic lymphocytes, such as cytotoxic T lymphocytes and Natural Killer cells, play an important role in viral infection control by maintaining immune homeostasis and the inflammatory response. The occurrence of apoptosis or functional exhaustion of cytotoxic lymphocytes causes lymphopenia, monocytopenia, and leukopenia.[13]

According to the description of inflammatory marker parameters, 77.1% of patients had procalcitonin level below 0.5 ng/ml, 86.5% had an increase in interleukin-6 of more than 7 pg/ml, 69.4% had an increase in ferritin level >159 ng/ml, and 84.1% had an increase in D-Dimer level of more than 500 ng/ml. Zhou et al in Wuhan concluded that the most frequent laboratory abnormalities found in patients with COVID-19, besides lymphopenia, leukopenia, and thrombocytopenia, were elevated inflammatory markers.[12]

An irregular and exaggerated immune response to SARS-CoV-2 infection activates several complex pathways involved in cytokine storm pathogenesis, which are the Renin-Angiotensin-Aldosterone System (RAAS), Janus kinases (JAK), the Signal Transducer and Activator of Transcription (STAT) pathway, and the complement activation pathway. Furthermore, there is also a cytokine release syndrome (CRS), which is a state of immune dysregulation and hyperinflammation thought to be due to T cell activation and characterized by an increase in inflammatory markers.[14]

The parameters of the chest radiograph showed that the median RALE score was 2, with a minimum score of 0, and the highest was 48. These results were in line with study from Sensusiati at Universitas Airlangga Hospital, which gained the median RALE score of 3. The Borghesi study in the United States also concluded that a high RALE score (>3) increased the risk of requiring ICU care or mortality.[15]

Furthermore, COVID-19 patients who were treated had the most common comorbidities of hypertension (7.8%) and DM (7.3%). This result was similar to the finding of Surendra's study in Jakarta, where hypertension was the most common comorbidity (19%), followed by DM (12%).[9] In Indonesia, the epidemiological transition resulted in an increase in noncommunicable diseases. The burden of hypertension and DM in Indonesia is high, with a national prevalence of 34.1% and 11.3%, respectively.[16]

Based on the number of comorbidities, 34.3% of patients have >1 comorbidity. The Leulseged study in Ethiopia found that 43.8% of COVID-19 patients had a history of one or more comorbidities.[17] Surendra in Jakarta found 69% patients without comorbidity, 20% with 1 comorbid, and 11% with >1 comorbidity.[9] A total of 31.8% of patients admitted to Dr. M. Djamil Hospital for the period January – March 2021 died, and the rest 68.2% survived. This result was not much different from the study by Ramatillah

in Jakarta which observed 27.8% of patients treated in hospitals could not survive.[18]

In this study, it was found that age significantly affected the outcome of COVID-19 patients (*P*<0.002), and that the risk of mortality increased with age. These results were consistent with the study of Signescosta et al, who concluded that old age was associated with increased mortality in COVID-19.[19] The same conclusion was also obtained by Borghesi et al, Huang et al, and Zhou et al.[12,15,20] Borghesi stated that men >50 years and women >80 years were the highest age groups at risk of suffering from severe COVID-19 symptoms.[15]

Increasing age is associated with physiological changes as part of the aging process, such as immunosenescence, which alters pathogen recognition and clearance due to a decrease in T cells and accumulation of memory T cells.[21] The aging process will trigger an imbalance of functions in various systems, including the immune system, making them more susceptible to inflammation and death. Patients over the age of 50 have higher ACE2 expression encoded by the ACE2 gene, which is associated with other risk factors such as decreased immunity, por organ function, or previous comorbidities that increase the risk of mortality.[22]

Although more men were hospitalized in this study than women, the percentage of men who died was higher (35.5%) than that of women (28.9%), but the difference was not statistically significant (*P*=0.337). Jin et al concluded that although the susceptibility of men and women to be infected with COVID-19 was the same, however, male sex was an important clinical risk factor for severity and death (*P*=0.016).[23] Women were less susceptible than men for reasons associated with innate immunity, steroid hormones, and other factors associated with the sex chromosomes. Immune regulatory genes encoded by the X chromosome in women will cause a decrease in viral load and a decrease in inflammation compared to men, in addition to higher CD4+ T cells and a better immune response. TLR7 levels in women are also higher, allowing a better immune response and increased resistance to viral infections than in men, so they have a better prognosis.[24]

Critical clinical severity showed a significant relationship with the risk of mortality in COVID-19 (*P*<0.001), with OR=31.92 (95% CI=14.81–68.81). Gau et al also observed a significant relationship between clinically critical severity and 28-day mortality of COVID-19 patients (*P*<0.05).[25] Oliveira stated that the mortality rate in critical COVID-19 in the ICU ranged from 50–65%, and in patients requiring mechanical ventilation, the mortality rate is 97%.[26] Critical severity is characterized by dysregulation of cytokine release, pneumonia, and acute lung injury, which can rapidly progress to acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC), multisystem failure, and death.[27]

Based on the parameters of the laboratory description, there was a relationship between hemoglobin and 28-day mortality of COVID-19 patients (*P*<0.05), the chance of 28-day mortality risk of COVID-19 patients in patients with Hb levels below 10 g/dl had an OR=2.43 (95% CI=1.24-4.75). Oh et al revelaed the same conclusion, that anemia at admission was an independent risk factor for death (OR=1.523; 95% CI=1.008-2.303; *P*=0.046). Anemia is independently associated with an increased likelihood of death in hospitalized patients with COVID-19.[27,28]

There was a relationship between leukocytes, neutrophils and monocytes with 28-day mortality of COVID-19 patients (*P*<0.05). The increase in total leukocyte count and neutrophil count had a significant correlation with the severity of the disease. One mechanism underlying this increase is bacterial or fungal comorbidity in a large number of patients infected with SARS-CoV-2 with poor outcomes.[28]

There was an association between 28-day mortality of COVID-19 patients (*P*<0.05) with; procalcitonin (PCT) >0.5 ng/ml (OR=11.23; 95% CI=5.62-22.43), interleukin-6 >7 pg/ml (OR=8.66; 95% CI=2.02–37.19), ferritin >159/ml (OR=5.86; 95% CI=2.65–12.97), and D-Dimer >500 ng/dl (OR=10.82; 95% CI=2.54–46.14). Similar findings were obtained by Zare et al and Deng et al.[28,29] Increased PCT levels were observed in severe SARS-CoV-2 infections that had bacterial

complications or higher levels of proinflammatory cytokines.[28] Excessive cytokine production can activate the coagulation pathway, induce DIC and multi-organ failure, with IL-6 and TNF- serving as the primary mediators of Cytokine Release Syndrome (CRS) in COVID-19. Deng et al obtained significantly higher amounts of ferritin in the critical group compared to the moderate and severe groups. Other inflammatory cytokines, such as interleukin (IL)-8, IL-10, C-reactive protein (CRP), and tumor necrosis factor (TNF)-α, were found to be positively correlated with ferritin and D-dimer concentrations.[30]

Based on the parameters of liver function and kidney function, there was a correlation between SGOT, ureum and creatinine with the 28-day mortality of COVID-19 (*P*<0.05). Higher risk was found in patients with SGOT levels >38 u/l with an OR of 2.63 (95% CI 1.52–4.57), in ureum >50 mg/dl with an OR8.58 (95% CI=4.49–16.35), and in creatinine >1.3 mg/dl with an OR=4.45 (95% CI=2.21–8.96). Potential mechanisms of liver dysfunction in COVID-19 include: (a) immune-related impairment due to a severe inflammatory response to infection, (b) direct cytotoxicity due to viral replication in ACE-2-expressing bile duct epithelial cells, (c) hypoxic hepatitis due to anoxia, and (d) drug-induced liver damage.[30]

Bao et al stated that the SGOT/SGPT ratio examination could be a strong predictive factor for early detection of liver damage and was positively correlated with COVID-19 patient mortality.[30] Acute kidney injury (AKI) in COVID-19 is causally related to the cytopathic effect of the virus or to the systemic inflammatory response and cytokine storm. These results were in line with study conclusion by Arikan which stated that AKI affected the increasing morbidity and mortality of COVID-19.[31]

Based on the parameters of blood gas analysis, there was a relationship between each PO_2, SO_2 and PO_2/FiO_2 with 28-day mortality in COVID-19 patients (*P*<0.05). The higher risk was found in patients who had PO_2 <80 mmHg with OR=8.29 (95% CI=4.51–15.22), SO_2 90% with OR=6.59 (95% CI=3.51–12.39), and PO_2/FiO_2 300 mmHg with OR=19.06 (95% CI=8.24–44.12). Gao et al also reported that SpO_2 <90% was strongly associated with mortality (OR=47.41; 95% CI=6.29–357.48) and concluded that PaO_2/FiO_2 <200 mmHg was associated with a higher 28-day and 60-day mortality risk (*P*<0.05).[25]

Based on the chest X-ray with RALE scores, we found a correlation between RALE scores and 28-day mortality in COVID-19 patients (*P*<0.05) with an OR=1.09 (95% CI=1.06–1.12). It was in line with study from Sensusiati that also obtained significant relationship between RALE scores and outcomes of COVID-19 patients (*P*<0.05); the risk of mortality for patients with high RALE scores increased 6.826 times compared to patients with lower scores (95% CI=2.076–22.444).[32] Cozi et al also concluded a significant correlation between RALE scores and patient outcomes. A RALE score of more than 15 points was associated with a higher risk of ICU admission.[33]

Chronic kidney disease, DM and hypertension were found to have association with 28-day mortality of COVID-19 patients (*P*<0.05), with each OR=4.71 (95% CI=2.06–10.78), OR=3.14 (95% CI=1.74–5.65) and OR=1.89 (95% CI=1.07–3.32), respectively. These results were similar to the study findings from Guan et al, which stated that comorbidities such as chronic renal failure, hypertension, and DM, were correlated with poor COVID-19 outcomes.[9,14]

Patients with hypertension exhibit endothelial dysfunction and immunometabolic modifications that contribute to high serum levels of inflammatory cytokines. Diabetes mellitus is prone to experience poor COVID-19 outcomes because of chronic inflammation in DM increases the risk of hyperinflammation and cytokine storms. The IL-6 and CRP were found to be significantly higher in patients with DM. In addition, hyperglycemia can interfere with immune responses and increase oxidative stress.[25,26] Meanwhile, chronic kidney disease is associated with an increased risk of pneumonia, increased ACE2 expression and increased susceptibility to hyperinflammation and cytokine storms in SARS-CoV-2 infection, resulting in an increased risk of mortality.[34]

The number of comorbidities was associated with 28-day mortality of COVID-19 patients (*P*<0.05).

Patients with comorbidity >1 had the highest risk factor, with an OR=4.65 (95% CI=2.23–9.69). Study by Surendra reported that the mortality rate for patients with no comorbidities was 38%, while patients with 1 comorbidity had a mortality rate of about 30%, and patients with more than 1 comorbidity had a mortality rate of about 32%.[9] Khedr et al pointed out that the number of comorbidities significantly increased the risk of death; patients with one comorbidity had HR=2 (95% CI=1.1–3.7), patients with two comorbidities had HR=2.6 (95% CI=1.4–4.7) while those with 3 or more comorbidities had HR=2.9 (95% CI=1.5–5.6).[35]

Critical clinical severity was found to be the most important risk factor for 28-day mortality of COVID-19 patients, with an OR= 8.47 (95% CI=2.55–28.14; *P* <0.001). Gao et al also concluded that critical clinical severity was an independent risk factor for 28-day and 60-day mortality of COVID-19 patients in China.[25] These findings supported previous studies that obtained critical clinical severity of COVID-19 pneumonia to be associated with a high mortality rate. The mortality rate in ICUs due to COVID-19 worldwide and in the United States range from 20–62%. In mechanically ventilated patients, mortality ranges from 50-97%. Patients classified as having critical clinical severity on admission have a much greater risk of mortality, because they have a higher indication of increased inflammation, a more serious risk of organ dysfunction, and a higher SOFA score, which can caused septic shock, followed by ARDS and multiorgan failure.[25,35]

LIMITATION

The limitations of this study are that this study did not assess the influence of therapeutic factors as one of the factors that can influence mortality.

CONCLUSION

Risk factors associated with 28-day mortality of COVID-19 patients at Dr. M. Djamil Hospital Padang were age >70 years, critical clinical severity, laboratory values: Hb <10 g/dl, leukocyte count >10.0x10^3/mm^3, neutrophil count >70%, monocyte count ≤8.0%, procalcitonin >0.5 ng/ml, interleukin-6 >7 pg/ml, ferritin level >159/ml, D-Dimer >500 ng/dl, SGOT level >38 u/l, ureum >50 mg/dl, creatinine >1.3 mg/dl, PO_2 <80 mmHg, SO_2% <90%, PO_2/FiO_2 300 mmHg, high RALE score, comorbidities: chronic renal failure, hypertension, type II DM and the number of comorbidities >1. The critical clinical severity was the most important risk factor for the 28-day mortality of COVID-19 patients at Dr. M. Djamil Hospital Padang.

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CONFLICT OF INTEREST

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Original Article

Remdesivir in COVID-19: A Retrospective Analysis of Remdesivir Effectiveness and the Relation with Blood Type Variation

Elsa Vera Denida Purba,[1] Retnosari Andrajati,[1] Nadia Farhanah Syafhan,[1] Aditya Wirawan[2]

[1]Faculty of Pharmacy, Universitas Indonesia, Depok, Indonesia
[2]Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Indonesia, Universitas Indonesia Hospital, Depok, Indonesia

Abstract

Background: Remdesivir has been proven effective for COVID-19 treatment. This research aims to identify the profile of the effectiveness of Remdesivir (RDV) therapy and its relationship to blood type variations in COVID-19 patients at Universitas Indonesia Hospital (RSUI).

Methods: Variations in blood types were examined for their influence on the effectiveness in COVID-19 infected patients with RDV as an antiviral treatment. Data for this study were acquired at RSUI using a retrospective cross-sectional method. The sample is infected patients with COVID-19 from January 2021 to December 2021 who received RDV therapy. The parameters of the effectiveness of the treatment was a reduction of minimally 2 points on the WHO Clinical Progression Scale after 14 days of Remdesivir administration.

Results: RDV effectiveness percentage shows 57.5% of patients experienced clinical improvement. The analysis results of the effect of blood type variations on clinical outcomes significantly affect the effectiveness of RDV therapy (OR=1.705; 95% CI=1.091–2.665; *P*=0.019) but insignificant in terms of mortality status (OR=0.654; 95% CI=0.383–1.117; *P*=0.120).

Conclusion: Blood type variations significantly affected the effectiveness of RDV therapy in infected COVID-19 patients.

Keywords: antiviral, blood type variations, COVID-19, clinical outcome, remdesivir

Corresponding Author:
Retnosari Andrajati | Faculty of Pharmacy, Universitas Indonesia, Depok, Indonesia | andrajati@farmasi.ui.ac.id

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INTRODUCTION

The new viral disease, COVID-19, was first discovered in 2019, specifically in Wuhan, China. From there, it expanded to nearly all the nations around the world.[1] On March 2nd, 2019, two patients from Jakarta were accused in the nation's first COVID-19 infection case in Indonesia. Then, the total number of patients confirmed positive for COVID-19 was 38,277, with 2,134 deaths on June 15, 2020. The number of patients infected by COVID-19 and the number of death increases every day.[2]

Direct and indirect contact are methods by that SARS-CoV-2 can be spread. Direct contact refers to person-to-person and droplet transmission, and indirect contact refers to contaminated objects and airborne transmission. A source of airborne transmission can also be spread from PPE (personal protective equipment).[3]

Pharmacological therapy in patients infected by COVID-19 is categorized based on the degree of severity. The severity of COVID-19 patients is grouped into severe to critical symptoms, moderate, mild, and asymptomatic. Difference symptoms need a different treatment.[4]

The FDA, commonly known as Food and Drug Administration, recently approved intravenous RDV as an antiviral treatment for adults and children with COVID-19 (aged 12 years and weight 40 kg). It is approved for the antiviral therapy of non-hospitalized COVID-19 patients (i.e., on day 3 with 7 days following the onset of symptoms), inpatients (i.e., on day 5), and antiviral therapy for mild symptoms to moderate symptoms in high-risk of COVID-19.[4] RDV is used for COVID-19 patients with moderate and severe symptoms in Indonesia.[5]

RDV (GS-5734) is the first FDA-approved therapy for severe COVID-19. RDV (GS-5734) is an analog of adenosine nucleotide that is active against a broad spectrum of single-stranded RNA viruses, including emerging and zoonotic coronaviruses such

as 2019-nCoV3, MERS-CoV, and SARS-CoV. The drug was first described in 2016 and was derived from a small molecule antiviral library to target emerging pathogenic RNA viruses. RDV is a monophosphoramidate whose action is blocking RNA production and inhibiting RNA polymerase proofreading.[6]

Referring to previous research, RDV is a pretty effective antiviral for COVID-19 treatment. Research conducted by Gupte in India shows that RDV administration can increase 84% of clinical improvement, while research by Olender reveals that the percentage of clinical improvement reaches 74.4% with RDV administration on day 14 compared to without RDV administration.[7,8]

Research on blood type variations concerning the severity, mortality rate, and risk of COVID-19 infection has been widely conducted. Patients with the O blood have the lowest risk of infection but have milder symptoms than other blood type variations.[9] Meanwhile, AB patients are a risk factor for high mortality.[10]

There has not been any significant research examining the variation of blood types and the effect on RDV antiviral effectiveness. However, the study conducted by Du et al examines the effect of blood type variations on propofol effectiveness and reveals that blood type variations affect the effectiveness of propofol.[11]

METHODS

This observational study used a cross-sectional design. Data sources were medical record data of COVID-19 inpatients at RSUI. Data were collected retrospectively using specified inclusion and exclusion criteria. The sample of this research was COVID-19 inpatients receiving RDV therapy in 2021.

The data to be collected includes blood type, patient demographics, and clinical outcomes. The effectiveness of the treatment was measured by WHO Clinical Progression Scale consisting of 5 levels of the patient's clinical condition with a score range of 0 (uninfected) – 10 (died). After analyzing the WHO Clinical Progression Scale, the effectiveness of therapy was analyzed for its relationship with the blood type variation. Data analysis covered descriptive analysis and inferential statistics using Chi-square.[1]

The University of Indonesia Hospital Ethics Committee approved the research protocol under number S-026/KETLIT/RSUI/VII/2022. Since there is no direct interaction with the patient, the Ethics Committee omitted the requirement for consent.

RESULTS

From January to December 2021, 295 out of 1542 confirmed Covid-19 patients were treated with RDV therapy. Then, 80 of 259 patients were randomly selected according to the inclusion and exclusion criteria.

