Original Article

Myocard Injury in COVID-19 Patients After Application OfUmbilical Cord Mesenchymal Stem Cell (UC-MSC) as Adjuvant Therapy in Persahabatan Hospital

Mega Juliana[1]*, Triya Damayanti[1], Yasmina Hanifah[2], Erlina Burhan[1]

[1]Department of Pulmonology and Respiratory Medicine Faculty of Medicine, Universitas Indonesia/ Persahabatan Hospital, Jakarta
[2]Department of Cardiology, Persahabatan Hospital, Jakarta

Corresponding Author:
Mega Juliana | Department of Pulmonology and Respiratory Medicine Faculty of Medicine, Universitas Indonesia/ Persahabatan Hospital, Jakarta | julianas.mega@gmail.com

Submitted: April 1st, 2023
Accepted: May 3rd, 2023
Published: June 28th, 2023

Respir Sci. 2023; 3(3): 185-99
https://doi.org/10.36497/respirsci.v3i3.89

Abstract

Background: Myocardial injury was a frequent cardiovascular manifestation of COVID-19 and associated with high mortality. Cell-based approaches, primarily using mesenchymal stem cell (MSC) has demonstrated safety and possible efficacy as adjuvant therapy in COVID-19 patients. This study aims to evaluate myocardial injury in patients with moderate-severe and critically ill COVID-19 after the application of umbilical cord mesenchymal stem cell (UC-MSC) as adjuvant therapy in Persahabatan hospital.

Method: This is a retrospective and prospective cohort study. A total of 28 subjects were allocated to 13 subjects in the control and 15 subjects in the experimental group. Subjects were given the standard treatment and UC-MSC or placebo. Myocardial injury is defined by an increase of troponin I >26 pg/ml. The biomarkers of troponin I, NT-proBNP and CRP was examined periodically. Cardiac pump evaluated by EF and TAPSE from echocardiography examination before and after UC-MSC application. The evaluation of myocardial injury, biomarkers, cardiac pump and 15-day mortality were observed between the two groups.

Results: The incidence of myocardial injury was 28,6% of total subjects. Subjects with worsening myocardial injury were higher in the control group (6 subjects) than the experimental group (4 subjects) although not statistically significant. The difference in biomarkers (troponin I, NT- pro-BNP and CRP), cardiac pump function (EF and TAPSE) and 15-day mortality between two groups were not statistically significant. There was a trend of decreasing troponin I, NT-proBNP and CRP in the experimental group.

Conclusion: UC-MSC application can be an option as adjuvant therapy in improving myocardial injury of moderate-severe and critically ill COVID-19 patients.

Keywords: COVID-19, mesenchymal stem cell, myocardial injury

INTRODUCTION

Coronavirus Disease 2019 (COVID-19) has become a pandemic, causing high and still increasing mortality and morbidity rates worldwide.[1] As of 23 July 2020, WHO reported 15,012,731 cases and 619,150 deaths (4.1% mortality rate) worldwide. Meanwhile, the Ministry of Health of the Republic of Indonesia said in July 2020 there were 95,418 cases and 4,665 deaths (mortality rate 4.9%).[2]

Myocardial injury is the most common cardiovascular manifestation of COVID-19 and is associated with a worse outcome. A meta-analysis by Zou et al reported a prevalence of myocardial injury of 24.4% in hospitalized patients with a fivefold increase in mortality compared to those without myocardial injury.[3] The Chinese Center for Disease Control and Prevention reports that cardiovascular comorbidities increase the mortality rate to 10.5% compared to the total mortality rate of 2.3%.[4]

Mesenchymal stem cell therapy (MSCs) has been reported as a new therapeutic strategy for COVID-19. Hirsch et al, through clinical trials and pilot projects, said that giving MSCs to patients with severe and critical degrees of COVID-19 pneumonia decreased intensive care rates and accelerated patient recovery.[5]

Leng et al reported that giving MSCs therapy to COVID-19 patients improved symptoms and lung function.[6] The mechanism of action of MSCs in COVID-19 is not fully known, but it is suspected to have immunomodulatory abilities so that it can suppress cytokine storms improve the microenvironment of lung tissue and capillary networks and prevent fibrosis.[7]

Therefore, researchers plan to conduct a study to evaluate myocardial injury in pneumonia patients with moderate-severe and critical COVID-19 receiving additional therapy for umbilical cord MSCs (UC-MSC) at Persahabatan Hospital.

