Original Article

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

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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

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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.

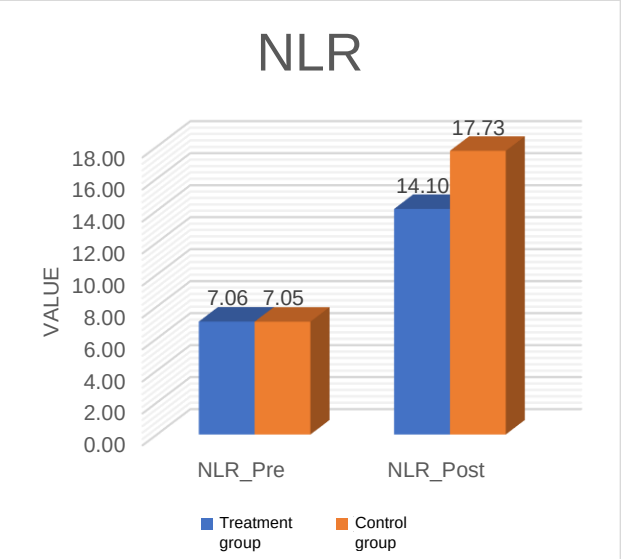

Fig 1. Bar chart of the NLR value of both groups

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).

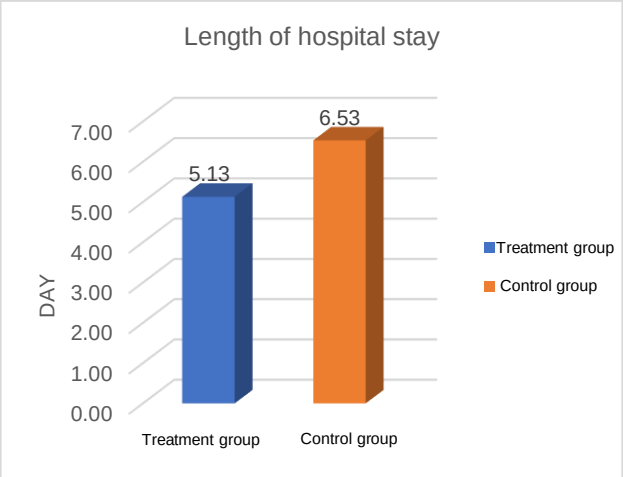

Fig 2. Difference in LHS between treatment and control group

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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