Table 1. Patient Demographics

Category	N (%)
Gender	
Male	51 (63.8%)
Female	29 (36.3%)
Blood type	
A	22 (27.5%)
AB	10 (12.5%)
B	15 (18.8%)
O	33 (41.3%)
Age, year (Mean±SD)	56.74±11.339
18-59	47 (58.8%)
≥60	33 (41.3%)
Oxygen therapy	
Yes	73 (91.3%)
No	7 (8.8%)
Number of comorbid	
0	4 (5.0%)
1	15 (18.8%)
>1	61 (76.3%)
History of comorbid	
No	4 (5.0%)
Yes	76 (95.0%)
Comorbid	
HT	47 (58.8%)
DM	44 (55.0%)
Respiratory disorders	5 (6.3%)
Immunity disorders	0 (0.0%)
Kidney disorders	21 (26.3%)
Obesity	12 (15.0%)
CVD	27 (33.8%)
HT+DM	23 (28.8%)
HT+CVD	13 (16.3%)
DM+CVD	13 (16.3%)
HT+DM+CVD	12 (15.0%)

Note: HT=Hypertension; DM=Diabetes Mellitus; CVD=Cardiovascular Disease

This research involved 80 COVID-19 patients who received RDV therapy. Based on gender, the

patients comprised 63.80% male and 36.30% female. The most common blood type is type O, with a percentage of 41.3%. The patients are dominated by patients aged 18-59 years (58.8%) and followed by those aged ≥ 60 years (Table 1).A total of 73 patients out of 80 patients received oxygen therapy with a percentage of 12.33% using intubation and ventilators. The results showed that 95.0% of patients had comorbidities, and 61 had more than one comorbid. The most common comorbid was HT (hypertension) (58.8%) then, followed by diabetes (55.0%), cardiovascular (33.8%), kidney disorders (26.3%), obesity (15.0%), and respiratory disorder (6.3%). The most common comorbid combinations were hypertension-diabetes, with an incidence of 28.2%, followed by hypertension-cardiovascular and diabetes-cardiovascular, with a percentage incidence of 16.3% each. The comorbid combination of hypertension, diabetes, and cardiovascular reached 15.0%.

The patients were given 200mg of RDV intravenously as the initial dose on the first day, followed by 100mg of RDV as a maintenance dose for the next four days. After 14 days of RDV therapy, the clinical improvement was checked based on the point decrease of the WHO Clinical Progression Scale score by minimally 2 points. Based on the clinical status of the patients, 46 patients (57.5%) of 80 patients experienced clinical improvement, and nine patients experienced a decrease of 1 score. A total of 14 patients (17.5%) died after the administration of RDV antiviral therapy, and one died before RDV therapy.

According to the graph (Figure 1), the variation in the percentage of blood types A and O in enhancing clinical outcomes and lowering deaths in COVID-19 patients receiving RDV antiviral medication was more significant than in individuals with blood types B and AB. The percentage, OR, and value of *P* of each blood type variation can be seen in the table of bivariate analysis results in Tables 4 and 5.

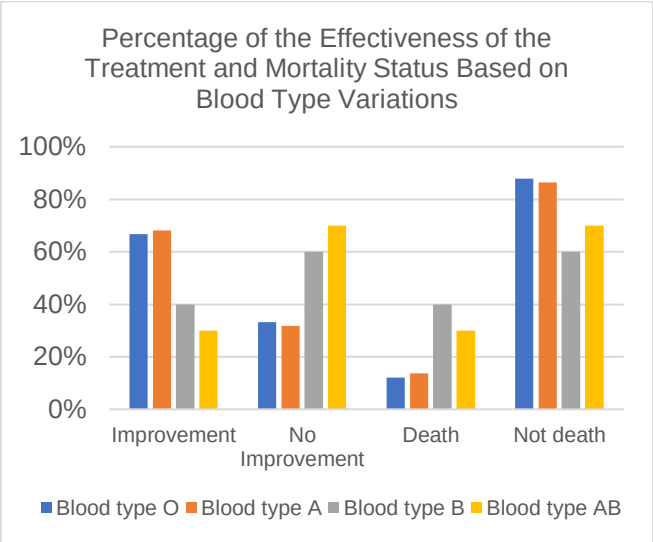

Figure 1. Percentage of the Effectiveness of the Treatment and Patient Mortality Status Based on Blood Type Variations

Table 2. General Characteristics of Patients Based on Blood Type Variations

Characteristics	Blood type				*P*
	A n (%)	B n (%)	AB n (%)	O n (%)	
Clinical outcome					
Improvement	15 (68.2%)	6 (40.0%)	3 (30.0%)	22 (66.7%)	0.068
No improvement	7 (31.8%)	9 (60.0%)	7 (70.0%)	11 (33.3%)	0.068
Death status					
Yes	3 (13.6%)	4 (26.7%)	3 (30.0%)	4 (12.1%)	0.416
No	19 (86.4%)	11 (73.3%)	7 (70.0%)	29 (87.9%)	0.416
Gender					
Male	16 (72.7%)	10 (66.7%)	4 (40.0%)	21 (63.6%)	0.353
Female	6 (27.3%)	5 (33.3%)	6 (60.0%)	12 (36.6%)	0.353
Number of comorbidities					
No comorbid	1 (4.5%)	1 (6.7%)	0 (0.0%)	2 (6.1%)	0.873
1 comorbid	4 (18.2%)	2 (13.3%)	1 (10.0%)	8 (24.2%)	0.873
>1 comorbid	17 (77.3%)	12 (80.0%)	9 (90.0%)	23 (69.7%)	0.873
Age (Mean±SD)	55.64±13.106	59.20±6.527	61.50±9.880	54.91±12.017	
Adult	12 (54.5%)	9 (60.0%)	5 (50.0%)	21 (63.6%)	0.847
Elderly	10 (45.5%)	6 (40.0%)	5 (50.0%)	12 (36.4%)	0.847

Note: SD=Standard Deviation

Table 3. Multivariate Analysis of the Effectiveness of RDV Antiviral Therapy

	Risk Factor	WHO Clinical Progression			Mortality Status		
		OR	95% CI	*P*	OR	95% CI	*P*
Crude	Blood Type.	1.705	1.091–2.665	0.019	0.654	0.383–1.117	0.12
Adjusted	Blood Type	1.32	0.751–2.319	0.334	0.832	0.398–1.738	0.625
	Gender	1.01	0.308–3.318	0.987	1.669	0.288–9.678	0.568
	Age, years	1.882	0.585–6.058	0.289	3.396	0.511–22.569	0.206
	Comorbid	0.486	0.066–3.572	0.478	2.779	0.228–33.890	0.423
	Hypertension	0.022	0.000–1.105	0.056	2.446	0.071–84.123	0.62
	Diabetes mellitus	0.012	0.000–0.676	0.032	0.719	0.018–28.522	0.86
	Respiratory disease	0.12	0.006–2.523	0.172	17.633	0.779–398.931	0.071
	CVD	0.005	0.000–0.443	0.02	39.226	0.607–2534.764	0.084
	Kidney diseases	0.311	0.072–1.346	0.118	3.77	0.692–20.552	0.125
	Obesity	0.203	0.024–1.723	0.144	17.513	1.502–204.196	0.022
	HT+DM	1.324	0.267–201.031	0.239	31.174	0.755–1286.656	0.07
	HT+CVD	57.57	2.078–1595.103	0.017	0.068	0.001–5.797	0.236
	DM+CVD	52.342	1.537–1781.904	0.028	0.741	0.012–44.149	0.236
	HT+DM+CVD	0.315	0.051–1.940	0.213	0.291	0.006–13.329	0.527

Note: HT=Hypertension; DM=Diabetes Mellitus; CVD=Cardiovascular Disease; CI=Confidence Interval; OR=Odd Ratio

Based on the statistical data in Table 2, patients infected with COVID-19 with blood type variation A (68.2%) and O (66.7%) have a higher percentage of improvement. Meanwhile, patients with blood type B (40.0%) and AB (30%) have a smaller percentage of improvement. The highest mortality status is found in patients with blood type AB (30.0%), followed by type B (26.7%), A (13.6%), and type O (12.1%).

The logistic regression analysis indicated that blood type variations had a significant effect on the effectiveness of RDV antiviral therapy (OR=1.705; 95% CI=1.091–2.665; *P*=0.019) but did not significantly affect mortality status (OR=0.654; 95% CI=0.383–1.117; *P*=0.120). Meanwhile, when influenced by other confounding variables, blood type variation did not significantly affect the effectiveness of RDV antiviral therapy (OR=1.320; 95% CI=0.751–2.319; *P*=0.334).

Based on the analysis result of the effect of blood type variations on the effectiveness of RDV therapy using the Chi-square test (Table 4), RDV therapy for COVID-19 patients with blood types variations A and O have effectiveness of 1.866 (95% CI=0.633–5.254; *P*=0.313) and 1.917 (95% CI=0.762–4,821; *P*=0.178) times better in improving patient clinical outcomes compared to other blood types.

The percentage of improvement in RDV therapy in COVID-19 patients with blood types A (68.2%) and O (66.7%) is higher than the other blood types (53.4% and 51.1%) with OR of 1.866 and 1.917, respectively. It can be said that blood type A has proven to have 1.866 times the effectiveness of RDV therapy, and blood type O has 1.917 times better in improving patient clinical outcomes compared to other blood types.

The percentage of improvement in COVID-19 patients with RDV antiviral therapy in patients with blood type B (40.0%) and type AB (30.0%) is lower than the other blood types (61.5% and 61.4%). On the other hand, the percentage of no improvement in COVID-19 patients with RDV antiviral therapy with blood type B (60.0%) and type AB (70.0%) is higher than the blood types (38.5% and 33.3%). Based on the Chi-square test results on the effect of blood type variations on the effectiveness of RDV therapy (Table 5), blood type O (OR=0.51; 95% CI=0.145–1.794; *P*=0.376) and blood type A (OR=0.675; 95% CI=0.169–2.691; *P*=0.747) are a protective factor in the possible mortality status.

The percentage of mortality status in blood type A (13.6%) and type O (12.19%) is lower than the other blood types (19.0% and 21.3%). This result indicates that individuals with blood types A and O have a reduced probability of death than those with non-A and non-O. The blood type non-A refers to blood types O, B, and AB, whereas blood types A, B, and AB are referred to as type non-O.

Table 4. Bivariate Analysis of the Effectiveness of RDV Antiviral Therapy based on the WHO Clinical Progression Scale

Blood Type		WHO Clinical Progression		OR	95% CI	*P*
		Improvement	No improvement			
		n (%)	n (%)			
A	Yes	15 (68.2%)	7 (31.8%)	1.866	0.633–5.254	0.313
	No	31 (53.4%)	27 (46.6%)			
B	Yes	6 (40.0%)	9 (60.0%)	0.417	0.132–1.313	0.155
	No	40 (61.5%)	25 (38.5%)			
AB	Yes	3 (30.0%)	7 (70.0%)	0.269	0.064–1.131	0.088
	No	43 (61.4%)	27 (38.6%)			
O	Yes	22 (66.7%)	11 (33.3%)	1.917	0.762–4.821	0.178
	No	24 (51.1%)	34 (48.9%)			

Note: CI=Confidence Interval; OR=Odd Ratio

Table 5. Bivariate Analysis of the Effectiveness of RDV Antiviral Therapy based on the Mortality Status

Blood Type		Mortality status		OR	95% CI	*P*
		Yes	No			
		n (%)	n (%)			
A	Yes	3 (13.6%)	19 (86.4%)	0.675	0.169–2.691	0.747
	No	11 (19.0%)	47 (81.0%)			
B	Yes	4 (26,7%)	11 (73.3%)	2	0.530–7.547	0.286
	No	10 (15.4%)	55 (84.6%)			
AB	Yes	3 (30.0%)	7 (70.0%)	2.299	0.514–10.280	0.368
	No	11 (15.7%)	59 (84.3%)			
O	Yes	4 (12.1%)	29 (87.9%)	0.51	0.145–1.794	0.376
	No	10 (21.3%)	31 (78.7%)			

Note: CI=Confidence Interval; OR=Odd Ratio

Unlike blood types A and O, patients with B and AB seemed to have no reduced probability of death than those with non-B and non-AB. The percentage of death patients in blood type B (26.7%) and type AB (30%) is higher than the other types (13.6%) and (12.1%). In blood types A and O, the mortality status is lower than type non-A and type non-B. This result is proven by the percentage of patients who did not die with blood type A (86.4%) and blood type O (87.9%) higher than the other types (81.0% and 78.7%).

DISCUSSION

Based on the collected data, the percentage of male patients (63.8%) is higher than females (36.3%). Based on Table 2, the general characteristics of patients are based on blood type variations, in general, male patients with different blood types have a higher percentage than female patients (*P*=0.353). This is consistent with previous research by Magdalena et al in a hospital in Malang that men are at a higher of getting infected by COVID-19 (OR=2.202; 95% CI=0.994–4,878; *P*=0.050).[12]

However, there is no difference in the proportion of gender among the COVID-19-infected patients, according to a comprehensive review analysis by Peckham et al that included 97 pieces of evaluated literature. However, compared to female patients, male patients had a higher probability of disease development to the severity and increased mortality risk.[13]

Viral load, oxygen therapy, and patient clinical improvement are essential parameters for the effectiveness of the treatment for COVID-19 patients. All aspects to see the effectiveness of the therapy can be seen in the WHO Clinical Progression Scale.[13] In our study, the analysis of the effectiveness of RDV therapy based on the WHO Clinical Progression Scale shows that 57.5% of COVID-19 patients experience improvement. This percentage is lower than the previous study by Olender et al, where the percentage of effectiveness of the treatment reaches 74.4%.[8]

Patients requiring oxygen therapy via a mechanical ventilator and ECMO were excluded from this study. This is one of the reasons why the effectiveness of RDV therapy is lower than in the previous study by Olender et al. Another study by

Henry B shows that patients using ECMO are predictors of death due to COVID-19.[14]

The examination result of the effect of the variation of blood type on RDV effectiveness shows that blood type variations have a significant effect on the effectiveness of RDV antiviral therapy (OR=1.705; 95% CI=1.091–2.665; *P*=0.019). The effect of variation of blood type on RDV effectiveness has not been specifically researched. However, there is research on the effect of blood type on effectiveness by Du et al in Mongolia. Du et al examine the anesthetic effect of propofol on patients with different blood types. The research reveals that variations in blood type may produce different effects on clinical outcomes.[11]

Examination results of the influence of blood type variations on clinical outcomes while influenced by covariates did not significantly lead to conclusive findings (OR=1.320; 95% CI=0.751–2.319; *P*=0.334). It may be due to the influence of comorbid factors that affect clinical outcomes, severity, and even death. Petrilli et al reveal that age and comorbidities strongly predict hospitalization, critical severity, and death.[15]

Obesity, cerebrovascular diseases, renal illness, respiratory issues, hypertension, CVD, diabetes mellitus, and malignancy are among the comorbidities that increase the likelihood of COVID-19's severity.[16] Gender and age are risk predictors for the severity degree and severity development of COVID-19, according to the comprehensive review study and meta-analysis conducted by Fathi et al Additionally, men who are older and have comorbid conditions are more likely to experience severe COVID-19 symptoms.[17]

In this study, the most significant comorbid in preventing the improvement of the patient's clinical condition is CVD, either single comorbid (*P*=0.02) or in combination with DM (*P*=0.028) and HT (*P*=0.017) and also DM as single comorbid without other combination (*P*=0.032). Meanwhile, obesity is a comorbid factor that elevated the probability of death in COVID-19 patients receiving RDV treatment (*P*=0.022). The patients who previously had comorbid CVD, as showed by Zhang et al are more prone to deterioration. According to Zhang et al, COVID-19 patients suffering from CVD are more likely to have impaired liver function, elevated blood creatinine, and lactate dehydrogenase (*P*<0.05).[18]

This also can affect the clinical outcome in patients. Like CVD, DM is also an inhibiting factor for improvement in COVID-19 patients with RDV therapy. Thus, DM is a comorbid COVID-19 that can be a predictor of acute lung damage and ARDS through an increase in ROS, IL-6, Inflammatory cytokines, and lipopolysaccharides that cause pulmonary fibrosis resulting in acute lung damage and ARDS. The two other potential mechanisms are the increased ROS production and the RAAS (renin-angiotensin-aldosterone system) activation by the viral. The increased angiotensin II expression results in insulin resistance, hyperglycemia, and damage to the endothelium of the vascular system. These all lead to CVD (cardiovascular disease), thromboembolism, DIC (disseminated intravascular coagulation), and mortality.[19]

Based on Table 3, obesity shows an increase the mortality (OR=17.513). Yu et al conducted a study on obese patients infected with COVID-19) in 43 hospitals in the US and found that obese patients decreased the likelihood of the length of stay (LOS) to less than 28 days compared to non-obese COVID-19 patients (*P*<0.001). However, this study shows that obesity is not significantly associated with mortality during 28 days of treatment.[20] Some studies related to obesity and COVID-19 also reveal that obesity significantly results in worse clinical outcomes and even has a higher risk factor for death than patients with average weight.[21–23]

In a study conducted in Istanbul, Sahin et al discovered that COVID-19 patients with average weight had lower levels of biomarkers of acute inflammation than obese patients did. Obesity was also identified as an independent predictor for the severity of COVID-19. In this cross-sectional investigation, obese patients frequently had pulmonary and hypoxic conditions. Besides, the hospitalization rate, the longer length of stay, the longer duration of ICU care, and the need for NIMV are more common in obese COVID-19 patients.[23]

According to the analysis, blood type O has the best chance of enhancing patients' clinical outcomes. The percentage of improvement in RDV therapy in patients infected with COVID-19 with blood type O (66.7%) is higher than other types (51.1%) with OR=1.917. This result indicates that RDV antiviral therapy in infected COVID-19 patients with blood type O is 1.917 times more effective in improving clinical outcomes than other types. The findings of several studies on the relationship between blood type and COVID-19 are compatible with this; for instance, Shibeeb et al and Zhao et al demonstrate that blood type O reduces the probability of infection and has milder symptoms than other blood types.[9,24]

This becomes one of the reasons that blood type O has the best chance of improving the clinical outcome. Considering that the sample size used in this study was relatively limited, further research is required on blood type's influence on the effectiveness of RDV therapy.

According to the statistical information in Table 2, the COVID-19 patients who are receiving RDV with variation blood type A (68.2%) also have a high proportion of improvement compared to those who have not improved (no improvement) with OR=1.866 (95% CI=0.633-5.254; *P*=0.313). Thus, it indicates that RDV antiviral therapy in infected COVID-19 patients with blood type A has 1.866 times more effective in improving clinical outcomes. Despite increasing the risk for COVID-19 infection, blood type A did not significantly impact the high degree of symptom severity.[25] Zietz et al explain that although type A increases the chance of getting COVID-19 infection, it also reduces the risk of being intubated. (ARD = -2.9; 95% CI=7.2–0.6) and death (ARD = -1.6; 95% CI=4.9–1.6).[10,26]

Different from blood types O and A, blood types B (40.0%) and AB (30%) have a low percentage of improvement compared to no improvement percentage. The highest mortality percentage is found in COVID-19 patients with blood type AB (30.0%), followed by type B (26.7%), type A (13.6%), and type O (12.1%). In a study conducted in New York, Zietz et al discovered that people with blood type AB had a high risk of mortality (ARD=1.4; 95% CI=6.9–8.9) and intubation (ARD=1.8; 95% CI=8.3–12.2) than other blood types. However, blood type B had a higher risk of intubation than other blood type variations (ARD=2.5; 95% CI=2.7–7.5).[10]

LIMITATIONS

The limitations in this study include the limited literature on the results of previous studies which were still small. Thus, this research has many weaknesses, both in terms of research results and analysis. In addition, there is also a limited number of samples, which was only 80 patients. Thus, further research is needed to see how variations in blood type have different effects on the effectiveness of Remdesivir.