METHOD

This retrospective and prospective cohort study was part of a multicenter, randomized controlled, and double-blind phase III clinical trial entitled "Application of UC-MSC as Adjuvant Therapy for Moderate-severe and Critical COVID-19 Pneumonia Patients". This research was conducted in the COVID-19 treatment room at Persahabatan Hospital from September 2020 to March 2021. The research sample was a reachable population that met the admission criteria.

Respondent acceptance criteria were patients aged 18-95 years, diagnosed with COVID-19 pneumonia confirmed by RT-PCR examination of throat swabs, meeting the requirements for moderate-severe and acute COVID-19 pneumonia, and willing to participate in the study by signing a consent form. While the criteria for rejection are having a history of malignancy, pregnant women, or a positive pregnancy test, or the subject has participated in other clinical trials in the last 3 months.

Samples were taken by consecutive sampling according to the acceptance criteria until the number of samples was fulfilled. Statistical tests were carried out using SPSS ver 25. The Shapiro-Wilk normality test assessed data distribution. Categorical data is assessed by the Chi-square test, while numerical data is evaluated by the unpaired T-test or the alternative test if the conditions are not fulfilled.

RESULTS

This study's sample size was 38 subjects who met the acceptance criteria. These subjects consisted of 13 subjects with a critical degree of COVID-19 pneumonia and 25 subjects with a moderate-severe degree of COVID-19 pneumonia. A total of 10 subjects in the moderate-severe degree were rejected because 7 subjects entered the critical criteria and 3 subjects underwent conversion before implantation.

Subjects were divided into 13 subjects in the treatment group and 15 in the control group. As long as post-implantation follow-up is given, no subject is lost to follow-up, so all subjects can be analyzed. The basic characteristics of the subject are shown in Table 1. There were no statistical differences in the subjects' characteristics between the two groups.

The incidence of myocardial injury in all subjects before implantation was found in 8 subjects (28.6%) consisting of 5 subjects (17.9%) from the treatment group and 3 subjects (10.7%) from the control group. Furthermore, an assessment of the characteristics of the subjects who experienced myocardial injury was carried out.

The female gender was found to be more numerous, namely 5 subjects (62.5%), and the age range in both age categories was obtained. The highest BMI was found in the obese group, namely, 4 subjects (50%), more critical level Covid-19 pneumonia, 6 subjects (75%), the number of comorbidities ≥3 and cardiovascular comorbidities found in 6 subjects (75%) and the mortality outcome were found in 6 subjects (75%). Furthermore, an assessment of myocardial injury after implantation was carried out. Changes in troponin I value were considered significant if there was an increase of >20% after implantation compared to before implantation.[8]

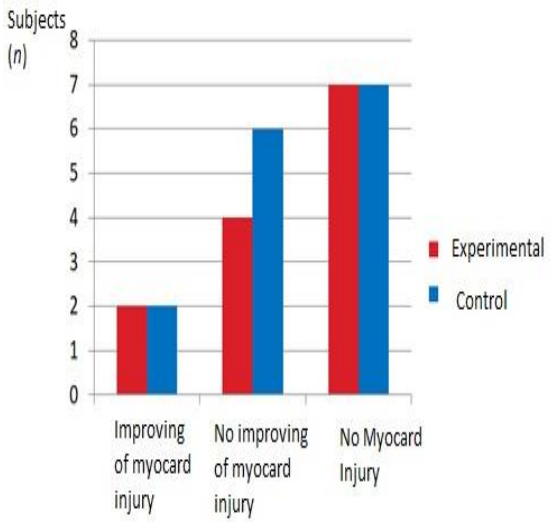

Figure 1. Evaluation of Myocardial Injury Both groups

Subjects were divided into three groups, the myocardial injury group improved, the myocardial injury group did not improve, and the myocardial injury did not.