CONCLUSION

Blood type variation significantly affects the effectiveness of RDV antiviral therapy (OR=1.705; 95% CI=1.091–2.665; *P*=0.019) based on the WHO Clinical Progression Scale. However, when influenced by other confounding variables, blood type variation does not significantly affect the effectiveness of RDV antiviral therapy (OR=1.320; 95% CI=0.751–2.319; *P*=0.334). RDV therapy in infected COVID-19 patients with blood types A and O has better effectiveness in improving the clinical outcome compared to other blood types. The sample size used in this study might have certain limitations. Therefore, more studies are required to determine how blood type affects the effectiveness of RDV therapy.

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CONFLICT OF INTEREST

The authors acknowledge that there are no substantial conflicting interests, such as financial, professional, or personal interests, which may

influence how the work reported in this publication is performed or presented.

AVAILABILITY OF DATA AND MATERIALS

On reasonable request, the corresponding author would provide you with the data and materials to support the findings of this study.

AUTHORS' CONTRIBUTION

The study was conceived and designed by EV, RA, NF, and AW. The data were gathered, examined, and interpreted by EV. The manuscript was written by EV, RA, NF, and AW. The final draft of the work was approved by all authors after a critical revision for significant intellectual substance.

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Original Article

High Levels of Leucocyte and Thrombocyte Increasing COVID-19 Mortality Rate in RSUP Dr. M. Djamil Padang

Maulana Muharam, Sabrina Ermayanti, Afriani

Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Andalas, RSUP Dr. M. Djamil, Padang, Indonesia

Abstract

Background: Different leukocytosis, leucopenia, thrombocytosis, thrombocytopenia, and clinical severity appear in COVID-19's cases. This study is aimed to identify the association between leucocyte, thrombocyte, and clinical severity in COVID-19's outcome.

Methods: A retrospective cohort study involving 121 patients with COVID-19 whom admitted from January to March 2021. Kruskal Wallis test was applied for analysis.

Results: The majority of participants were female (55.4%), aged between 18-49 years old (42.1%), and had comorbidities (81.8%). Most participants had a normal range of leucocyte (57.9%), thrombocyte (62.8%), and moderate clinical severity (67.8%). Subjects with full recovery were 79.3%, with sequelae such as weakness, and/or shortness of breath 3.3%, and deceased 17.4%. Leucocyte and thrombocyte had an association with COVID-19 outcome (P=0.045 and P=0.030 respectively). Clinical severity had no association with COVID-19 outcome (P=0.304).

Conclusion: Leucocyte and thrombocyte have an association with COVID-19 outcome. Clinical severity has no association with COVID-19 outcome.

Keywords: clinical severity, COVID-19, leucocyte, thrombocyte

Corresponding Author:
Maulana Muharam | Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Andalas, RSUP Dr. M. Djamil, Padang, Indonesia | parupadang@yahoo.com

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INTRODUCTION

Various inflammatory responses may occur in COVID-19 and cause different symptoms, ranging from fever, hypoxia, acute respiratory distress syndrome (ARDS), shock and death.[1] Various studies have shown haematological and immunological changes in COVID-19 patients.[2]

The recovery rate among COVID-19 patient is fairly high, reaching >97%.[3] Patients who have been declared cured may still have symptoms in the form of weakness and shortness of breath. The study of Docherty et al in UK reported that only 12.6% of patients who were declared cured had no symptoms. Complaints of shortness of breath in patients with COVID-19 can be related to inflammation, organ damage, or effects of long treatment, especially in ICU.[4]

Specific parameters are essential for predicting clinical outcome of COVID-19 patients, in addition to providing appropriate treatment, resource efficiency, as well as reducing mortality in patients with COVID-19.[5] Changes in leucocyte, thrombocyte values, and clinical severity may facilitate in classifying and estimating outcomes of patients with COVID-19 in order to provide immediate therapy and improve outcomes.[6] Therefore, this study examined the association between leucocyte, thrombocyte, and clinical severity with COVID-19 outcomes.

METHODS

An analytical study with a retrospective cohort design was conducted from January 2021 to November 2021 at RSUP Dr M Djamil Padang. The population of this study was all COVID-19 patients who were admitted in RSUP Dr M Djamil Padang from January 1 to March 31, 2021 with inclusion criteria of aged 18 years, having medical record data in the form of routine haematology laboratory examination results, clinical degree, and description of clinical condition when leaving the red-zone

isolation room, as well as negative result of Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) examination when the patient was allowed to leave the red-zone treatment room. Patients who had undergone treatment in other hospitals for more than 1 day were not included in this study. This study received approval from the Research Ethics Committee of RSUP Dr. M Djamil Padang on April 23, 2021. The data were analysed using the Kruskal Wallis test to see the association between the variables in this study.

RESULTS

There were originally 192 COVID-19 patients involved in this study from January 1 through March 31, 2021. A total of 71 samples (36.98%) were excluded, among others due to incomplete clinical grade data (6 people), being hospitalized for more than one day before (59 people), and discharged at their own request (6 people), so that only 121 samples (63.02%) were included in this study. Characteristics of participants are defined in Table 1.

Table 1. Characteristics of Confirmed COVID-19 Patients Treated at RSUP Dr. M. Djamil Padang

Patient Characteristics	N (%)
Gender	
Female	67 (55.4%)
Male	54 (44.6%)
Age; median (interquartile), years	53 (18–80)
18–49	51 (42.1%)
50–59	34 (28.1%)
0–69	23 (19%)
>70	13 (10.7%)
Comorbidities	
Yes	99 (81.8%)
No	22 (18.2%)
Leucocyte; median (intequartile)	7,640 (940–87,500)
Low	25 (20.7%)
Normal	70 (57.9%)
High	26 (21.5%)
Thrombocyte; Mean ± SD	235,190.08 ± 99,812.016
Low	19 (15.7%)
Normal	76 (62.8%)
High	26 (21.5%)
Clinical Severity	
Mild	21 (17.4%)
Moderate	82 (67.8%)
Severe	2 (1.7%)
Critical	16 (13.2%)
Outcome	
Recovered	96 (79.3%)
Recovered with Sequelae	4 (3.3%)
Died	21 (17.4%)

It was revealed that 55.4% of patients with confirmed COVID-19 and treated at RSUP Dr M Djamil Padang were females. The average age was 53 years, with majority of them was in the 18–49 years age group (42.1%). There were 81.8% patients with comorbidities. The median value of leucocyte in the study was 7.640/mm^3 (940-87500/mm^3), most of them (57.9%) were in the group with normal leucocyte levels. The mean of thrombocyte was 235,190.08±99,812,016/mm^3, most of them (62.8%) were in the group with normal thrombocyte levels.

Majority of participants had moderate clinical severity at 67.8%, with a cure rate of 79.3% for COVID-19 patients. 92% of confirmed COVID-19 patients whom were treated at RSUP Dr M Djamil Padang with low leucocyte levels were recovered. High leucocyte levels indicated a low cure rate in confirmed COVID-19 patients.

This study found that high mortality rate in confirmed COVID-19 patients was associated with high leucocyte levels. Based on the Kruskal-Wallis test, there was a significant correlation between leucocyte levels and the outcome of COVID-19 with *P*=0.045 (Table 2).

Table 2. Association between Leucocyte and Outcome in Confirmed COVID-19 Patients at RSUP Dr. M. Djamil Padang

Leucocyte	Recovered	Recovered with Sequelae	Died	*P*
Low	23 (92.0%)	1 (4.0%)	1 (4.0%)	0.045
Normal	56 (80.0%)	3 (4.3%)	11 (15.7%)	
High	17 (65.4%)	0 (0.0%)	9 (34.6%)	

High thrombocyte levels indicated a low cure rate in confirmed COVID-19 patients, as well as high mortality rate. The Kruskal-Wallis test showed that there was a significant association between thrombocyte levels and the outcome of COVID-19 with *P*=0.030 (Table 3).

Table 3. Association between Thrombocyte and Outcome in Confirmed COVID-19 Patients at RSUP Dr. M. Djamil Padang

Thrombocyte	Recovered	Recovered with Sequelae	Died	*P*
Low	15 (78.9%)	1 (5.3%)	3 (15.8%)	0.030
Normal	65 (85.5%)	2 (2.6%)	9 (11.8%)	
High	16 (61.5%)	1 (3.8%)	9 (34.6%)	

The highest mortality rate was found in the critical clinical severity group at 25%. The cure rate

in severe clinical severity was 100%, with only 2 subjects. The clinical severity of patients with COVID-19 at the time of admission in this study did not have a significant association with the outcome of COVID-19, with p-value = 0.304 (table 4).

Table 4. Association between Clinical Severity and Outcome for Confirmed COVID-19 Patients at RSUP Dr. M. Djamil Padang

Clinical Severity	Recovered	Recovered with Sequelae	Died	*P*
Mild	16 (76.2%)	0 (0.0%)	5 (23.8%)	0.304
Moderate	68 (82.9%)	2 (2.4%)	12 (14.6%)	
Severe	2 (100.0%)	0 (0.0%)	0 (0.0%)	
Critical	10 (62.5%)	2 (12.5%)	4 (25.0%)	

DISCUSSION

There were more women affected by COVID-19 patients whom treated at RSUP Dr. M. Djamil Padang from January 2021 to March 2021 than men (55.4% vs 44.6%). Similar results were reported by Mardewi's study in Bali, there were more women affected by COVID-19 than men (53.9% vs 46.1%).[6] Different result was reported by Priya et al's study in India which had more men with COVID-19 than women, by 60%.[7]

Research by Long et al in China revealed that number of men and women with COVID-19 was identical, amounting to 49.8%.[8] Majority of men are affected by COVID-19 possibly due to androgen hormonal activity via the Transmembrane Protease Serine 2 (TMPRSS2) pathway, which facilitates binding of SARS-CoV2 to the ACE-2 receptor.[9] Chen et al's study reported that in East Asian women, the expression of ACE2 receptors was higher, so they were more likely to be exposed to COVID-19.[10]

Most participants in this study were 18–49 years old (42.1%) and only a few numbers of patient were >70 years (10.7%). Subkhan et al. revealed that patients with COVID-19 were mostly dominated by people aged 34 to 59 years old, but severe cases were mostly occurred at the age of >60 years with comorbidities.[1] This study is following the study by Huang et al. The COVID-19 which reported that patients were mostly 25–49 years old (49%).[11]

Jie et al meta-analysis study in 11 countries also revealed that majority of COVID-19 patients had an average age of 40 years (95% CI=42.8–50.6).[12] This situation is possible since the age group of 18–49 year is productive age with high mobility. Ghiffari's study in Jakarta affirmed a relationship between population mobility and an increase in the number of confirmed cases of COVID-19.[13] Older people who suffer from COVID-19 are more susceptible to worsening clinical conditions, even death, due to decreased function of T cells and B cells, and excessive cytokine production, which causes a prolonged inflammatory response.[14]

81.8% participants of this study had comorbidities. Several comorbidities were found in participants, including stroke (3 people), hypertension (31 people), cardiovascular disease (19 people), chronic lung disease (6 people), malignancy (8 people), kidney disease (14 people), diabetes (18 people), and pregnancy (15 people). Comorbidities in COVID-19 in the form of chronic diseases may trigger chronic inflammation, increased ACE2 expression, and impaired immunity, making them susceptible to infection including SARS-CoV2.[14]

Patients with age >65 years and comorbidities, especially cardiovascular and diabetes, had better prognosis. Patients with poor well-being, 20.3% of them require ICU care, may experience multi-organ failure and increased mortality.[12,15] Long's study also stated that comorbidities were associated with clinical deterioration of COVID-19.[8]

A total of 57.9% of patients with COVID-19 in this study had normal range of leucocyte levels. High leucocyte levels was found in 21.5% of COVID-19 patients. The mean of leucocyte levels in mild clinical severity was 6470:3195/mm^3, moderate clinical severity was 7220:4998/mm^3, severe clinical severity was 5530/mm^3 and 8680/mm^3, and critical clinical severity was 10570:10368/mm^3. High leucocyte levels accompanied by a decrease in lymphocytes in patients with COVID-19 may lead to clinical deterioration. This is thought to be the result of increased levels of neutrophils in response to cytokines and chemokines due to endothelial damage, which also releases cytokines and chemokines. The repetition of this process increases the release of stored neutrophils from the bone

marrow and increases the levels of cytokines and chemokines in the body. This situation is exacerbated by the binding of the virus to lymphocytes which reduces the production of CD4+ and CD8+, in an effort to avoid the body's immune response.[16]

The high leucocyte levels in COVID-19 patients in this study had a significant relationship with death as an outcome. The study of Li et al revealed an increase in leucocyte levels among patients with severe and critical clinical severity, as well as in patients died from COVID-19.[17] A study by Zhao et al reported that patients with high leucocyte levels 17.3% of them required oxygen supplementation, and 46.2% required ICU care; the mortality rate was also higher than patients without an increase in leucocyte levels (19.2% vs. 5.8%).[18]

A study by Alamin et al in Saudi also reported high levels of leucocyte in patients died from COVID-19.[19] An increase in leucocyte, especially neutrophils, is usually accompanied by an increase in systemic inflammatory responses such as IL-6 serums. The increase in serum IL-6 then increases the differentiation of Th17 cells from T cells. Increase in Th17 cells consequently increases the release of reserves and activation of neutrophils, increasing cytokines, which can trigger a cytokine storm, tissue damage, resulting in severe pneumonia, and even death.[18]

Most patients had normal range of thrombocyte levels (62.8%). Patients with mild clinical severity had a mean of thrombocyte count of 279714±110635/mm^3, moderate clinical severity with 231658±94042/mm^3, severe clinical severity with 285000/mm^3 and 293000/mm^3, and critical clinical severity with 188125±100135/mm^3. Low thrombocyte levels were found in 15.7% of patients, while high thrombocyte levels were found in 21.5% of patients. Research by Li et al in China showed that low thrombocyte levels were associated with inflammatory process and death in patients with COVID-19.[20]

Study by Lippi et al through a meta-analysis found that low thrombocyte levels were closely related to severe and critical clinical severity.[21] Research by Lanini et al in Italy found that thrombocyte levels at the beginning of patient's treatment was not related to the clinical severity and outcome of COVID-19, although the decrease in thrombocyte levels found in the treatment indicated a poor prognosis.[22]

Low thrombocyte levels are thought to occur due to the use of thrombocyte in repair of microvascular endothelial damage due to SARS-CoV2 virus infection and subsequent inflammation. The use of thrombocyte is seen as a microthrombus event that triggers ARDS and multiorgan failure, and is associated with clinical deterioration.[23] A study by Yang et al reported that SARS-COV2 infection caused diffuse alveolar damage, which then trapped megakaryocytes, and prevented platelets release from megakaryocytes.[24] Increased thrombocyte levels in COVID-19 are thought to be due to inflammation with an increase in various cytokines, including IL-6 which increases the expression of Thrombopoietin messenger ribonucleic acid (TmRNA) in the liver, thrombopoietin levels, and thrombocyte levels in the blood.[25]

Most of the COVID-19 patients whom treated at RSUP Dr M Djamil Padang were in the moderate clinical severity group (67.8%). The group with severe clinical severity was the group with the least number of treatments (1.7%), while the critical clinical ones were 13.2%. This study did not find a significant association between clinical severity and COVID-19 outcomes. Study by Li et al in China revealed that clinical severity was associated with mortality, although it was not statistically related.[26]

Eastin et al's study found that an increase in mortality was related to clinical severity, in which 52.4% of patients with critical clinical severity were dead, and 38.1% of them still required mechanical ventilation.[27] There were 79.3% patients with COVID-19 who were fully recovered, 3.3% were recovered with sequelae, and 17.4% were deceased. Research by Nalbandian et al reported that respiratory complaints after COVID-19 in Wuhan reached 76%. As many as 42-66% of patients still complained about shortness of breath up to 100 days after being declared cured.[28]

Most deaths were seen in the critical clinical severity (25%), followed by mild clinical severity at 23.8%. The severe clinical severity in this study was 0%. There was no case of death in severe clinical severity group since there were only 2 participants, so it could not provide a severe clinical severity outcome. The number of deaths in mild clinical severity in this study may be related to comorbidities, 15 of the 21 people with mild clinical severity had comorbidities, and 5 deceased patients had comorbidities, including coronary artery disease (CAD) in 3 people, and pregnancy in 2 people. Drew and Adisasmita's research in East Jakarta, as well as Hidayani's literature study, confirmed an increased risk of mortality in COVID-19 with comorbidities.[29,30] Similar results were reported by Priya's study, in which patients with COVID-19 ad comorbidities had 3 times higher mortality rate than COVID-19 patients without comorbidities. This figure could be higher if the patient with COVID-19 has a combination of comorbidities.[7]

LIMITATIONS

This study found a significant association between high levels of leucocyte, thrombocyte, and the outcome of patients with COVID 19. However, there may be other factors affecting the levels of leucocyte and thrombocyte that were not assessed in detail, so there may be bias in this study.

CONCLUSION

Most of COVID-19 patients whom treated at RSUP Dr M Djamil Padang were women with age ranged between 18–49 years, had comorbidities, normal range of leucocyte and thrombocyte levels, and moderate clinical severity. This study found a significant association between high levels of leucocyte and thrombocyte with the outcome of COVID-19. This study did not find a significant association between clinical severity and COVID-19 outcomes. High levels of leucocyte and/or thrombocyte can be considered as components of monitoring in patients with COVID-19. Further study is suggested to develop an assessment of leucocyte and thrombocyte as predictors of COVID-19 outcome.

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CONFLICT OF INTEREST

None.

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Original Article

Increasing Serum Levels of Nephronectin Based on Exposure Duration of Marble Dust in Industry Workers

Jimmy Akbar, Triwahju Astuti, Ngakan Putu Parsama Putra, Fitri Indah Sari

Department of Pulmonology and Respiratory Medicine
Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia

Abstract

Background: Exposure to silica dust is still an occupational health problem worldwide. The marble industry is one of the industries at risk of causing respiratory disease in its workers. Exposure to marble dust in the airways triggers pulmonary fibrosis via nephronectin (Npnt) as an α8β1 integrin ligand, which is an extracellular matrix protein. The purpose of this study is to look at how serum nephronectin (NPNT) levels change over time after being exposed to marble dust.

Methods: This was a cross sectional analytical study of marble industry workers. A significant difference test is carried out on 4 groups of subjects (n=50), including marble industry workers with exposure durations of 1-5 years (n=12), 6-10 years (n=14) and >10 years (n=14), as well as non-marble industry workers (unexposed) as control subjects (n=10). A correlation test was performed to see the relationship between duration of exposure and serum Npnt levels.

Results: The median age value in the exposed group was 40.5 (20-67) years. There was a significant difference (*P*=0.012) in the median Npnt level of the exposed group [1.699 (0.22–5.27) ng/mL] and the non-exposed group [0.678 (0.21–1.96) ng/mL]. The median value of nephronectin levels in the 10 years exposed group [2.4710 (1.74–5.27) ng/mL] were significantly different with both the 1–5 years exposed group (*P*=0.0001) with a median value of 0.6960 (0.22–2.27) ng /mL and the 6–10 years exposed group (*P*=0.039) with a median value of 1.0480 (0.27–4.29) ng/mL. There was a significant (*P*=0.0001) positive relationship (r=0.633) between the length of exposure and the level of Npnt.

Conclusion: The duration of marble dust exposure had a significant effect on serum Npnt levels. The longer the marble industry workers were exposed to marble dust, the higher the serum Nephronectin level.

Keywords: nephronectin, silica, marble

Corresponding Author:
Jimmy Akbar | Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Brawijaya, Indonesia | jimmyakbar@student.ub.ac.id

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INTRODUCTION

Silica dust exposure is still an occupational health problem worldwide. It was estimated that 2 million people in the United States and 3 million people in Europe were exposed to silica dust in their work environment.[1] In Asia, it was estimated that more than 23 million people in China and 10 million people in India were exposed to silica dust in their work environment. Currently, there is no national data on the prevalence of occupational diseases due to the inhalation of silica dust in Indonesia. Studies conducted in a cement factory showed a radiological suspicion of silicosis of 0.5%. A study conducted at a cement factory in West Java showed that the incidence of silicosis was 2.06% in 1990–2003.[2]

Marble is also a raw material for tiles, tables, and floors. This industry produces fine dust, which is a source of occupational health problems worldwide. Marble industry workers are most at risk of exposure to marble dust, which contains calcium carbonate and silica.[3] Continuous exposure to marble dust can reduce lung function and cause various lung diseases, such as chronic obstructive pulmonary disease (COPD) and silicosis.[4]

Silicosis is caused by the chronic inhalation of large amounts of dust from an environment containing silica particles. Pathological changes in silicosis include the formation of irreversible silicosis nodules and excessive extracellular matrix (ECM) deposition, leading to pulmonary insufficiency. Although the etiology of silicosis remains unclear, various emerging studies have shown that several specific types of cells and cytokines play an essential role in the process of silicosis.[5]

Nephronectin (Npnt) is an α8β1 integrin ligand, which is an extracellular matrix protein. Nephronectin is expressed in various tissues and organs: kidney, lung, choroid plexus, tongue, jawbone, dental epithelium, and facial bone.[6] A study by Lee et al found that nephronectin (Npnt) levels were elevated in silicosis patients. This result indicates that Npnt plays a role in the initiation and progression of silica-induced pulmonary fibrosis. In addition, decreased lung function (%FEV_1) is also associated with high Npnt levels. Lee also found that Npnt was associated with the late phase of pulmonary fibrosis.[7] Based on the description above, this study aimed to analyze changes in nephronectin levels according to the duration of marble dust exposure in industry workers.

METHODS

This was a cross-sectional analytic study with the subjects of marble industry workers in Tulungagung, Indonesia. Samples were obtained through stratified random sampling that met the inclusion and exclusion criteria. Fifty subjects were obtained and divided into four treatment groups. Inclusion criteria included men aged 18–70 years who worked at least one year in the marble industry and signed the informed consent. Exclusion criteria were workers with a history of chronic lung disease and workers with malignancy, growth disorders, connective tissue diseases, as determined based on interviews and physical examinations.

The collected data were characteristics and clinical history of patients using patient data research forms, respiratory signs and symptoms using chest x-ray (CXR) photos as the tools for evidence, and serum Npnt levels using 3 ml of the subject's blood specimen. The Human Nephronectin ELISA Kit (Cat. No. E5745Hu) was utilized to measure serum Nephronectin levels. The plate has been pre-coated with Human Npnt antibody. The Npnt present in the sample was added and binded to antibodies coated on the wells. The biotinylated Human Npnt Antibody was then added and binded to Npnt in the sample. The next step was to add Streptavidin-HRP to the Biotinylated Npnt antibody. After incubation, unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added, and color developed in proportion to the amount of Human Npnt. The reaction was finally terminated by the addition of acidic stop solution, and absorbance was measured at 450 nm.

A significant difference test was conducted for serum Npnt levels in 4 groups of subjects, namely marble industry workers with consecutive exposures of 1–5 years (12 subjects), 6–10 years (14 subjects), >10 years (14 subjects), and healthy subjects who were not marble industry workers (unexposed) as control subjects (10 subjects). Analysis of the difference was performed using one-way ANOVA if the data was normal, followed by post-hoc analysis with Bonferroni. If the data was not normal, a non-parametric test with the Kruskal-Wallis test was carried out, and it was significant if $P \leq 0.05$.

A correlation test of serum Npnt levels was also conducted in 4 groups of subjects using linear regression analysis. If the normality of the data was not fulfilled, a non-parametric test with an ordinal regression test was carried out, and the magnitude of the correlation was expressed by r (-1 to +1).

RESULTS

The mean age of the subjects was 40.08±10.99 years. Based on the length of exposure, the shortest exposure time was one year, and the longest was 39 years. Most workers had an exposure duration of 6–10 years (35%) and more than ten years (35%). Most of the workers (55%) were smokers with a mild Brinkman Index. Most subjects used masks as personal protective equipment (PPE), but they were not according to standards (45%).

In this case, the use of masks that met the standards was surgical mask covered with cloth mask. According to the subject's CXR, 90% of the subjects had normal CXR, while the other 10% had abnormal X-Ray. The abnormal CXR was in the form of chronic bronchitis in 5% of the subjects, hillar thickening in 2.5% of the subjects, and minimal pleural effusion in 2.5% of the subjects. Patient characteristics are described in Table 1.

Table 1. Characteristics of Subjects (n=40)

Characteristics	N (%)
Age, years [median (min-max)]	40.5 (20–67)
Working duration (years)	
1-5 years	12 (30.0)
6-10 years	14 (35.0)
>10 years	14 (35.0)
Cumulative duration of exposure (hours-years)	
Smoking	
Smokers	22 (55.0)
Ex-smokers	5 (12.5)
Non-smokers	13 (32.5)
Brinkmann Index (n=22)	
Mild (0–199)	20 (90.9)
Moderate (200–599)	2 (9.09)
Severe (>600)	0 (0.0)
Use of Personal Protective Equipment (PPE)	
Never use any masks	0 (0.0)
Sometimes use non-standard masks	8 (12.5)
Always use non-standard masks	17 (42.5)
Always use standard mask	18 (45.0)
Clinical Symptoms of Respiratory Disorders	
No symptoms	40 (100)
No symptoms for more than 2 weeks	0 (0.0)
Chest X-ray	
Normal lung	36 (90.0)
Abnormality findings	4 (10.0)

There was a significant increase (*P*=0.012) in Npnt levels among marble industry workers compared to non-marble industry workers (control subjects). The mean value of Npnt levels among marble industry workers (1.7517±1.20218 ng/mL) was significantly higher than the Npnt levels of control subjects (0.8014±0.61660 ng/mL). The mean serum Npnt levels are described in Figure 1.

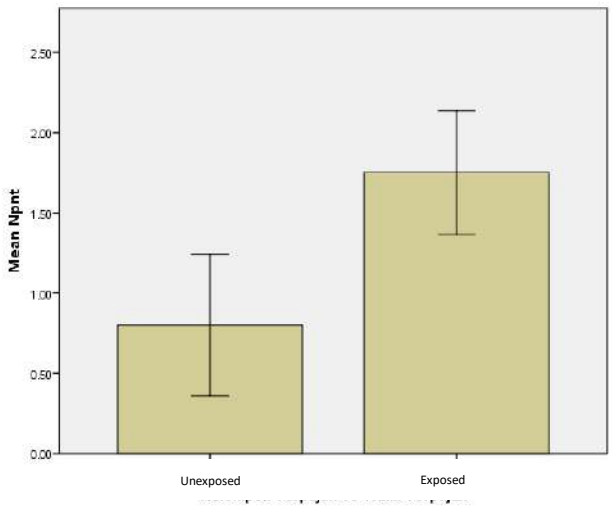

Figure 1. Comparison of the Mean Value of Serum Nephronectin Levels between the Exposed Group and the Unexposed Group

There was a significant result from the comparison of the mean Npnt level between the ten years exposed group and 1–5 years exposed group (*P*<0.05) and the 6–10 years exposed group (*P*=0.039). This result indicates that the mean Npnt level of the >10 years exposed group was significantly different (*P*=0.0001) from the other three groups, including the unexposed group.

Table 2. Nephronectin levels in the exposed groups and the control group

Exposure	N	Median (Min-Max)
Unexposed	10	0,6780 (0,21–1,96) ng/mL
Exposure 1–5 years	12	0.6960 (0.22–2.27) ng/mL
Exposure 6–10 years	14	1.0480 (0.27–4.29) ng/mL
Exposure >10 years	14	2.4710 (1.74–5.27) ng/mL

However, there was no significant result for the comparison of mean Npnt level between the 1–5 years exposed group and the 6–10 years exposed group (*P*=0.08), between the 1–5 years exposed group and control group (*P*=0.922), and between the 6-10 years exposed group and control group (*P*=0.089).

The results were shown to be significant only at >10 years of exposure compared to other groups. Nephronectin levels in the exposed groups and control group and the results of comparison test are described in Table 2 and Table 3.

Table 3. Comparison of Nephronectin Levels' Median between Four Exposure Groups

Comparison of Npnt levels		*P*
Control	Exposed 1–5 years	0.922
Control	Exposed 6–10 years	0.089
Control	Exposed ≥10 years	0.0001*)
Exposed 1–5 years	Exposed 6–10 years	0.080
Exposed 1–5 years	Exposed ≥10 years	0.0001*)
Exposed 6–10 years	Exposed ≥10 years	0.039*)

Note: **P*<0.05 are significant

The duration of exposure and cumulative duration of exposure had a very close relationship (*P*=0.0001) with Npnt levels. As a result, the duration of exposure to marble dust and the cumulative duration of exposure to marble dust had a significant impact on Npnt levels in marble industry workers. This study also showed that the duration of exposure and the cumulative duration of exposure were directly proportional to the Npnt levels (positive correlation value 0.633 for the duration of exposure and 0.633 for the cumulative duration of exposure).

As a result, the longer a worker is exposed to marble dust, the higher the Npnt serum level of the worker. Conversely, the shorter a worker is exposed to marble dust, the lower the serum Npnt level. The duration of exposure and the cumulative duration of exposure are depicted in Figure 2 and Figure 3.

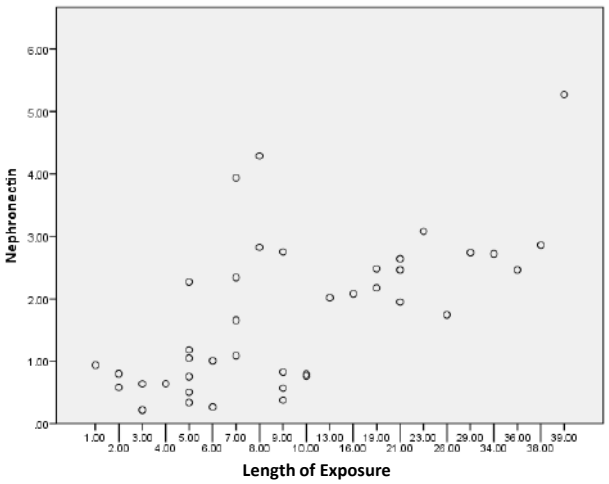

Figure 2. Scatter Plot of Length Exposure to Nephronectin Levels

To remove the potential bias within this study, we analyze the 3 confounding variables of age, smoking behavior, the use of PPE, and duration of exposure as independent variables. The findings revealed that all four combined variables had a significant impact (*P*=0.0001) on Nephronectin levels in marble industry workers. The duration of exposure and smoking behavior significantly (*P*=0.0001 and *P*=0.048 with R=0.470) affected Npnt levels in marble industry workers, where the duration of exposure had a stronger effect than smoking behavior.

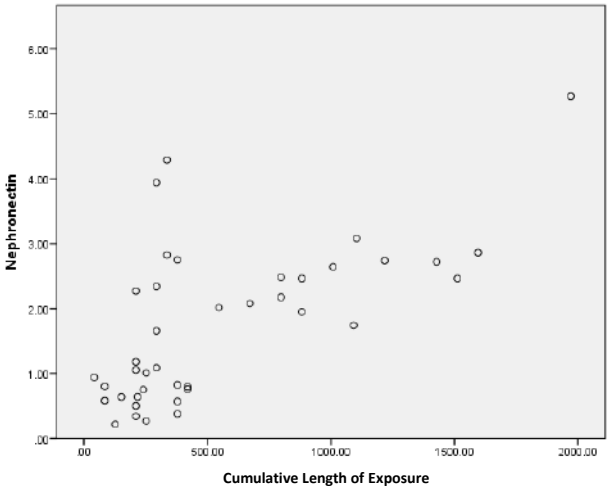

Figure 3. Scatter Plot of Cumulative Length of Exposure to Nephronectin Level

Thus, the four independent variables in the multiple regression model influenced 47% of the data diversity on Nephronectin levels, while other factors outside the model only influenced 53%. On the other hand, the age of workers and the use of PPE had no significant effect on Npnt levels (*P*=0.0795 and *P*=0.582).

In this study, we also want to know whether changes in nephronectin levels are followed by abnormalities in the CXR of marble industry workers. Based on logistic regression analysis, the significance value of the simultaneous test was 0.481, and the partial test had a significance value of 0.505. Therefore, the levels of Npnt had no significant effect on the CXR of the marble industry workers.

The duration of exposure did not significantly affect the CXR of the marble industry workers in this study. The logistic regression analysis results found that the significance value of the simultaneous and partial test of the variable length of exposure was 0.511 and 0.499. Therefore, it could be concluded that the duration of exposure had no significant effect on the CXR of the marble industry workers.

DISCUSSION

The subjects in this study were men aged 18 to 67 years with a mean age of 40.08±10.99 years. In a previous study conducted by Khoiroh, who also studied marble workers as subjects, the mean age of marble industry workers was around 46–55 years old.[8] However, study by Ahmed et al obtained that the average age of marble industry workers was 29,92±6,19 years old.[9] Productive human resources in Indonesia have entered the working age or productive age range that is 15 to 64 years. This data shows that the workers in the marble industry are classified as being of productive age.

Workers with 1–5 years of exposure to marble dust comprised 12 subjects (30%) of the subjects, those with 6–10 years of exposure comprised 14 subjects (35%), and those with >10 years of exposure also comprised 14 subjects (35%). These results are consistent with study by Khoiroh, where most marble industry workers have worked for 13 to

19 years, and in study by El-Gammal et al for 5 to 35 years.[3,8]

The number of smokers among the subjects were 22 subjects (55%). Eryani in 2015 showed that several factors affected lung function capacities: age, gender, years of service, length of work, work history, disease history, nutritional status, smoking habits, and exercise habits.[10] This study follows study by Wijayain 2019, where 83.6% of stone processing workers exposed to silica dust had a smoking habit.[2]

Research from Fathmaulida in 2013 also obtained that smoking habits were found in limestone processing workers, who smoked 13 cigarettes per day. However, the smoking habit and lung disorders in these workers did not reveal a significant relationship.[11] Several findings have shown that smokers exposed to silica dust were more likely to develop clinical silicosis than non-smokers exposed to the same dose.[12] Thus, education for workers about smoking cessation is important to reduce the adverse effects of silica dust exposure on health.

The majority of study subjects (45%) always wore masks, but not in accordance with the standard. This finding is similar to the research conducted by Hutomo in 2016 to see the level of knowledge regarding the use of PPE for furniture industry workers in Jepara, Indonesia. In this study, it was observed that most of the respondents used masks as PPE (47.6%), although their knowledge of the types of masks was not good (46%).[13]

All subjects did not experience clinical symptoms of respiratory distress. Unlike what Sahrun discovered in 2018 that mining, metal, and ceramic workers who spend about 8 hours per day inhaling 3500L of air, including dust particles or other contaminants at work, will be exposed to clinical manifestations of lung disease.[14]

There was a significant increase in Npnt levels among marble industry workers compared to control subjects (P=0.012). These findings are consistent with study from Lee, et al., who discovered that subjects exposed to silica dust had higher levels of Nephronectin than normal patients (who were not exposed). Nephronectin also plays an essential role in inducing and developing pulmonary fibrosis due to silicosis.[7]

There is a relationship between the differences in serum levels of Npnt according to the duration of exposure to silica dust in marble workers. In this study, the results were shown to be significant at >10 years of exposure. The main factors that play a role in the pathogenesis of silicosis are dust particles and the body's response, especially the respiratory tract, to these dust particles. Chemical composition, physical properties, dose, and duration of exposure determine whether or not silicosis can occur easily. The amount of inhaled crystalline silica depends on the concentration and particle size (<5μm) as well as individual susceptibility.[10]

The most common crystalline forms of silica in the workplace include quartz, tridymite, and cristobalite. Quartz contains the highest free silica, so workers exposed to these crystals experience a fast latency period.[15] Workers with high silica exposure categories are 30 times more likely to die than workers with low or no crystalline silica exposure.[16]

These results showed that the duration of exposure and the cumulative duration of exposure to marble dust significantly affected Npnt levels in marble industry workers. This study also showed that the longer a worker is exposed to silica dust, the higher the Npnt serum level of the worker. This follows research from Alonso, et al. in Spain, which pointed out that the duration of exposure to silica dust for 15–20 years had a significant effect on the incidence of silicosis.[17] According to procedures, improper PPE rules may be to blame for the shorter duration of marble workers in Indonesia. Thus, in order to have high levels of Npnt as an indicator of silicosis, the exposure time is shorter, starting at the 10th year of exposure as opposed to the study from Alonso, which began at the 15th year.[17]

The four independent variables, namely duration of exposure, age, history of smoking, and use of PPE, significantly affected Npnt levels in marble industry workers (P=0.0001). The duration of exposure and smoking had a significant effect on the levels of Npnt in marble industry workers, and the

duration of exposure had a stronger effect than smoking (*P*=0.0001 and *P*=0.048 with R=0.470). These findings are supported by study from Lee, et al. in Japan, which revealed a link between Npnt levels in subjects exposed to silica dust, but no relationship between age and Npnt levels.[7] Wijaya, et al. observed that 83.6% of stone processing workers exposed to silica dust had smoking habit. However, there were no significant results in the same study between smoking habits and serum TGF-β1 as a biomarker of silica in the blood. This was due to the small proportion of subjects in the study.[2]

In contrast, smoking can cause an increase in serum TGF-β1 levels due to the immunosuppressive effect of TGF-β1 on the immune system. Smokers who smoke 20 cigarettes per day have higher mean serum TGF-β1 level than non-smokers and smokers who smoke less than 20 cigarettes per day. Serum TGF-β1 levels increase in tandem with cigarette consumption.[18]

This study concluded that the Npnt levels had no significant effect on the CXR of marble industry workers. The CXR is one of the essential tools in detecting pneumoconiosis (asbestosis, silicosis, and pneumoconiosis in coal miners). On exposure to silica dust, the development of opacity with a diameter of more than 1 cm will be seen. The standard of the CXR interpretation method has been determined by the International Labour Organization (ILO).[19]

Even though the standard has been used, there is still variability between readers of the CXR results. Radiographs may also be less sensitive to early-stage changes produced by exposure to dust. For example, it was estimated that about 20% of asbestos-exposed workers with pulmonary fibrosis on pathological examination do not show any abnormal changes detected on radiographs. CXR alone is inadequate to serve as a surveillance tool or to detect occupational lung disease. Bronchitis is difficult to be detected on CXR. Emphysema is accurately detected only at an advanced stage.[19]

In a study conducted by Lopes in 2008, there was a more significant difference between specialist doctors who read CXR based on the results of small opacity readings. The inter-reader variability is lower at significant opacity. However, despite these limitations, CXR is still an efficient tool for follow-up evaluation of workers exposed to silica, as it is an inexpensive procedure and subjects are exposed to only low doses of radiation. This study obtained that the diagnosis of silicosis using a CT scan is better than a CXR for the early detection of the early phase of the disease and detection of progressive massive fibrosis.[20]

A study conducted by Austin in 2021 also stated that CXR alone was not sufficient to detect occupational lung disease. It was recommended to use CT (Computed Tomography) scan to diagnose occupational lung disease because CT scan sensitivity was higher for early detection of disease and had better accuracy for determining disease patterns.[21]

This study proved that the length of exposure did not significantly affect the CXR of marble industry workers. However, this could be explained because the assessment of CXR did not follow the ILO standard and used regular assessment in the hospital. This result was not in line with the study conducted by Mitra in 2015 on stone crushing factory workers in Lakshmi, India, which revealed that the longer the duration of exposure, the higher the prevalence of CXR with statistically significant positive silicosis (*P*<0.05).[22]

LIMITATIONS

This study had several limitations, namely that the number of sample subjects used was still small. Moreover, the recommended radiological examination for the diagnosis of pulmonary fibrosis as part of the ILO standards has not been carried out. In addition, a pulmonary function test was not executed in this study where the lung test is a better approach to describing the subject's respiratory function, because the study was conducted during the second wave of the pandemic. Finally, the

acquired risk factors, such as a history of smoking and the use of PPE, were not homogeneous, which could affect the results.