Table 1. Basic Characteristics of Subject

Characteristic	Treatment (n=13)		Control (n=15)		P
	n	%	n	%	
Gender					
Male	8	61.5	10	66.7	1.0[f]
Female	5	38.5	5	33.3	
Age					
40-60	7	53.8	10	66.7	0.48[cs]
>60	6	46.1	5	33.3	
Body Mass Index					
Normal (18.5-25)	8	61.5	5	33.3	
Fat (25.1-27)	1	7.7	3	20	0.25[ks]
Obesity (>27)	4	30.8	7	46.7	
Number of Comorbid					
0	1	7.7	0	0	
1	3	23.1	7	46.7	0.26[ks]
2	4	30.8	6	40	
≥3	5	38.5	2	13.3	
Type of Comorbid					
Hypertension	7	25.9	9	36	
Diabetes Mellitus type II	8	29.6	7	28	
Obesity	4	14.8	7	28	
Congestive Heart Failure	4	14.8	0	0	0.77[ks]
Coronary Artery Disease	2	7.4	0	0	
Ex-Pulmonary Tuberculosis	2	7.4	1	4	
Old Cardiovascular diseases	1	3.7	1	4	
Levelst					
Critical	6	46.2	7	46.7	0.97[cs]
Moderate-Severe	7	53.8	8	53.3	
Oxygen Assistance					
Mechanical Ventilation	6	46.2	7	46.7	1.0[ks]
High Flow Nasal Cannula	6	46.2	8	53.3	
Nassal Canula	1	7.6	0	0	
Compilation					
Hospital-acquired Pneumonia	9	69.2	11	73.3	
Acute Kidney Injury	9	69.2	4	26.7	
Urinary Tract Infection	4	30.8	3	20	0.75[ks]
Systemic Candidiasis	1	7.7	0	0	
Diabetic Ketoasidosis	1	7.7	2	13.3	
Other Adjuvant Therapy					
Tocilizumab	1	7.6	0	0	0.35[cs]
Intravenous immunoglobulin (IVIG)	0	0	1	6.7	

Note: [f]Fisher's test; [cs]Chi Square Test; [ks]Kolmogorov Smirnov Test

Figure 2 shows the myocardial injury subjects improved, and no myocardial injuries were the same in both groups, namely 2 subjects and 7 subjects. While subjects with myocardial injuries did not improve, there were more in the control group, namely 6 subjects, compared to the treatment group, namely 4 subjects.

Table 2. Differences in Troponin I, NT-proBNP and CRP values of the two groups before implantation

Biomarkers/Group	Before Implementation Median (min-max)	P	After Implantation Median (min-max)	P
Troponin I				
Treatment	9.5 (0.6-179.5)	0.29[m]	10.3 (1-469.4)	0.84[m]
Control	5.6 (0.6-5.986)		16.9 (1.3-2.160.4)	
proBNP				
Treatment	336 (5-11.188)	0.7[m]	430.2 (16-10.866)	0.64[m]
Control	157.8 (38.7-10.273)		197.7 (19.2-9.287)	
CRP				
Treatment	336 (5.0-11.188)	0.66[m]	83.5 (2.8-469.4)	0.13[m]
Control	157.8 (38.7-10.273)		105 (10.3-245)	