CONCLUSION

The duration of exposure to marble dust had a significant effect on serum Nephronectin levels. The longer the marble industry workers were exposed to marble dust, the higher the serum Nephronectin level.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

None.

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Literature Review

Pathophysiology of Hemoptysis in Lung Disease

Airin Aldiani, Mohamad Fahmi Alatas

Department of Pulmonology and Respiratory Medicine
Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

Abstract

Hemoptysis is expectoration of blood with or without sputum originating from the lower airway. Even minor symptom of hemoptysis is a distressful for the patient. This condition is an alarming symptom that may lead to massive bleeding and life-threatening condition. When evaluating a patient presenting with expectoration of blood, one must determine the source of the bleeding and whether the patient is presenting with true hemoptysis or pseudo hemoptysis. Based on the underlying disease, hemoptysis can occur through several pathological mechanisms leading to bronchial artery rupture. Mostly, hemoptysis is caused by bronchiectasis, pulmonary tuberculosis, lung cancer, and pulmonary fungal infections. The incidence rate of the causative diseases may differ depending on geographical location. Pulmonary vascularization consists of two circulation pathways namely the pulmonary and bronchial circulation, each of which has its own role. Around 90% of hemoptysis cases are caused by the collapse of bronchial arteries due to increased pressure in its circuit. It is important to understand the pathophysiology and pathomechanism of hemoptysis for further management of diseases and clinical manifestation.

Keywords: bronchial circulation, hemoptysis, pathophysiology hemoptysis, pulmonary hemorrhage

Corresponding Author:
Airin Aldiani | Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, University of Indonesia | airin.aldiani@gmail.com

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INTRODUCTION

Hemoptysis is derived from ancient Greek words *haema* means blood, and *ptysis* means spitting. Hemoptysis could be found in daily practice as a symptom of lower respiratory problems.[1,2] Hemoptysis occurs in 10% of patients with chronic lung disease. Although 90% of hemoptysis cases are self-limiting diseases, hemoptysis is a life-threatening condition that requires prompt diagnosis and treatment.[3]

Asphyxia is the leading cause of death in hemoptysis. In addition, in hemoptysis, cardiovascular collapse is often among the cause of death. The mortality rate from untreated massive hemoptysis is more than 50%.[4] Passage of relatively small amounts of blood is a symptom that should still be watched out for. Regardless of the quantity, the cause of hemoptysis must be identified immediately. It is important to initiate adequate treatment and to avoid fatal complications.[4,5]

Hemoptysis is often caused by bleeding from the bronchial circulation due to inflammatory process in the airway. Infection is the leading cause of hemoptysis in 60-70% cases. Inflammation and edema of airway can lead to rupture of surface blood vessels. Common causes of hemoptysis are classified into: infectious diseases, neoplastic, vascular, autoimmune and drug-related. The etiology of hemoptysis can affect treatment strategies. Detailed history-taking and careful physical examination are necessary to provide certain underlying diagnosis. This article aims to provide an in-depth literature review on hemoptysis, assessing its causes and pathophysiologic mechanisms. Also this article provided with details about anatomy of bronchial arteries which are responsible for hemoptysis.[4,6]

HEMOPTYSIS

Hemoptysis is defined as expectoration of blood from the lower respiratory tract or expectoration of sputum accompanied by bloody spots. In diagnosing hemoptysis, it is important to distinguish lower respiratory tract bleeding from

nasopharyngeal bleeding and gastrointestinal tract (pseudohemoptysis).[4,5]

Table 1. List of diseases causing hemoptysis[4]

Diseases Causing Hemoptysis	
Causes of hemoptysis from small vessel diseases	
Immunologic and vasculitis disease	1. Acute lung allograft rejection
	2. Antiphospholipid antibody syndrome
	3. Behçet disease
	4. Goodpasture's Syndrome
	5. Henoch-Schönlein Purpura
	6. Isolated Pulmonary Capillaritis
	7. Microscopic polyarteritis
	8. Mixed cryoglobulinemia Wegener granulomatosis
Cardiovascular diseases	Mitral stenosis
Coagulation Diseases	1. Latrogenic (anticoagulant/thrombolytic agents)
	2. Coagulopathies
Others	1. Diffuse alveolar damage
	2. Lymphangioleiomyomatosis
	3. Pulmonary capillary hemangiomatosis
	4. Pulmonary hemosiderosis
	5. Tuberous sclerosis
	6. Veno-occlusive diseases
Causes of hemoptysis from large vessel diseases	
Infectious diseases	1. Abscess
	2. Bronchitis (acute or chronic)
	3. Bronchiectasis
	4. Fungal infection
	5. Parasitic infection
	6. Pneumonia
	7. Tuberculosis or nontuberculous mycobacteria
Cardiovascular diseases	1. Arteriovenous malformation
	2. Bronchial artery aneurysm
	3. Bronchovascular fistula
	4. Congestive heart failure
	5. Pulmonary embolism or infarction
	6. Pulmonary hypertension
	7. Right-sided endocarditis
	8. Thoracic aortic aneurysm rupture or dissection
	9. Septic pulmonary embolism
Congenital diseases	1. Cystic fibrosis
	2. Pseudosequestration
	3. Pulmonary artery atresia or stenosis
Neoplastic diseases	1. Bronchial adenoma
	2. Lung metastasis
	3. Primary lung cancer
Vasculitic diseases	1. Behçet disease/Hughes-Stovin syndrome
	2. Lupus pneumonitis
	3. Takayasu arteritis
	4. Wegener's granulomatosis

Table 2. List of diseases causing hemoptysis (Cont.)

Diseases Causing Hemoptysis	
Causes of hemoptysis from large vessel diseases	
Others	1. Chronic obstructive airway disease
	2. Drug
	3. Foreign body
	4. Iatrogenic (Swan-Ganz catheter)
	5. Interstitial fibrosis
	6. Lung contusion
	7. Pulmonary endometriosis
	8. Trauma
	9. Dieulafoy's disease or the bronchus
	10. Cryptogenic hemoptysis

Based on the underlying disease, hemoptysis is the result of several pathological mechanisms. The causes of hemoptysis are divided into parenchymal disease, airway disease, and vascular disease. Hemoptysis originates from both great and small blood vessel.[4,5] Bleeding from small vessels is caused by immunologic, cardiovascular vasculitis, and coagulation disorders. Bleeding from large vessels is often caused by tuberculosis (TB), bronchiectasis, fungal infections, and malignancy (Table 1).[4]

Massive hemoptysis may increase risk of mortality up to 80% in untreated patients.[7] Various studies have defined the range of massive hemoptysis differently from 100 mL to more than 1000 mL in 24 hours.[3,4,8] Initial estimation of blood loss is frequently incorrect; therefore, hospitalized patients' blood loss should be monitored daily. There is a lack of universal consensus on the quantification and severity of hemoptysis. National Respiratory Referral Hospital, Persahabatan, using Busroh Criteria for massive hemoptysis:

a) Coughing up blood ≥600 mL per 24 hours and persisting under observation
b) Coughing up blood ≥250 mL but <600 mL per 24 hours and hemoglobin (Hb) level <10 g/dL, with ongoing bleeding
c) Coughing up blood >250 mL but <600 mL per 24 hours, Hb level >10 g/dL and within 48 hours conservative treatment the bleeding has not stopped.[9]

Each case of massive hemoptysis does not only depend on the quantity of blood loss but also on the mechanism of the blood expulsion and the pre-

existing pulmonary dysfunction. Death from asphyxia occurs long before massive blood loss or before hemorrhagic shock develops. This is mainly due to low tracheobronchial volume (150-200 mL) so even a small amount of blood loss can alter the balance of gas exchange in the lungs.[3] In addition, the anatomic dead space in the main airway is around 100-200 mL, so the volume-based definition of massive hemoptysis is more relevant and can be life-threatening.[10]

LUNG VASCULARIZATION

Lung organs have two pathways to supply blood. Approximately 90-99% of gas exchange and tissue perfusion is carried out by the pulmonary arteries, and the remaining 1-5% is derived from the bronchial arteries.[3,4,11] The pulmonary arteries originate from the right ventricle and branch into billions of pulmonary capillaries, and enclose the alveoli to allow gas exchange. Meanwhile, the bronchial arteries originate from the descending aorta and are arranged parallel to the bronchi and supply blood through its branches. The bronchial arteries arise from the aorta or intercostal arteries. Bronchial arteries are part of the systemic circulation with high resistance and low capacitance system. The bronchial arteries receive approximately 2% of cardiac output (Figure 1).[2,4,5,11]

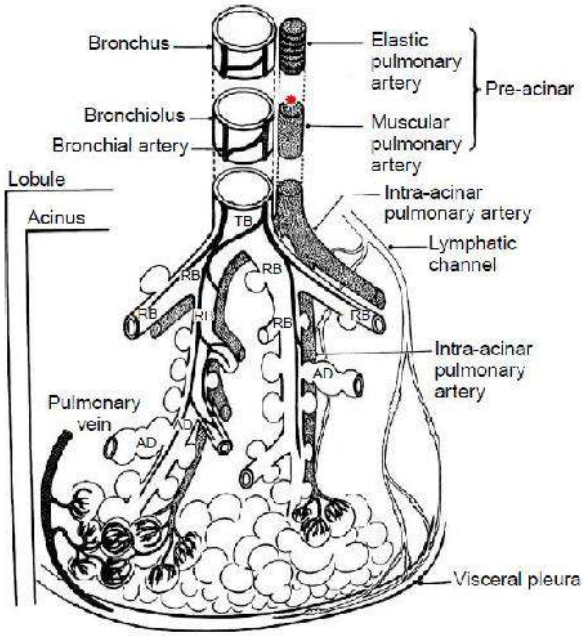

Figure 1. Schematic of blood supply in the lung[12]

Bronchopulmonary anastomoses connect the bronchial and pulmonary arteries at the level of alveoli and respiratory bronchioles. Blood drainage in bronchial arteries occurs due to the passage of blood from the bronchial veins to the right atrium and from the pulmonary veins to the left atrium (Figure 1).[4,11,13] Several disorders may damage this mechanism, including:[3]

a) Hypoxic vasoconstriction.
b) Pulmonary artery thrombosis or thromboembolism.
c) Vasculitis.
d) Chronic inflammatory or malignant disease.
e) Pulmonary vascular malformations.

In this condition, compensation occurs in the pulmonary arteries resulting in dilatation of the bronchial arteries and their anastomoses. Thus, the percentage of cardiac output flowing through the bronchial arteries increases.[11] In pulmonary disease conditions such as systemic hypoxemia, alveolar hypoxia, pulmonary infarction, and various chronic inflammations in the chest cavity, the bronchial arteries dilate to supply oxygenated blood to the ischemic area. The walls of the bronchial arteries are thinner and more fragile, as well as the burden of systemic arterial pressure and disturbances due to chronic disease may lead to tearing or bleeding from the airways, manifesting as hemoptysis.[2–4,11]

The intensity of coughing and the amount of expelled blood in each patient vary depending on the degree of pulmonary impairment or the impact of the underlying disease and the type of blood circulation involved.[1]

Study showed that about 90% of hemoptysis originates from the bronchial arteries, about 5% from the pulmonary arteries, and the other 5% from non-bronchial systemic arteries.[2–4] When bleeding occurs between the three locations, sensory receptors can be irritated via afferent nerves from the cough reflex, namely cranial nerves V, X, XII, and superior laryngeal nerves. Then through the efferent nerves, including spinal nerves and recurrent laryngeal larynx, the blood is expelled with or without other secretions.[5]

PATHOPHYSIOLOGY OF HEMOPTYSIS IN TUBERCULOSIS

In lung tuberculosis (TB), several mechanisms can induce hemoptysis. Hemoptysis occurs in both active and inactive lesions and depends on the size.[1] Proliferation and enlargement of bronchial arteries can be found in pulmonary infections such as bronchiectasis, pneumonia, and tuberculosis. So it can be concluded that bronchial arteries have an important role in lung infection.[11]

Infection by *Mycobacterium tuberculosis* is one of the most common causes of hemoptysis worldwide. Hemoptysis in the presence of TB is primarily caused by several etiopathologies, including bronchiectasis, aspergillomas, broncholiths, reactivation of TB, scar carcinoma, chronic bronchitis, pulmonary cavity's colonization, and vascular abnormalities such as pseudoaneurysms.[11,14] In active infection, cavitary lesion with inflammation may cause bronchial and alveolar ulceration that leads to necrotizing and erosion surrounding bronchial and alveolar blood vessels.[1,11]

Post TB sequelae with ectasis, hypervascularization, cavitary lesion, dilated bronchial blood vessel, and collateral anastomosis is usually found in inactive TB with hemoptysis.[1] In sequalae of TB infection, hemoptysis results from remodeling of lung parenchymal and vasculature. Recurrent or chronic infection and inflammation lead to permanent damage and dilatation not only on the bronchial lumen but also its arteries. Infection and/or inflammation presented in normal anastomoses between bronchial and pulmonary blood vessels become larger resulting in heavier blood flow through dilated bronchial arteries. Thus, the bronchial vessels become hypertrophied and ectatic. The new and collateral blood vessel made by angiogenic growth factors namely vascular endothelial growth factor (VEGF) is thin walled which is more prone to rupture. Hypervascularity, structural disturbances, and infection exposure in blood vessel make it more prominent to bleeding.[15]

Tuberculous cavities usually develop from caseous lesions that have liquefied and necrotic. Most of the cavities have collagen and spongy tissue on the inner and outer layers, and there is granulation tissue and capillaries between them. There is an incomplete thrombosis of the central vein, which can easily be affected by TB infection and cause hemoptysis. Pulmonary TB lesions can usually block blood vessels that carry blood at high pressure, causing damage and rupture of Rasmussen's aneurysm.[16]

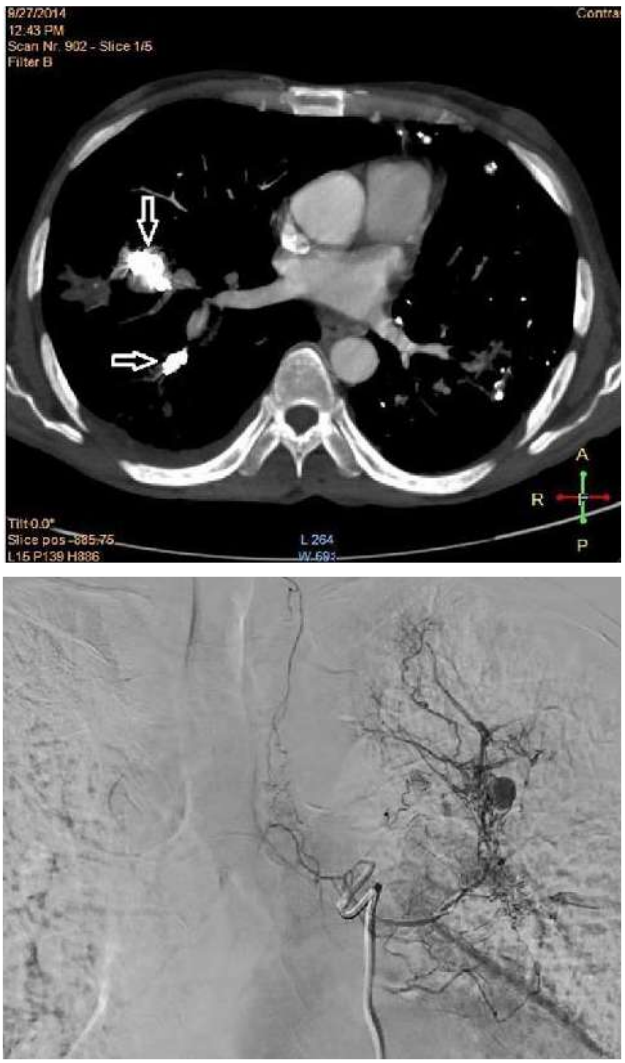

Figure 2. Rasmussen's Aneurysm[14,18]

Rasmussen's aneurysm was discovered by a Danish doctor named Fritz Valdemar Rasmussen. Rasmussen aneurysms have been described as pulmonary vessels penetrating the cavity wall of pulmonary TB, with aneurysmal enlargement occurring in 5% of patients with chronic cavitary tuberculosis (Figure 2). Cavities in pulmonary TB are formed due to the mechanism of TB immunopathogenesis which causes granulation tissue to form cavities. The inner walls of blood

vessels (adventitia and media) around the cavity are also coated with a layer of fibrinogen leading to thinning of the vessel wall and subsequent aneurysm formation and rupture leading to hemoptysis.[14,16–20]

Frailty of the bronchial and pulmonary artery walls is caused by granulation connective tissue replacing the tunica media and adventia. Then over time, it is replaced by a layer of fibrin, causing erosion of the arterial wall, resulting in a pseudoaneurysm. At this time, nutritional intake begins to be disturbed, resulting in hypoxic vasoconstriction mechanisms, intravascular thrombosis and edematous compression. This condition may lead to massive hemoptysis. Under normal conditions, an increase in intraarterial pressure can be compensated by the body. However, in chronic inflammatory conditions such as pulmonary TB there is an increase in pulmonary artery pressure.[14,17–19]

Hemoptysis may occur due to increased pulmonary artery pressure or compensation through the bronchial arteries. When the pulmonary artery pressure increases, the bronchial arteries dilate so that their thinner and more fragile surfaces are more susceptible to rupture or tear. Hemoptysis ranks second as one of the causes of death in patients with pulmonary tuberculosis. Death in hemoptysis is often caused by asphyxia, hemorrhagic shock, or both, so hemoptysis should not be underestimated. Pulmonary tuberculosis with hemoptysis can also increase the risk of TB transmission due to a greater bacterial load. In addition, hemoptysis can also cause complications, including pulmonary atelectasis. Rasmussen's aneurysm is a rare sequela of pulmonary tuberculosis but may result in life-threatening condition of hemoptysis.[14,16,17]

BRONCHIECTASIS

Bronchiectasis is an abnormal dilatation in bronchus and bronchioles caused by destruction of bronchial cartilage due to recurrent infection. Fibrosis of lung tissue also leads to bronchiectasis. Repeated bacterial infection leads to change in the surrounding bronchial arteries, hypertrophy, distortion, aneurysm formation, pulmonary vascular anastomosis, fistula formation or increased vascularization. Any of the above arterial rupture can cause massive, rapid, and fatal hemoptysis.[1,11,21]

In patients with bronchiectasis, hemoptysis is caused by hypertrophy of the bronchial arteries resulting in rupture of the bronchial arteries into the bronchial lumen. The bronchial arteries are branches of the thoracic aorta which possess higher systemic pressure than the pulmonary vessels. Normally, the bronchial arteries are present in the bronchi. In bronchiectasis, the pulmonary artery remains patent but as the disease progresses a thrombus form in the pulmonary artery. Then, recanalization occurs through bronchopulmonary anastomoses with dilated bronchial arteries. The modality for diagnosing bronchiectasis is high-resolution computed tomography (HRCT), showing ectasis in the bronchi and fusion of the bronchial arteries or the "signet-ring sign" (Figure 3).[2,11]

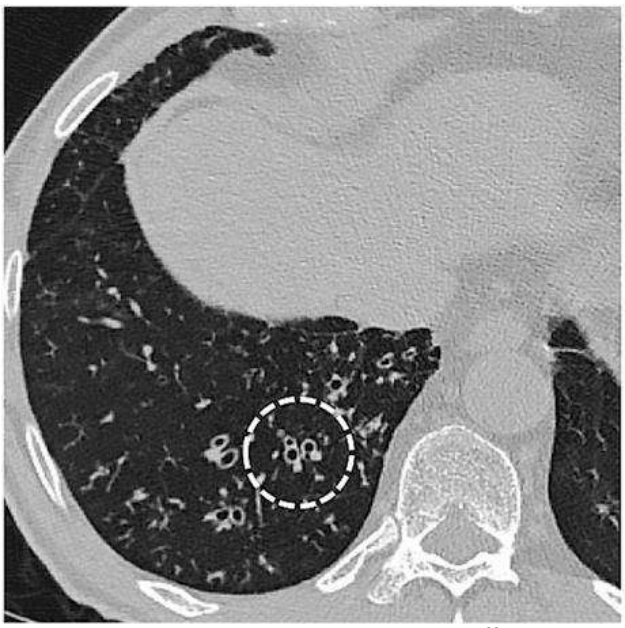

Figure 3. Signet ring signs from HRCT[22]

LUNG CANCER

Lung neoplastic lesions causing hemoptysis are divided into primary and metastatic lesions. Symptoms of hemoptysis in lung cancer patients are estimated to be 7-35%. Approximately 20-60% of primary lung cancer patients will eventually develop symptoms of hemoptysis, and 3% of them die due to massive hemoptysis. Hemoptysis in lung cancer metastases occurs only if the lesion is located in the

endobronchial lumen.[1,23]

Hemoptysis is generally divided into massive and sub-massive (non-life-threatening) hemoptysis. The incidence rates of massive and sub-massive hemoptysis are 3.3% and 16%, respectively. Massive hemoptysis usually depends on the type and location of the tumor. Massive hemoptysis is often caused by squamous cell tumors located in a central area or major airway. In sub-massive hemoptysis, there is no known cause yet. Sub-massive hemoptysis is more common in about 98% of cases. In contrast to squamous cell carcinoma (SCC), this type of adenocarcinoma cancer often originates in the peripheral areas. Hu et al (2013) showed that central lesions of lung tumors with symptoms of hemoptysis had the worst prognosis. It is because the tumor's location is easier to invade large blood vessels, although it is usually easier to detect with simpler investigations.[24]

Nichols et al (2012) stated that hemoptysis and SCC were significantly related. In terms of histological cell types, SCC is a type of tumor that often forms cavities and then invades blood vessels, cutting off blood supply and causing ischemic necrosis. However, SCC can fatally invade large blood vessels because of its central location.[24,25]

Therefore, SCC is contraindicated with bevacizumab therapy due to the inhibition of vascular endothelial growth factor (VEGF), which can lead to more frequent of hemoptysis. From the analysis results, VEGF expression, extra-tumor microvessel density (MVD), tumor necrosis, vascular invasion, and coagulation function described vascular invasion. All are important mechanisms of hemoptysis in lung adenocarcinoma. It can be concluded that the cancer cells in lung cancer patients with hemoptysis symptoms are more likely to invade other organs. In addition, hemoptysis can also be used as an independent prognostic factor with poor outcomes in lung cancer patients.[24,25]

PULMONARY MYCOSES

Pulmonary mycoses with hemoptysis symptoms include aspergillosis, coccidioidomycosis, and pulmonary histoplasmosis. Angioinvasion of fungal elements can cause structural damage to parenchymal and blood vessels, causing pulmonary infarction and bleeding. Aspergillosis is a type of pulmonary mycoses that often causes hemoptysis and it is estimated that 90% of people with aspergillosis have experienced at least one episode of hemoptysis in their lifetime. Hemoptysis is the most common symptom manifestation in patients with aspergilloma with an incidence rate of about 50-90%. The condition is rare but can be mild to life-threatening. The estimated mortality rate from massive hemoptysis is 38%. The dilated bronchial artery or intercostal artery is often surrounded by the cavity, which is highly susceptible to rupture and leads to massive bleeding.[1,11,21]

The pathogenesis of hemoptysis in pulmonary aspergillosis is local vascular invasion of the preexisting lung cavity and cystic spaces by *Aspergillus fumigatus*. Collateral vessels are formed between the bronchial and systemic arteries of the chest wall. The anastomosis causes an increase in blood supply to the infected tissue and mechanical friction between the fungus ball and the walls of the blood vessels around the cavity can be a predisposing factor for massive hemoptysis. The pathogenesis of hemoptysis in patients with pulmonary histoplasmosis and coccidiomycosis are through calcification of local lymph nodes. Calcification causes erosion of adjacent vascular vessels such as the bronchial arteries and surrounding tissues. Other several mechanisms of hemoptysis in fungal lung infections include 1) release of endotoxin by fungi accompanied by hemolytic material and 2) the length of the destructive process in the lungs due to infection in the pulmonary arteries causes prolonged inflammation so that the pulmonary arteries are always open.[1,26,27]

CONCLUSIONS

Hemoptysis must be distinguished from bleeding from organs other than the lungs (pseudohemoptysis). Mostly caused by a collapse of

the bronchial circulation due to the formation of bronchopulmonary anastomoses as a compensatory mechanism for increased pulmonary intraarterial pressure. Underlying lung disease may be the basis for the different mechanisms and pathophysiology of hemoptysis. Treatment strategies can change according to the etiology, and the primary types of treatments include medical management, embolization, and surgery.

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CONFLICT OF INTEREST

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Literature Review

The Role Extracorporeal Membrane Oxygenation in Acute Respiratory Distress Syndrome

Widhy Yudistira Nalapraya[1,2], Menaldi Rasmin[2], Zuswayudha Sjamsu[3]

[1]Faculty of Medicine, Universitas Islam Bandung, Bandung, Indonesia
[2]Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Indonesia, RSUP Persahabatan, Jakarta, Indonesia
[3]Department of Anesthesia, Harapan Kita Hospital, Jakarta, Indonesia

Abstract

Treatment of oxygen therapy should be based on appropriate indication and dose of administration. British Thoracic Society (BTS) recommends oxygen therapy were administered with a 94–98% saturation target in most acute patients and for patients with hypercapnic respiratory failure, BTS recommends 88-92 saturation target or spesific saturation target. Acute respiratory distress syndrome (ARDS) is characterized by acute, diffuse, inflammatory lung injury leading to increased capillary permeability in the alveolus with clinical manifestations of hypoxemia and bilateral opafication. The use of extracorporeal membrane oxygenation (ECMO) along with mechanical ventilator can be useful for ARDS patients and can improve survival.

Keywords: acute respiratory distresss syndrome (ARDS), Extracorporeal Membrane Oxygenation (ECMO)

Corresponding Author:
Widhy Yudistira Nalapraya | Faculty of Medicine, Universitas Islam Bandung, Bandung, Indonesia, Persahabatan Hospital, Jakarta, Indonesia | widhyyudistira@gmail.com

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INTRODUCTION

Oxygen is a therapy that is often used in emergencies in the medical field. Thirty-four percent of patients receive oxygen therapy on their way to the hospital, and 15–17% of inpatients receive long-term oxygen therapy. The wrong perception is about the safety of oxygen therapy. Many people are not aware of the dangers of hyperoxemia; many believe that oxygen therapy is used to relieve shortness of breath even without hypoxemia. There is no evidence to suggest a benefit of oxygen administration in normothermia or mild hypoxemia. Shortness of breath can occur not only due to cardiorespiratory disease. However, it can also occur in metabolic acidosis, anxiety, and pain, so oxygen therapy is not indicated in these cases.[1]

Optimal oxygen therapy at the time of patient delivery is of paramount importance in addition to assessment by physicians in the emergency department and treatment of disease. Most patients need to get adequate oxygen therapy according to the patient's needs. Too little oxygen can result in hypoxia. Inadequate administration of oxygen therapy can cause cardiac arrhythmias, tissue damage, kidney failure, and brain damage. Giving excess oxygen therapy can also result in death in patients with respiratory failure.[2]

The British Thoracic Society (BTS) recommends that oxygen therapy be administered with a saturation target of 94–98% in most acute patients and a saturation target of 88–92% or specific targets for patients suffering from or at risk of developing hypercapnic respiratory failure. Patients with cystic fibrosis frequently experience exacerbations, and their characteristics resemble those of chronic obstructive pulmonary disease (COPD), namely hypoxemia, hypercapnia, and acidosis. Management of oxygen therapy in cystic fibrosis using a non-invasive ventilator (NIV) is significant in severe cases; NIV is needed to reduce

symptoms of increased respiratory effort and shortness of breath and help clear the airways.[3] Extracorporeal membrane oxygenation (ECMO) is a method of assistance living outside the body; its efficacy has been proven and accepted for managing respiratory and cardiopulmonary failure in the neonatal and pediatric population. In the adult population, there is still much debate regarding the advantages of using ECMO but it has an advantage in the postcardiotomy heart failure population.[4]

PHYSIOLOGICAL EXTRACORPOREAL MEMBRANE OXYGENATION

Diffusion law

Ficks' law explains the process of diffusion through the tissue; namely, the speed of air exchange through the tissue will be directly proportional to the tissue surface area and the difference in partial air pressure between the two sides and inversely proportional to the thickness of the tissue. The surface area of the blood gas barrier in the lungs is around 50 – 100 m^2, and the thickness is 0.3 microns, so the barrier is ideal for the diffusion process. The velocity of air exchange is proportional to the diffusion constant, which is influenced by the tissues and gas components in the air. The diffusion constant is proportional to the solubility of air and inversely proportional to the square root of the molecular weight.[5]

Diffusion process

The diffusion process based on Ficks' law has three principles. First, the rate of gas diffusion through the tissue is directly proportional to the area of the tissue and inversely proportional to the thickness of the tissue. Second, the rate of diffusion of gases is directly proportional to the difference in partial pressure. Third, the rate of gas diffusion is directly proportional to the solubility of the gas in the tissue but is not related to the molecular weight of the gas. This statement explains that the diffusion speed of carbon dioxide (CO2) is 20 times faster than oxygen (O2) because CO2 has a more excellent solubility than O2, even though it has almost the same molecular weight.

ACUTE RESPIRATORY DISTRESS SYNDROME

Acute respiratory distress syndrome (ARDS) is an inflammatory lung injury that causes an acute and widespread increase in capillary permeability in the alveoli. Increased lung weight and reduced oxygenation to lung tissue seen in clinical manifestations of hypoxemia and bilateral opacity on chest X-rays are associated with decreased lung compliance, increased venous flow velocity, and increased physiological dead space. Acute respiratory distress syndrome is a life-threatening condition. This condition can be caused by pulmonary disorders such as pneumonia and aspiration or nonpulmonary disorders such as sepsis, pancreatitis, and trauma, which cause nonhydrostatic pulmonary edema.[6]

Table 1. Berlin criteria for ARDS[7]

Criteria	Rational
Onset within 7 days of clinical cause or new or worsening respiratory symptoms	*Acute respiratory distress syndrome* may occur within 72 hours in the majority of patients at risk for the syndrome and within 1 week in all patients at risk.
Bilateral opacities consistent with pulmonary edema on chest X-ray or chest CT	The Berlin criteria are clearer in making the criteria for opacity i.e., not an effusion, atelectasis of the lung or lobe, not a mass or nodule
Degree of severity ARDS	
Light	PaO_2/FiO_2 201–300 mmHg, Mortalities 27%
Moderate	PaO_2/FiO_2 101–200 mmHg, Mortalities 32%
Heavy	$PaO_2/FiO_2 \leq 100$, mortalities 45%
Minimum PEEP setting or Continuous Positive Airway Pressure (CPAP). Assessment of PaO_2/FiO_2 in invasive mechanical ventilation.	In the use of *high flow nasal cannula* (flow ≥ 45 L/minute); The need for a high PEEP setting does not increase the severity of ARDS on the Berlin criteria

The prevalence of ARDS in the United States reaches 200,000 patients annually and contributes to 75,000 deaths annually. This figure is higher than breast cancer and Human Immunodeficiency Virus (HIV) infection. Worldwide data states that ARDS affects 3 million patients annually, contributing to

10% of Intensive Care Unit (ICU) care and 24% of ARDS patients receive mechanical ventilation in the ICU. Supportive therapy with mechanical ventilation remains the mainstay of management for ARDS, although the mortality rate remains high at 35–46%. The mortality rate is related to the degree of lung injury and the onset of ARDS.[6]

The diagnosis of ARDS continues to be updated to date. ARDS criteria in 1967 are based on clinical syndromes of the severity of shortness of breath, tachypnea, and cyanosis that persists on oxygen therapy accompanied by decreased lung compliance and bilateral wide infiltrates on chest radiographs. In 1994, the definition of ARDS, according to the American-European Consensus Conference (AECC), was the acute onset of hypoxemia with bilateral infiltrates on chest X-ray with no clinical evidence of left atrial hypertension when pulmonary capillary wedge pressure (PCWP) ≤18 mmHg was measured. The degree of hypoxemia is measured by the arterial partial pressure of oxygen (PaO_2) divided by the fraction of inspired oxygen (FiO_2). ≤200 mmHg is considered ARDS, while the limit for Acute Lung Injury (ALI) uses the criteria (PaO_2/FIO_2) ≤300 mmHg.[6]

The 2012 Berlin definition established clinical criteria, namely the degree of shortness of breath worsening within 7 days with minimal positive end-expiratory pressure (PEEP) settings, bilateral opacity on chest X-ray and chest computed tomography (CT), and PaO_2/FiO_2 ≤ 200 to diagnose ARDS. PaO_2/FiO_2 criteria are divided into three levels: mild criteria if PaO_2/FiO_2 is 201–300 mmHg, moderate criteria for PaO_2/FiO_2 is 101–200 mmHg, and criteria for severe PaO_2/FiO_2 ≤100 mmHg. The cause of ARDS, whose onset appears seven days is pneumonia or sepsis. ARDS symptoms can also be found in other diseases called mimic ARDS, but the onset of ARDS appears more slowly.[7]

ARDS Pathogenesis

Injury in the distal part of the lung to the alveoli can be caused by direct or indirect causes that cause microvascular injury. In the exudative phase, alveolar macrophages are activated to release inflammatory mediators and chemokines, resulting in neutrophils and monocytes congregating at the injury site.[7]

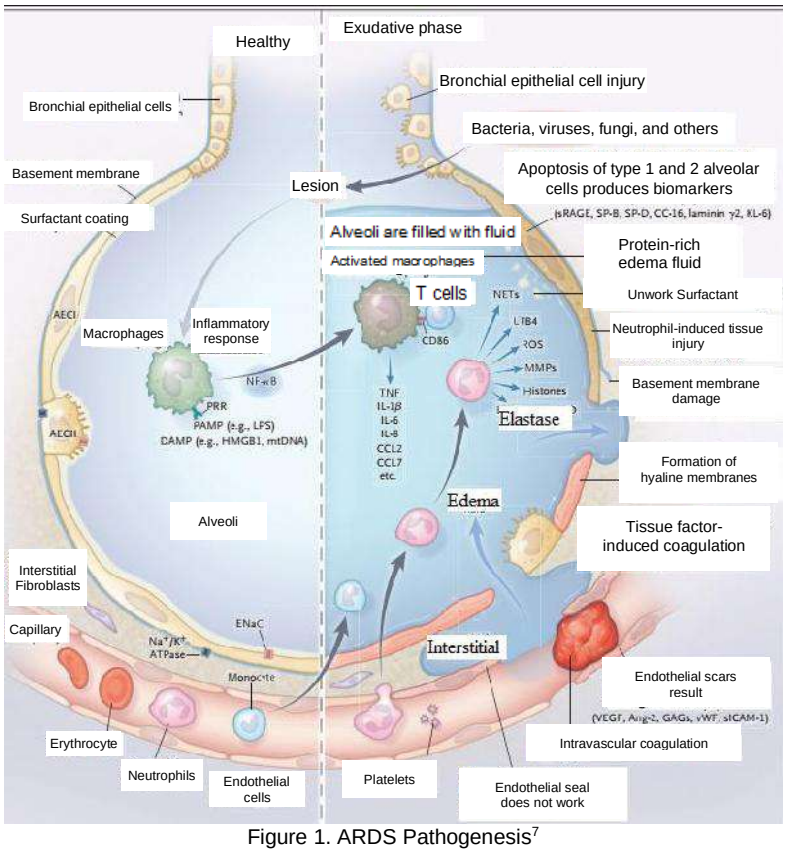


Figure 1. ARDS Pathogenesis[7]