Note: [m]Mann Whitney Test

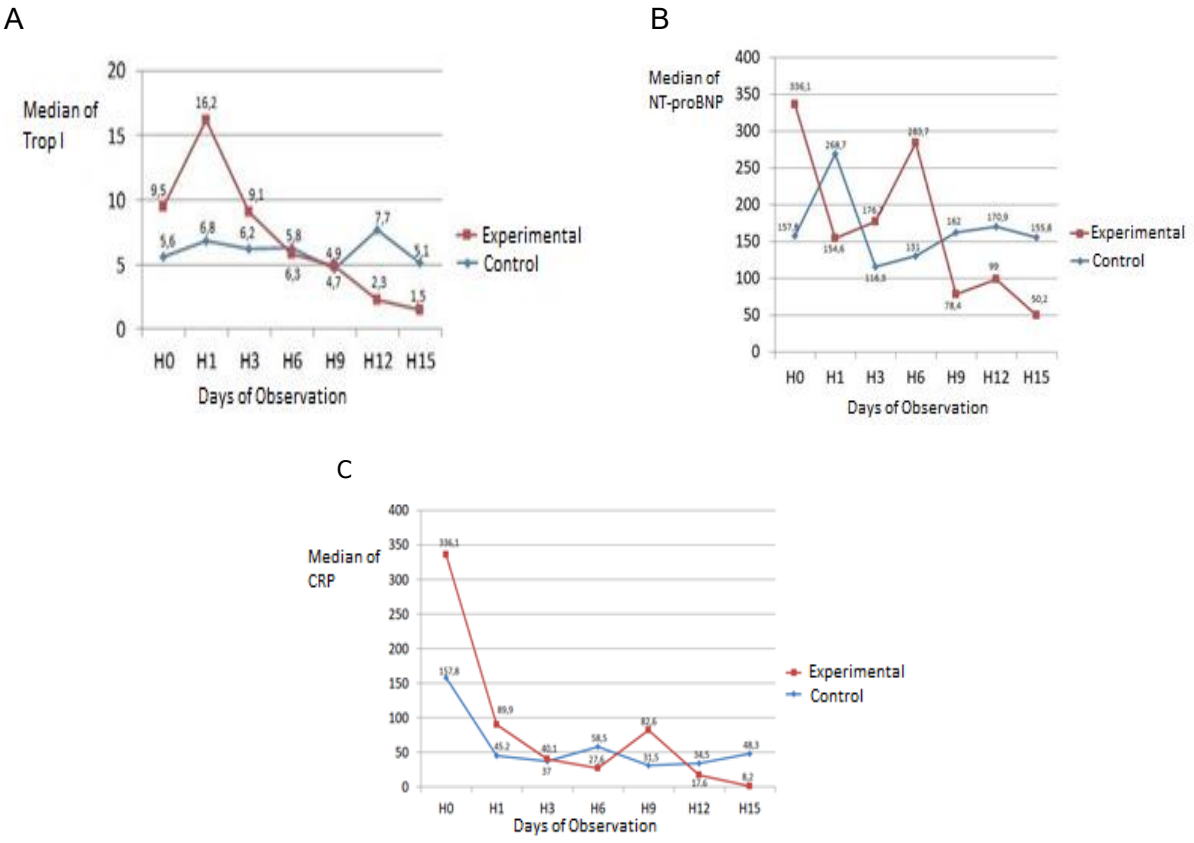

Figure 2. Graph of the trendline of the biomarker values of the two groups on each day of observation: A) Troponin I, B) NT-proBNP, and C) CRP.

In this study, three biomarkers were assessed, two markers related to cardiac pathology, namely troponin I and Nt-proBNP, and one reactive protein in acute phase inflammation, namely CRP. Measurements were taken before implantation (H0) and then periodically after implantation, namely on day 1, day 3, day 6, day 9, day 12, and day 15. The normality test of the three data shows that the data distribution is not normal. The result analysis of the values of the three biomarkers showed no statistically significant relationship between the two

groups before and after implantation (Table 2).

Figure 2A below shows the trend of changes in the median troponin I value in the two subject groups on each observation day. The graph shows that the trend of changes in the median troponin I value in the treatment group tends to decrease more compared to the control group. The median value of the treatment group started at 9.5 pg/dl, then increased on the next observation day to 16.2 pg/dl, and then decreased until the end of the observation day, which was 1.5 pg/dl. At the same time, those in the control group tended to settle down, starting from 5.6 pg/dl and decreasing slightly to 5.1 pg/dl at the end of the observation day.

Figure 2B shows the trend of changes in the median NT-proBNP values of the two groups. It can be seen that the median NT-proBNP value in both groups is still within the normal range. The treatment group started with a value of 336.1 pg/dl and tended to decrease until the end of the observation day, which reached a value of 50.2 pg/dl, although there was one more increase on the other day's 6th observation. Meanwhile, the median NT-proBNP value in the control group tended to increase, especially from the 3rd day of observation to the end of the observation.

Figure 2C below shows the difference in the trend of changes in the median CRP value in the two groups. The graph shows that the CRP value of the treatment group tended to decrease, starting from 336.1 mg/L up to 8.2 mg/L on the last day of observation. Meanwhile, the control group tended to settle down, starting from 157.8 to 48.3 mg/L on the last day of observation.

An echocardiographic examination was performed before and after implantation by assessing ejection fraction (EF), which reflects the pumping function of the left heart, and Tricuspid annular plane systolic excursion (TAPSE), which describes the pumping function of the right heart. Due to limited research resources, only 13 subjects could undergo a complete examination for EF scores (5 subjects in the treatment group and 8 subjects in the control group), and for TAPSE scores, 12 subjects (5 subjects in the treatment group and 7 subjects in the control group).

A total of 13 subjects died, 2 subjects returned from treatment before post-implantation echocardiography and 1 subject's TAPSE value could not be evaluated due to the operator's limited field of view when using level 3 PPE during the examination. The analysis results showed no significant difference in the EF and TAPSE scores in the two groups of subjects before and after implantation (Table 3).

In this study, the clinical outcomes of the subjects were assessed, namely, alive or dead. There were 15 living subjects (53.6%) of the subjects, with 6 people in the treatment group and 9 subjects in the control group. In comparison, the subjects who died were 13 subjects (46.4%) of the total subjects, with the total number of subjects in the treatment group found to be larger, namely, 7 subjects (53.8%) compared to 6 subjects (46.7%) from the control group.

Table 3. Differences in EF and TAPSE values for the two groups

Biomarkers/Group	Before Implementation Median (min-max)	P	After Implantation Median (min-max)	P
EF				
Treatment	68 (55-75)	0,67[m]	55 (50-83)	0,4[m]
Control	69.5 (64-79)		66,5 (55-80)	
TAPSE				
Treatment	2,4 (1,9-3)	0,31[m]	2,4 (1,8-3)	0,39[m]
Control	2,0 (1,8-2,5)		2,2 (1,9-2,5)	

Note: [m]Mann Whitney Test

The difference in subject outcomes between the two groups was not statistically significant, with value of P=0.74. Then, survival analysis was carried out using the Kaplan-Meier method to assess the number and time of survival of the subjects in both groups.

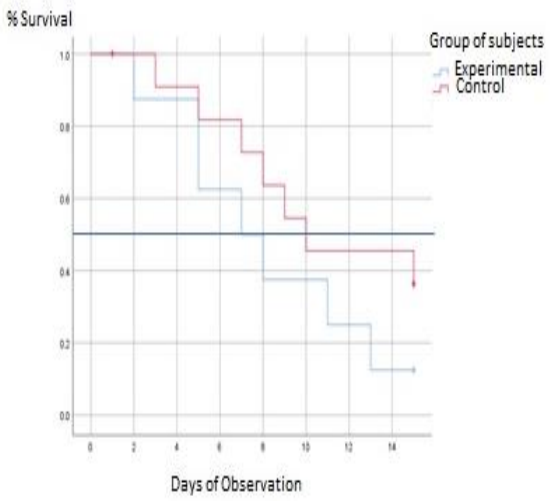

Figure 3. Kaplan Meier survival curve between treatment and control groups

In Figure 4, it can be seen that the treatment group tends to form a steeper curve than the control group. The median survival value of 50% was obtained in the shorter treatment group, which was 7 days, compared to the control group, which was 10 days. The relationship between the two was not statistically significant, with P=0.92 (log-rank) and a hazard ratio (HR)=1.05.