Activated neutrophils play a vital role in causing alveolar tissue injury because they produce toxic mediators which cause loss of insulating function so that the interstitial and interalveolar layers are filled with fluid. Tissue-derived tumour necrosis factor (TNF) causes platelet aggregation, micro thrombus formation, interalveolar coagulation, and the formation of hyaline membranes (Figure 1).[7]

Management

The current management of ARDS is still relatively limited; the final stage of management is mechanical ventilation with the ultimate goal of minimizing the incidence of ventilator-induced lung injury (VILI). VILI events are iatrogenic and cause secondary injury with systemic inflammation leading to multi-organ failure. The ARDS management algorithm starts with optimizing ventilation that does not injure the lungs and increasing interventions based on the physiology of gas exchange. Additional management is individual, based on the cause and availability of facilities at the place of care. Current management of ARDS includes extracorporeal carbon dioxide removal (ECCO2R), prone position, administration of statins, and high-frequency oscillatory ventilation (HFOV).[6]

Prevention of lung injury can reduce morbidity and mortality due to ARDS. Platelets play a role in the injury process, so it is hypothesized that anti-platelet anti-aggregation therapy can prevent ARDS in high-risk patients. VILI events can occur even though the lung-protective ventilation method has been used. This method aims to reduce tidal volume and can reduce the incidence of VILI. This method can increase carbon dioxide and cause respiratory acidosis, so $ECCO_2R$ is needed, which can remove CO_2 from the blood through gas exchange devices outside the body.[6]

The incidence of VILI can be reduced by placing the patient in a prone position. This position facilitates the expansion of the lungs simultaneously so that the mechanical stress load can be distributed evenly in all parts of the lung. Several studies have shown that the prone position can benefit ARDS patients. A multicentre randomized controlled trial (RCT) of ARDS patients with PaO_2/FiO_2 ≤150 mmHg stated that the prone position for at least 16 hours/day significantly reduced mortality in 90 days with a hazard ratio (HR) value 0.44 (95% CI=0.29–0.67).[6]

Table 2. Risk factors for ARDS[7]

Direct lung injury risk factors	Indirect lung injury risk factors
1. Pneumonia (bacterial, virus, fungal, or opportunistic)	1. Sepsis (source of infection outside the lungs)
2. Gastric fluid aspiration	2. Hemorrhagic shock and extra thoracic trauma
3. Pulmonary blunt trauma	3. Pancreatitis
4. Inhalation injury	4. Big burn
5. Sink	5. Drug overdose
	6. Transfusion reaction
	7. *Cardiopulmonary bypass*
	8. Edema after lung transplantation

EXTRACORPOREAL MEMBRANE OXYGENATION

The definition of extracorporeal membrane oxygenation (ECMO) is a life support device outside the body that has an external artificial circuit and functions to carry venous blood from the patient for gas exchange in an oxygenator so that the blood becomes rich in oxygen and carbon dioxide can be removed. The blood is put back into the body.[8] Extracorporeal membrane oxygenation is a method that is often used in extracorporeal life support (ECLS). The ECLS system is a temporary mechanical technology.[4]

Tools included in ECLS include extracorporeal CO_2 removal ($ECCO_2R$), cardiopulmonary bypass support (CPS), and ECMO. Extracorporeal CO_2 removal is used to remove the partial pressure of carbon dioxide in hypercapnia respiratory failure. Cardiopulmonary bypass support can be used to maintain oxygenation and perfusion but can only be used for a few hours due to the limited half-life of the membrane oxygenator.[4]

ECMO devices require vascular access, connecting tubes, blood pumps, and gas exchange devices. Vascular access can be divided into veins or veins-arterials, depending on the physiological needs that occur in the patient. In adult patients, ECMO is often used in severe, acute, and irreversible

cardiopulmonary heart failure cases. The principle of the ECMO device differs from the cardiopulmonary bypass (CPB) device in several ways. On the CPS device, the heart is stopped, and perfusion occurs at a very slow rate of 2 L/min, so anticoagulation with heparin prevents thrombus formation. The ECMO device does not require heparin due to the high blood flow of 4 L/minute. Besides that, the ECMO device can be used for up to several weeks, while the CPS is only for a few hours.[4]

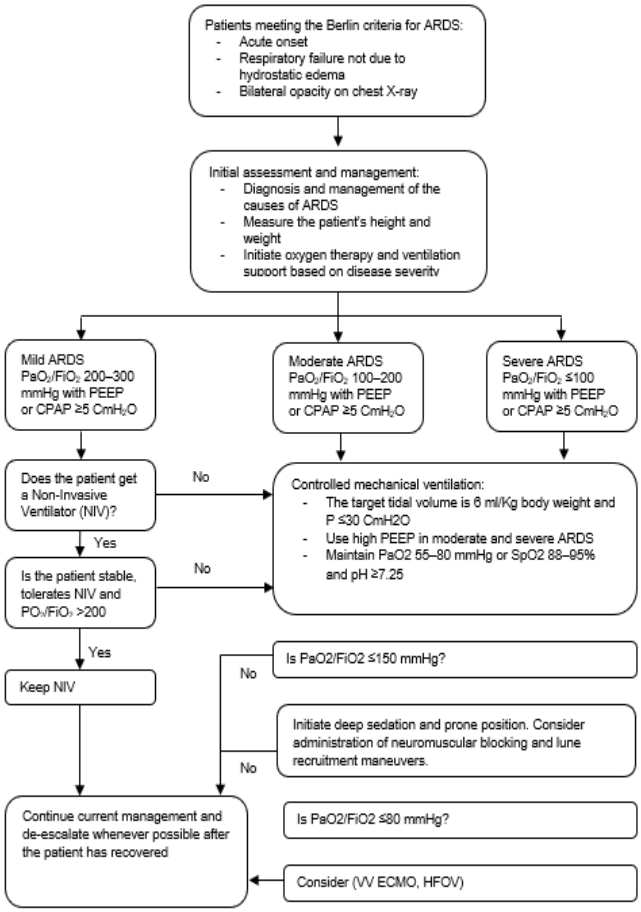


Figure 2. ARDS Management Algorithm[7]

The goal of ECMO is the successful gas exchange between oxygen and CO_2. Oxygen is exchanged through the membrane oxygenator which depends on the thickness of the blood layer, membrane material, FiO_2, and hemoglobin concentration. Excess volume and poor device flow can affect oxygen exchange thereby creating a ventilation-perfusion mismatch in the oxygenator device according to the natural processes of the lungs. CO_2 exchange, like the lungs, is affected by surface area, blood flow, and gas exchange rates. Gas exchange is measured in L/min gas flow through the oxygenator membrane. It's different with children. In adults the membrane oxygenator adds CO_2 to prevent excessive CO_2 loss and the development of respiratory alkalosis.[4]

The extracorporeal circuit has been assembled to meet the need for full gas exchange support, although certain patients only require CO2 removal. The circuit consists of:[9]

a) *Blood flow for heart support*. Using the venoarterial system, the selected circuit components can sustain a blood flow of 3 L/m2/min (neonates 100 ml/kg/min, children 80 ml/kg/min, adults 60 ml/kg/min). The target for measuring optimal systemic perfusion after installing the ECMO device is 70% venous saturation.

b) *Blood flow and gas exchange for respiratory failure*. Using the venous-arterial system or veins. Pulmonary membrane apparatus and blood flow can maintain oxygen flow and remove CO_2 in proportion to the patient's normal metabolic rate (neonates 6 ml/kg/minute, children 4–5 ml/kg/minute/ and adults 3 ml/kg/minute). A normal metabolic rate can be achieved with a venous system of 120 ml/kg/min for neonates and 60–80 ml/kg/min for adults. Oxygen delivery is determined by blood flow, hemoglobin concentration, hemoglobin saturation, and gas content in the lung membranes. CO_2 discharge occurs when the circuit is set for total support. Circuits regulated for CO_2 excretion may use venous-arterial, venous, or arterial-venous access with a blood flow of up to 25% cardiac output (CO) sufficient to remove CO_2 produced by metabolism, ie, 3–6 ml/kg/min. CO_2 output is determined from blood flow, gas sweep rate, PCO_2, and gas content in the lung membranes.

c) *Circuit components*. The circuit consists of a blood pump, lung membrane, connecting tube, and additional equipment such as heaters, monitors, and alarms.

d) *The pump*. The pump used can maintain the patient's total blood flow. The pump consists of various systems depending on the specifications

(modified roller with pressure control, centrifugal pump, peristaltic pump). Extracorporeal Life Support Organization (ELSO) recommendations for suction pressure not to exceed -300 mmHg. An aspiration pressure of more than this value is used when venous occlusion occurs, and the adjustment is carried out by a servo-controlled sensor that has a sensor inside the pump. The outlet pressure does not exceed 400mmHg, and the pump must have a battery that lasts at least one hour. This is necessary when there is a loss of power. The pump and circuit shall have an alarm mechanism intended to prevent arterial-to-venous backflow when using the venous-arterial mode in the event of a power failure.

e) *Lung membranes*. Gas exchange across the lung membranes occurs in the perforated layers of the dense silicone rubber membrane. The rate of contact between the blood flow and the surface area of the membrane determines the oxygen-carrying capacity. When using a total support system, blood that is 75% desaturated can reach a full saturation of 95% per minute. In the venous mode, blood recirculation is possible when the incoming blood is above 75% saturation. In this condition, oxygen flow per unit of blood flow is reduced, and it is necessary to regulate high blood flow. Cannula readjustment and increased hematocrit levels are required to achieve the required oxygen

f) *Sweep gas.* At the time of removal of CO_2 blood flow is regulated as low as 500 ml/min/m2.

g) *Circuit*. Circuits must be sterile with an isotonic electrolyte fluid content referring to normal extracellular fluids, namely potassium levels of 4-5 meq/L. The liquid circulates through the reserve bag until the bubbles disappear. The circulation process can be accelerated by adding 100% CO2 before filling the liquid in the circuit.

h) *Heater.* Heaters are needed to maintain body and blood temperature at a certain level. The heater requires a container filled with water, and the heating machine will be passed by a hose from the circuit so that the temperature is maintained at $<40^0$C.

i) *Monitors*. The monitor is set to assess circuit function and has an alarm on an abnormal condition.

j) *Blood tube.* The length and diameter of the tube determine the resistance to the blood flow pressure. The selected hose can drain venous blood and avoid high pressure when the blood flows back into the body. Blood will flow through a 1-meter-long tube at a pressure of 100 mm Hg. The diameter of the hose is 3/16 inches is capable of flowing blood at a rate of 1.2 L/minute, ¼ inch is capable of flowing blood at a rate of 2.5 L/minute, 3/8 inches is capable of flowing blood at a rate of 5 L/minute and ½ inch is capable of flowing blood at a rate of 10 L/min.

The system in ECMO has two systems, namely the veins (V-V) and the veins-arteries (VA). The V-V system is used for respiratory failure, while the VA system is used for heart or combined heart-lung failure. The V-V ECMO system produces oxygenated return blood resulting in high oxygen content and low CO2 in the right atrium. Arterial oxygen partial pressure and hemoglobin oxygen saturation are determined by the mixing effect of oxygenated return blood from the ECMO circuit to the right heart and deoxygenated blood from the bronchial veins, coronary sinus, and vena cava. Measurable pulmonary recovery can be characterized by the improvement of mixed venous oxygenation or systemic oxygen saturation during weaning from ECMO, indicating the lungs' ability to exchange gases. The Venoarterial extracorporeal membrane oxygenation (V-A SECMO) system can support patients who have lost partial or total function of the heart and lungs due to the blood flow provided by the ECMO circuit.

Complications from ECMO can be complications during cannulation or while on ECMO support. Complications can occur as bleeding, stroke, limb ischemia, thrombosis and infection. Research shows complications occur in 1:50 patients using ECMO, with bleeding and infection as the most common complications. Bleeding complications occur during cannulation or are caused by using anticoagulation while using ECMO. The V-A system

requires more aggressive anticoagulation because of the high risk of arterial thrombosis. Other complications, such as hemolysis, pulmonary edema, and leg ischemia, can be prevented by changing the cannula placement regularly.[10]

Table 3. Indications and contraindications[4]

Indications	Contraindications
1. Murray score ≥3	1. Irreversible heart and lung disease
2. Severe hypercapnia with a pH <7.2	2. Age >65 years
3. PaO_2:FiO_2 <50-100mmHg	3. Malignant disease
4. *Alveolar-arterial oxygen gradient* >600 mmHg without cardiogenic pulmonary edema	4. A serious brain injury
5. *Transpulmonary shunts >30%*	5. Mechanical ventilation >5–10 days
	6. Multiple traumas with high risk of bleeding

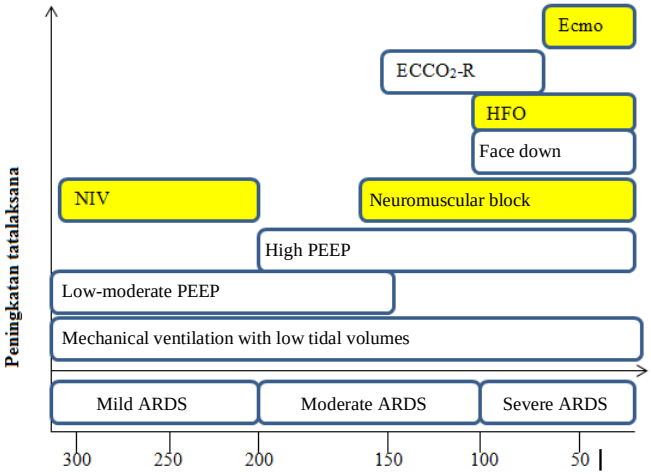
Extracorporeal Membrane Oxygenation in Acute Respiratory Distress Syndrome

The main indications for using ECMO are acute respiratory failure caused by a reversible process such as ARDS and as support before lung transplantation. Using a ventilator with positive pressure ventilation can cause VILI, oxidative stress, and lung injury, souse of ECMO can rest the lungs and is more protective than the ventilator. At the time of the H1N1 pandemic, the incidence of ARDS greatly increased, and patients treated with ECMO achieved a high survival rate of 79%. The multicenter conventional ventilatory support versus extracorporeal membrane oxygenation (CESAR) study using the RCT method compared ECMO with conventional therapy in patients with severe ARDS. The results of this study stated that the mortality rate was not significantly different, and the use of a single ECMO could not improve the outcome of patients with severe ARDS.[10]

The ongoing *extracorporeal membrane oxygenation for severe acute respiratory distress syndrome* (EOLIA) study evaluates early use of ECMO 3 hours before mechanical ventilation can improve the outcome of patients with ARDS.[10]

The Ministry of Labor's study of 49 ARDS patients with a median PaO_2/FiO_2 of 69, the median number of days on a ventilator before two days of ECMO cannulation and a median use of ECMO for 311 hours showed that 38 patients (78%) were successfully decannulated and survived. This study shows that the use of ECMO in combination with mechanical ventilation can increase the survival rate of patients with ARDS.[11] The American Thoracic Society (ATS) guidelines for the management of ARDS recommend setting mechanical ventilation using a low tidal volume of 4–8 ml/kgBB/breath, using inspiratory pressure lowest, namely the target <30 cmH_2O for severe ARDS patients (PaO_2/FiO_2 <100), prone position for at least 12 hours/day, not using HFOV and recommending the use of ECMO.[12]

Based on epidemiological data, ECMO can be considered a treatment for patients with hypoxemic-type respiratory failure. Data from Germany show the use of ECMO in respiratory failure starting in 2007, with a frequency of 2.4 cases per 100,000 population. The duration of ECMO use for ARDS patients varies. The CESAR study with a median of 9 days, and research in Korea showed a median of 7.4 days, and patients could be weaned. The study by Seiler et al. reported an average of 8 days of ECMO use before patients required mechanical ventilation. ECMO international registration data shows that as many as 22% of patients use ECMO with a duration of up to 14 days.[13]



Figuew 3. ARDS Management Algorithm implementing the Berlin criteria[14]

Figure 3 shows the treatment strategy according to the Berlin criteria, namely mild ARDS using NIV if it fails to use mechanical ventilation with low tidal volumes and low-moderate PEEP, for

moderate-grade ARDS using mechanical ventilation with low tidal volumes. Still, PEEP is set high and can even use additional neuromuscular block anesthesia at an advanced stage, namely severe ARDS using mechanical ventilation with low tidal volume using high PEEP added prone position (prone) is recommended using Extra Corporeal CO_2 Removal ($ECCO_2$-R). Still, if not available ECMO can be used.[14]

CONCLUSION

Giving oxygen therapy should be based on indications and the patient's oxygen needs. Acute respiratory distress syndrome can be caused by various conditions that cause diffusion disorders so that oxygenation to the tissues is reduced. The use of ECMO, together with mechanical ventilation, can be beneficial for ARDS patients and ~~can~~ increase patient survival. Appropriate management of hypoxemia and management of the causes of ARDS will reduce patient mortality.

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Literature Review

p21 Genetic Modification as a Tumor-Suppressor Gene: A Future Target in Lung Cancer Therapy?

Adityo Wibowo[1], Silvana Saputri[2]

[1]Department of Respiratory Medicine, Juntendo University Graduate School of Medicine, Japan
[2]Faculty of Medicine, Universitas Indonesia

Abstract

Lung cancer is one of the leading causes of cancer death worldwide. Although early diagnosis/screening methods and treatment strategies have developed, lung cancer patients' survival rates remain low. However, resistance to chemotherapy and radiotherapy causes tumor recurrence and worsening of the disease, thus being the lead cause of treatment failure. In the growth cycle of lung cancer cells, the highest p21WAF1/CIP1 gene expression was found in early-stage lung cancer and played a role in lung cancer progression. In addition, the correlation between CDK inhibitors and patient survival showed that inactivation of the p21WAF1/CIP1 and p16INK4a genes was associated with lower overall survival and poor prognosis. This review will focus on the role of genetic Modification in lincRNA-p21 in lung cancer therapy and the implication of a combination therapeutic approach.

Keywords: p21WAF1/CIP1, lung cancer, genetic modification therapy

Corresponding Author:
Adityo Wibowo | Department of Respiratory Medicine, Juntendo University Graduate School of Medicine, Japan | aditpulmo@gmail.com

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INTRODUCTION

Lung cancer is one of the most rapidly progressive types of cancer, with a high mortality rate worldwide, with an estimated 1.76 million deaths annually.[1,2] Lung cancer is classified into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NCSLC is the most frequent form of lung cancer, contributing to 85-90% of all cases. It is divided into squamous cell carcinoma (SQCLC) and adenocarcinoma (ADC), and large cell carcinoma (LCC).[2]

The gradual process of lung cancer caused by the activation of oncogenes and the inactivation of tumor suppressor genes plays an essential role in developing malignant transformation and cancer cells' resistance to therapy. The problem of low overall survival rates of lung cancer patients needs a better concern of lung cancer management. Identification of particular biomarkers, including cell cycle intervention, is required for effective targeted therapy to improve lung cancer patients' prognoses.[1]

CELL CYCLE AND TUMORIGENESIS

Tumorigenesis is associated with abnormal cell proliferation and programmed cell death (apoptosis) disruption.[3] Dysregulation of cell cycle development is the key to cancer cells. The cell cycle is divided into phases: G1, S, G2, and M (mitosis), each regulated by cyclins and cyclin-dependent kinases (CDKs). For example, the G1 phase entering the S phase is regulated by cyclin D/CDK4 and cyclin E/CDK6, and G2 phase to the M phase is regulated by cyclin A/CDK2 and cyclin A/cdc2 (CDK1). Cyclin or CDK activity is regulated by CDK inhibitors and the tumor suppressor protein, p53.[4]

This setting helps determine whether the cell will divide, remain silent, or quiescent (the condition of the cell not dividing but can return to the cell cycle) in response to external stimuli. During the cell cycle, each type of cell has distinct characteristics.