DISCUSSION

Myocardial injury is one of the most common cardiovascular manifestations of COVID-19 pneumonia and is associated with poor clinical outcomes. The management of COVID-19 pneumonia using a cell-based approach, particularly the UC-MSC, has been reported to show its safety and potential as an additional therapy for COVID-19 pneumonia.

In this study, the distribution of males was 18 subjects (64.3%) while females were 10 subjects (35.7%) with an age distribution in the range of 40-60 years of 17 people (60.7%) and over 60 years as many as 11 people (39.3%). The distribution of gender and age of these subjects follows the meta-analysis reported by Abate et al, who found the male prevalence of COVID-19 was higher, namely 55% compared to 45% for women.[9]

Karyono et al reported epidemiological data on COVID-19 patients in Indonesia and found that there were more males than females, namely 54.6%, with the highest age distribution in the 18-59 year range.[10] The prevalence of COVID-19, which is more in the male gender, is because men are generally more active

working outside the home than women, making it possible to interact with other people more often and for longer. Adults and the elderly are more at risk of being infected with COVID-19 due to a decrease in the body's immune system with increasing age. The CDC noted that over 40 years of age are at double the risk of infection and fifteen times the need for hospital care, while age >60 is one of the most severe clinical predictors according to WHO.[11]

The most common types of comorbidities were hypertension (57.1%), diabetes mellitus (53.6%), and obesity (39.3%). Research by Zhou et al also reported almost the same thing; namely, the most common comorbidities were hypertension, diabetes mellitus, and followed by CAD.[12] Likewise, with data in Indonesia, the most commonly found comorbidities are hypertension, diabetes mellitus, and cardiovascular disease.[10]

Kompaniyes et al reported on the American CDC page that as many as 50.7% of patients who were hospitalized from March 2020 to December 2020 were obese, and 28.3% were in the fat category.[11] In this study, the number of comorbidities in the treatment group was higher than in the control group. The number of comorbidities ≥3 was found in 5 subjects in the treatment group and 2 subjects in the control group. This difference can affect the research output.

The incidence of myocardial injury in this study was 28.5%, with 46.2% in the critical group and 13.3% in the moderate-severe group. This figure is not much different from that obtained by other studies. Based on several studies, the incidence of myocardial injury in COVID-19 generally has a fairly wide range, namely 7.2-40.9%.[13] A meta-analysis by Fu et al reported that the incidence of myocardial injury in subjects was 22%. In contrast, it was higher in subjects with severe COVID-19, namely 42%.[14]

In this study, there were more subjects with myocardial injury in women (62.7%) than in men (37.5%). These results differ from those reported by other studies in general, which found that the male sex was more numerous.[14] The mechanism of why the incidence is higher in males is still unclear. Allegedly, several genes related to the immune system located on the X chromosome cause an increase in the ability to recognize and eliminate antigens.[15] The different results in this study could be because the female subjects who experienced myocardial injury had other risk factors, namely age over 50 years (100%), number of comorbidities ≥3 (40%), critical degree of COVID-19 (60%) and have cardiovascular comorbidities (80%).

Other characteristics obtained in this study include age, BMI, number of comorbidities, and history of cardiovascular comorbidities consistent with other studies. Old age is a risk factor for myocardial injury. Fu et al reported that the group of subjects over 60 years had a significantly different prevalence of myocardial injury compared to the group aged less than 60 years.[14] Efros et al reported that subjects with myocardial injury had a history of

significant cardiovascular comorbidities, including hypertension, heart failure, ischemic heart disease, atrial fibrillation, and non-cardiovascular comorbidities, including diabetes mellitus, cerebrovascular disease, chronic kidney failure, and COPD.[16]

In this study, we found poor outcomes in patients with myocardial injury. Six subjects (75%) had critical clinical manifestations of COVID-19; the outcome was death. This is also consistent with the results of other studies, which report myocardial injury as a predictor of poor outcomes in COVID-19 patients. The results of a meta-analysis by Santoso et al showed that myocardial injury was significantly associated with higher mortality (RR=7.95), severe clinical COVID-19 (RR=13.8), and a higher need for ICU care (RR=7.94).[17] Efros et al reported significant differences in deaths and the need for mechanical ventilation in patients with myocardial injury compared to those without myocardial injury, with hazard ratios of 4.32 and 1.96.[16]