Embryonic stem cells, germ cells, and cancer cells proliferate, but they also undergo cell cycle arrest and go dormant in unpleasant conditions. Cells have a sophisticated management system to synchronize these events. Serious problems such as autoimmune diseases and carcinogenesis can result if these pathways are compromised.[5]

RECENT UPDATES IN LUNG CANCER THERAPY

Chemotherapy is still one of the most commonly used methods for treating malignancies. In general, chemotherapeutic agents contain taxane, platinum, and pemetrexed. Taxane, such as paclitaxel, inhibits microtubules and prevents cell division. Platinum, for example, cisplatin, binds to DNA purines, preventing replication and transcription. Pemetrexed inhibits an enzyme required for synthesizing the essential components of DNA. Targeted therapy, namely tyrosine-kinase inhibitors (TKIs), inhibits constitutive activation of cancer cell kinase signaling pathways and causes cancer cell apoptosis.[2]

The newest class of systemic therapies for cancer are immune checkpoint inhibitors (ICIs), such as pembrolizumab, atezolizumab, and nivolumab, which act via the programmed death ligand 1/2 (PD-L1 and PD-L2) pathway on cancer cells and the programmed death 1 (PD-L2) receptor PD-1) on T cells, where PD-L1 and PD-L2 are thought to suppress the immune system. ICI immunotherapy will prevent suppression of the immune system by cancer cells; as a result, cancer cells are detected by the immune system and undergo cytotoxic death.[6]

Lung cancer therapy depends on the type of cancer, stage, functional status of the patient, comorbidities, and molecular characteristics of the disease.[7] In early-stage SCLC, treatment includes surgery and additional platinum-based chemotherapy or concurrent chemo-radiation. Treatment of SCLC patients with metastases includes systemic chemotherapy with or without immunotherapy.[8]

In NSCLC, surgical resection is the first line at stages I and II, namely surgery at stage IA, surgery with/without chemotherapy at stage IB, and surgery (lobectomy or sub-lobar resection) with chemotherapy at stage II.[9] In patients who cannot be candidates for surgical resection, therapy focuses on stereotactic body radiation therapy (SBRT) or definitive radiotherapy.[7]

Stage III NSCLC tumors cannot be resected, and staging is performed during resection. In stage IIIA, treatment includes adjuvant chemotherapy, although chemotherapy and radiation are the more common options. In stage IIIB, chemotherapy, and radiation are used. In stage IV, therapy includes palliative chemotherapy and radiation. Current NSCLC therapy focuses more on targeted therapy based on mutational status than chemotherapy.[9] For example, in patients with positive epidermal growth factor receptor (EGFR), TKIs such as gefitinib, erlotinib, osimertinib, or afatinib may be used.[7] Other mutations among ROS1, BRAF, RET, TRK, MET, and KRAS genes may be present and specific inhibitors are required for integrated therapy.[9]

p21 GENE AND CANCER CELL

The development of p21 and the Evolution of Cancer Cell

p21 (encoded as the CDKN1A gene) is a CDK inhibitor that inhibits the cell cycle in the G1/S phase and phosphorylation of retinoblastoma proteins. p21 is a commonly used term with various other names, such as cyclin-dependent kinase inhibitory protein-1 [CDKN1A] or $p21^{WAF1/CIP1,}$ due to its diverse functions. At the beginning of its discovery, p21 was identified as a tumor suppressor in various cancer cells, including brain, lung, and colon malignancies, and it was correlated to carcinogenesis and metastasis.[3,5] p21 is a p53 target gene whose expression is promoted by wild-type p53 but not by mutant p53. Hence, p21 is also called wild-type activating factor-1 (WAF1).[5]

Since its early discovery as a CDK inhibitor, p21 has been a critical regulator in various cell processes, including G1/S phase cell cycle development, cell proliferation, DNA damage, and cell damage. The initial study found that p21 binding

to CDK and suppression of CDK interactions with other substrates slowed cell cycle progression in the G1/S phase.[3] In addition, p21 is also correlated to cellular susceptibility to Transforming Growth Factor-beta (TGF-beta), which helps to explain its function in cancer formation—considering the role of TGF-beta in the stages of malignant progression (pre-malignancy phase, malignant development, invasive-dissemination, and metastatic colonization).[5]

Previous research has also demonstrated that the lack of p21 changes keratinocyte proliferation and differentiation and promotes race-tumors' development. p21 is also linked to tumor migration and invasion. Cyclin D1 collaborates with p21 to regulate TGF-beta-mediated cancer cell migration and local tumor invasion. p21-activated kinase (PAK) promotes cancer cell proliferation, migration, and invasion via extracellular signaling and AKT-dependent pathways.[3]

The location of p21 and the state of the p53 protein determines the controversial aspect of p21.[3,5] In pediatric and adult cancers, p53 is the most mutated tumor-inhibiting protein; p53 promotes p21 expression in response to cellular stress to prevent the replication of damaged DNA.[3,5,10] Several circumstances explain why the p21 expression pattern is not p53-dependent on normal tissue growth, cell differentiation, or following serum stimulation.[4] This difference in p21 induction via p53-dependent and p53-independent pathways is specifically related to the role of p21 in tumor development.[3] That said, the effect of p21 on tumor evolution or cancer progression is highly dependent on the status of the p53 protein in cancer cells.[5]

Tumor cells are genomically unstable cells susceptible to mutations that can develop an aggressive phenotype and ultimately lead to metastasis. The pivotal point controls cell cycle progression and is believed to be critical to maintaining genome stability. $P21^{CIP1/WAF1}$ is a CDK inhibitor, so it functions in cell cycle arrest, where P21 can work together or inactivate the cyclin E-CDK2 complex, which plays a role in the cell cycle from G1 to S phase. In addition to its role in cell cycle arrest in the G1/S phase, p21 can also inhibit proliferating cell nuclear antigen (PCNA) during the S phase to slow down DNA synthesis and repair.[10]

Regulation of p53 on p21 makes p21 play a role in inhibiting tumorigenesis; hence p21 is considered an anti-oncogene. Various studies have proved this. In vitro studies have shown that p21 expression negatively affects the malignancy of various cancer cells (skin cancer, TET, adult T cell leukemia [ATLL] by suppressing growth and triggering apoptosis.[5] In vivo studies showed that upregulation of p21 in breast cancer cells led to cell cycle arrest and inhibited the invasion of breast cancer cells. In contrast, the knockdown of p21 increased cell proliferation and suppressed cancer cell invasion.[10] p21 suppresses tumor growth by inhibiting cyclin-kinase complexes, proliferation cell nuclear antigens (PCNA), transcription factors, and coactivators. Otherwise, By reducing the accumulation of DNA damage, p21 may indicate the appearance of tumor evolution, which leads to cancer progression.[5]

Another study in breast cancer cells demonstrated that expression of the cytosolic act-phosphorylated form of p21 (in the cytoplasm) of mouse breast epithelium accelerated tumor progression, suggesting an oncogenic role of cytoplasmic p21 that differs from its anti-proliferative role in the nucleus.[10] Thus, depending on its location, p21 can become an oncogenic or tumor suppressor protein. Controversy about p21 in cancer evolution poses a challenge in determining the right balance in which p21 can play a selective role in inhibiting cancer.[10]

Expression of p21 in Cancer Stem Cells

Several studies have found that p21 expression is associated with resting or late differentiation in tumor cells. p21 was reported to be the main factor for maintaining stem cells/progenitor cells because an increase in p21 mRNA can inhibit the development of progenitor cells. Under normal and stable conditions, large amounts of p21 expression were found in differentiated hematopoietic and red blood cells. Meanwhile,

decreased p21 levels caused the proliferation of hematopoietic stem cells. Therefore, maintaining stem cells in a resting state is very important to prevent premature stem cell depletion.[3,5]

p21 is also related to cancer stem cells (CSC). CSCs are a subpopulation of tumor cells capable of initiating tumors and promoting tumor heterogeneity. CSCs are formed from the accumulation of mutations occurring spontaneously in stem cells and progenitor cells during a person's lifetime. Normal stem cells can become CSC through genetic/epigenetic changes and mutations. CSCs have an excellent ability to self-renewal and maintain their ability to differentiate across multiple lineages. Several studies have shown an association of p21 expression with CSCs in several tumors.[3]

P21 has been reported to attenuate Ras- and c-Myc-dependent epithelial-mesenchymal transition of breast tumors and in vivo attenuates CSC-like gene expression. In prostate cancer, CSC dormancy and recurrence are regulated by bone morphogenetic protein seven via the p38/NDRG1/p21 signaling axis. In ovarian cancer, cell stems are suppressed by p21-regulating mRNA and miRNA. These studies demonstrated the role of p21 in stem cells.[3]

Currently, CSCs in the lung have been extensively studied. According to various studies, the characteristics of pulmonary CSCs include self-renewal ability, multipotent differentiation, tumorigenic potential, expression of stem cell markers, high invasiveness, proliferation as tumor plane, chemoresistance, radioresistance to hypoxia, resistance to apoptosis, and inactivity.[11,12]

The most common hypothesis regarding pulmonary CSCs states that CSCs are formed from tissue-specific normal stem cells in the tissue of origin. However, identifying stem cell origin in the lung is difficult to determine because the epithelium of the trachea and bronchi is quiescent and has a low proliferative fraction. Consequently, cells at specific anatomic sites in the lung were used to simplify the origin of pulmonary CSCs. SQCLC is associated with basal cells in the proximal airways, namely the trachea and bronchi, which exhibit stem cell-like activity. SCLC was associated with Clara cells and neuroendocrine cells, suggesting the presence of stem properties. Meanwhile, ADCs were associated with normal stem cells from the bronchoalveolar branching area.[13]

LincRNA-p21 and Cancer Development

mRNA is a small part of all RNA, with only 3-7% of the total RNA mass in the cell. Nevertheless, mRNA has always been the focus of most of the research. On the other hand, noncoding mRNA has received less appreciation regarding its functional regulatory activity. Although long noncoding RNA (lncRNA) accounts for only a tiny part of the total noncoding RNA (by mass and by the number of molecules), Cancer development has been linked to lncRNA. Long intergenic noncoding RNA-p21 (LincRNA-p21), the target of wild-type p53, is located 15 kb upstream of the p21 gene and regulates gene expression at both transcriptional and post-transcriptional levels. Upregulated by p53, LincRNA-p21 regulates the expression of p53 target genes by physical interaction with heterogeneous nuclear ribonucleoprotein (hnRNP K), which acts as a critical repressor.[5]

LincRNA-p21 regulates cell proliferation, response to DNA damage, and apoptosis through a regulatory role in the expression of p53 target genes. LincRNA-21 maintains reprogramming through several mechanisms; for example, LincRNA-p21 preserves CpG and H3K9me3 methylation in the pluripotency gene promoter, limiting somatic cell reprogramming. LincRNA-p21 also regulates the Warburg effect, so it has an essential role in the metabolism of cancer cells; currently, many research focuses are discussing the role of lincRNA-p21 associated with cancer development. P21-associated noncoding RNA DNA damage-activated (PANDA) is one of the lncRNA located 5 kb upstream of the p21 gene that regulates pro-apoptosis and aging genes through stabilization p53. In response to DNA damage, p53 attaches to the p21 transcriptional start site and activates PANDA and p21 transcription.[5]

p21 Gene-Targeted Therapy Against Lung Cancer

Conventional treatment methods for lung cancer were surgery, chemotherapy, and radiotherapy. However, resistance to chemotherapy and radiotherapy causes tumor recurrence and worsening of the disease, thus being the leading cause of treatment failure. One of the causes of resistance to chemotherapy and radiotherapy is CSC. In addition, the high mutation rate in NSCLC makes therapy more focused on targeted therapy.[14] However, targeted therapy, including TKI, is bound to face resistance and mutations in cell cycle regulators. Therefore, it is essential to focus on cell cycle regulatory genes as a strategy for lung cancer therapy and prevention.[4]

Gene regulation is mainly used for research purposes. Several gene regulatory techniques for manipulating gene expression (knocking out, mutating, or silencing) have been developed:

a. TALENs (transcription activator-like effector nucleases).
b. CRISPR (clustered regularly interspaced short palindromic repeats).
c. rAAV (recombinant adeno-associated virus).
d. ZFNs (zinc finger nucleases).
e. Homologous recombination.
f. Small interference RNA using lentivirus or adenovirus infection.

To study tumor growth, apoptosis, and cell cycle arrest in cancer cells, several versions of p21 have been created in vitro and in vivo models. Gene editing to alter the amount of p21 expression can be used as an additional therapy to suppress carcinogenic characteristics or inhibit treatment resistance in specific cancers.

p21, in general, has two roles closely related to the development of cancer cells. Research that discusses the function of p21 as a tumor suppressor was carried out in a study by B. Shamloo et al.[5] The survey results using a p21-deficient mouse model proved to be susceptible to the formation of hematopoietic, epithelial, and endothelial tumors. Another study also showed that p21-deficient mice injected with the carcinogen azoxymethane showed faster growth of premalignant lesions. Reverse proofing by transduction of adenovirus to increase p21 gene expression in prostate cancer cells has proven that the p21 gene can induce apoptosis and decrease tumor size in mice. The same results were shown by performing an in vitro test on cervical cancer cells that could not increase after p21 overexpression. Cell proliferation and tumor growth decreased significantly after introducing p21 and p53 via nanoparticle injection into a mouse model. These findings highlight the complexities of p21 in cancer therapy and the significance of a multimodal therapeutic strategy.[5]

p21 has an essential role in regulating G1/S and G2; therefore, p21 plays a role in cancer therapy.[3] An increase in p21 causes the cessation of the cancer cell cycle in the G1 or G2/M phase to stop the cell cycle.[5] Research with epigenetic Modification of p21 using histone hypermethylation method showed a significant picture in inhibiting the development of lung cancer cells. The expression of the p21 gene has been reported as a clinical marker of tumor progression. The resulting increase in protein levels indicates a higher overall survival rate in lung cancer patients.

Conversely, the results show that rapid tumor progression is associated with lower levels of p21 in patients with thoracic malignancies. We have seen in vitro studies on cancer cells modified with lentiviral infection. Research conducted using short hairpin RNA to suppress the SKP2 gene proved effective in increasing the expression of the p21 gene.[15]

Based on research conducted by Zhao et al.,[16] it is known that p21 is needed in NSCLC therapy to increase sensitivity to gefitinib. In human ovarian cancer cells, p21 increases the cytotoxic effect of cisplatin, while in hepatoma cells, exogenous p21 expression inhibits cell growth and increases cisplatin sensitivity.[3]

In the growth cycle of lung cancer cells, overexpression of cyclin D1 and underexpression of $p16^{INK4a}$ at an early stage were found to have a significant effect on poor prognosis. The $p21^{WAF1/CIP1}$ gene expression was generally decreased in advanced lung cancer, whereas the highest

expression was found in early-stage lung cancer. In addition, the association between CDK inhibitors and patient survival showed that inactivation of the $p21^{WAF1/CIP1}$ and $p16^{INK4a}$ genes was associated with lower overall survival and poor prognosis.[4]

Research on the CDK inhibitor gene targeting the p16 gene was carried out to see the mechanism of action of cell cycle arrest due to increased p21 expression. In tumor cells with fast progression, increased expression of p21 will affect cell growth and prevent proliferation.[17] A study was conducted to determine the effect of the p21 gene by inhibiting the TCAB1 gene in lung cancer cells. TCAB1 is one of the structural and essential component proteins in NSCLC. The role of TCAB1 in tumors is related to impaired telomere function and impaired mRNA formation. Overexpression of TCAB1 promotes cancer cell proliferation, while suppression of the TCAB1 gene will inhibit cancer cell growth by inducing growth arrest and cell development and stimulating apoptosis. In vitro experiments against TCAB1 gene suppression effect on increasing p21 levels in A549 cells (lung adenocarcinoma). Increasing p21 levels in cells that do not have the TCAB1 gene causes cancer cells to become senescent and unable to divide.[18]

Furthermore, previous studies have been conducted to prove that the p21 gene is required in the senescence and apoptosis of cancer cells in vitro. The expression of p21 in this study was produced indirectly by suppressing the miR-34a gene. miR-34a is a tumor suppressor inhibitor implicated in many tumors, including lung tumors. So far, its regulation is related to the p53 gene, but in this study, it was found that p53 levels were not directly related to miR-34a but had a different relationship with p21. Briefly, these results suggest that miR-34a might upregulate p21 and tell that p21 is a major effector of the induction of senescence and apoptosis of NSCLC cells.[19]

CONCLUSION

Lung cancer is one of the most diagnosed cancers and the leading cause of cancer death worldwide. Although early diagnosis/screening methods and treatment strategies have developed, lung cancer patient survival remains low. The development of more efficient therapeutic targets is needed to improve patient prognosis. Lung cancer therapy depends on the type of cancer, stage, functional status of the patient, comorbidities, and molecular characteristics of the disease. Traditional treatment methods for lung cancer include surgery, radiotherapy, and chemotherapy. However, resistance to chemotherapy and radiotherapy causes tumor recurrence and worsening of the disease, thus being the leading cause of treatment failure. One of the causes of resistance to chemotherapy and radiotherapy is CSC. In addition, the high mutation rate in NSCLC makes therapy more focused on targeted therapy.

$P21^{CIP1/WAF1}$ is a CDK inhibitor, so it stops the cell cycle and can be an oncogenic or tumor suppressor protein. P21 is also associated with Cancer stem cells (CSC). As a CDK inhibitor, P21 has an essential role in the cell cycle, where an increase in P21 causes the arrest of the cancer cell cycle in the G1 or G2/M phase, thereby stopping the cell cycle. In the growth cycle of lung cancer cells, the highest $p21^{WAF1/CIP1}$ gene expression was found in early-stage lung cancer and decreased in advanced lung cancer. The association between CDK inhibitors and patient survival rates also showed that inactivation of the $p21^{WAF1/CIP1}$ and $p16^{INK4a}$ genes was associated with lower overall survival and poor prognosis. Various studies have shown that increased p21 expression, which is generated indirectly through the p16 gene and suppression of the TCAB1 protein and miR-34a gene, indicates that increased p21 expression prevents proliferation and induces senescence and apoptosis of NSCLC cells. Therefore, the function of p21 in stopping the cell cycle is expected to become a more efficient target therapy for lung cancer.

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