In this study, it was found that subjects with myocardial injuries did not improve; more were found in controls, namely, 6 subjects (40%), compared to the treatment group, namely 4 subjects (30.8%), although this was not statistically significant. Mesenchymal stem cells have the potential to act as immunomodulators in the inflammatory process that occurs in myocardial injury through their ability to differentiate into cardiomyocyte cells, inhibit T-cell activation, proliferation, and maturation so that they can suppress the inflammatory process and their ability to reduce ischemic processes through paracrine signaling effects.[18] There has been no previous research that specifically assessed the benefits of giving UC-MSC to myocardial injury and heart pump function in COVID-19 patients.

High troponin I, NT-proBNP, and CRP values predict poor outcomes, a higher risk of death, and ICU admission in COVID-19 pneumonia patients. CRP is a non-specific acute phase protein secreted by the liver. Clinically, CRP is used as a biomarker for inflammatory or infectious conditions. The increase in CRP is directly related to the severity of the infectious disease. Hodges et al reported that increased CRP is associated with disease progression and the extent of lung lesions in COVID-19 pneumonia.[19]

Meanwhile, troponin I and NT-proBNP are biomarkers of cardiac pathology. The inflammatory process in COVID-19 can induce cardiac injury. Respiratory disorders that occur also reduce oxygenation in the heart muscle. In addition, the binding of the SARS-Cov2 virus to the ACE2 receptor will cause the release of pro-inflammatory angiotensin, which facilitates the secretion of NT-proBNP.[8]

Mesenchymal stem cells act as immunomodulators with their ability to suppress cytokine storm processes and coagulopathy, protect alveolar epithelial cells and vascular endothelium, and are proangiogenetic and antimicrobial. Mesenchymal stem cells will suppress TNF-α and induce macrophage differentiation

into M2, which is anti-inflammatory. In the next process, it will reduce the release of pro-inflammatory proteins.[8]

This study showed a trend of decreasing troponin I, NT-proBNP, and CRP values in the treatment group compared to the control group, although this was not statistically significant. This result is consistent with several other studies. Shu et al and Guo et al reported a significant difference in CRP values in COVID-19 subjects who were given UC-MSC compared to controls.[20,21]

Liang et al reported a case report of a 75-year-old woman with COVID-19 who was given UC-SMC in addition to standard therapy with an initial proBNP value of 4,012 pg/ml, which decreased significantly to the normal range on day 12 after implantation.[22] Leng et al reported evaluating troponin I values in COVID-19 pneumonia patients who received UC-MSC starting to experience improvement on the 6th day after implantation.[6]

The median EF value of the two groups in this study was lower after implantation than before. This can illustrate decreased left heart function in patients after COVID-19 infection. Heart failure is one of the comorbidities often found in COVID-19 patients and is one of the cardiovascular manifestations of COVID-19 pneumonia. COVID-19 infection can cause an acute exacerbation in patients with a history of heart failure or the onset of new heart failure in patients without a history of heart failure.[23]

The mechanism of decreased cardiac function in COVID-19 occurs through several causes, namely (1) activation of the sympathetic nervous system in infection will cause stress cardiomyopathy, (2) an increase in the body's metabolic demand to fight infection, which is not compensated by the available supply so that it can cause ischemia cardiac muscle, (3) release of proinflammatory cytokines, which can cause acute injury to the heart muscle, (4) coagulation dysfunction, which will cause ischemia of the heart muscle, (5) the occurrence of ARDS, which will cause pulmonary hypertension to right heart failure, (6) involvement of kidney dysfunction which will cause fluid retention thereby increasing the workload of the heart, and (7) septic conditions in severe infections will cause cardiac dysfunction.[23]

There was no previous research on administering UC-MSC to COVID-19 pneumonia patients that assessed heart function parameters. Previous studies conducted before the pandemic reported the benefits of giving MSCs to improve heart function. An early study of MSCs administration in acute myocardial infarction by Chen et al reported the safety of MSCs and an increase in the left ventricle (LV) of 14% in the treatment group.[24] Another meta-analysis by Jeong et al reported an increase in LV of 3.8% and a six-minute walk test of patients after 2 years of therapy.[25]

This study showed no significant difference in EF or TAPSE scores in the two groups of subjects before and after implantation. Of the 13 subjects (5 treatment groups and 8 control groups) with complete echocardiographic data, 3

subjects (60%) were aged >60 years in the treatment group compared to 3 subjects (37.5%) in the control group, 2 subjects (40%) had comorbidities ≥3 in the treatment group compared to 1 subject (12.5%) in the control group, and 2 subjects (40%) had CHF comorbidities, whereas 8 subjects in the control group did not some have comorbid CHF.

Differences in age factors, the number of cardiovascular comorbidities, and comorbidities between the two groups can affect the study results. In addition, due to limited research resources, echocardiographic examinations could only be carried out at a different time than observation.

In this study, one subject in the treatment group had comorbid CHF with a very low pre-implantation EF value, namely 15%, which was classified as severe dysfunction. Treatment is still given according to a predetermined protocol. After implantation, this subject had an arrhythmia later resolved with medical management.

In addition, there was also one subject in the treatment group with the oldest age, namely 71 years, and with a history of comorbid CHF and arrhythmia. After the arrhythmic condition is resolved, treatment is still given according to the protocol. Evaluation of these two patients showed improvement, and no adverse events were found until the subject was discharged. So in this study, we found that administering UC-MSC was relatively safe in COVID-19 pneumonia patients who were >70 years old, had a history of arrhythmias, or had a history of CHF with severe dysfunction.

In this study, the subject outcomes at the end of the observation day (H15) were the number of deaths slightly higher in the treatment group, namely 7 subjects (53.8%) compared to the control group, namely 6 people (46.7%) with a median survival value of shorter in the treatment group, namely 7 days compared to the control group, namely 10 days, which was not statistically significant. This result differs from several other studies, which found that the mortality rate in the treatment group was lower than in the control group.

A pilot study on administering UC-MSC to COVID-19 patients was conducted by Leng et al in China. Subject criteria and the dose of UC-MSC given in this study were the same as those of Leng et al. Leng et al reported that all subjects in the treatment group (7 subjects) lived, while one in the control group died.[6]

However, Leng et al did not describe each subject's comorbid history. Meanwhile, in this study, the number of comorbidities was ≥3 in 5 subjects in the treatment group, while only 2 subjects were in the control group, although the difference was not statistically significant. Biswas et al reported that comorbidities in COVID-19 patients could increase the risk of death, namely cardiovascular disease (RR=3.05), respiratory disease (RR=2.74), diabetes mellitus (RR=1.97), and hypertension (RR=1.95).[26]

Three other studies conducted by COVID-19 patients 19 degrees severe to

critical also reported different results from this study. Sanchez-Guijo et al reported a mortality rate of 2 out of 13 subjects (15%) on the 16th post-implantation day, Guo et al reported 4 of 31 subjects who died (12.9%), and Shu et al reported no death at subjects who were given SPM while in The three studies have a protocol for administering UC-MSC that differs from this study.[21,27]

Sanchez-Guijo et al provided MSC sourced from adipose tissue at a dose of $0.98x10^6$/kg BW 2-3 times the dose with an interval of 3 days. Guo et al gave an MSC dose of 10^6 cells/kg BW with a frequency of 1-3 times the dose. These two studies did not divide subjects into two groups, namely treatment, and control, so the patient outcomes could not be compared.[21,27] Shu et al gave an MSC dose of $2x10^6$ cells/kg BW with an observation duration of 28 days, and the basic characteristics of the subjects were only 41% had comorbidities, namely hypertension and diabetes mellitus.[20]

Two other phase II clinical studies reported results that differed from this study. Lanzoni et al reported fewer deaths in the treatment group, namely 2 subjects, compared to the control group, namely 7 subjects (P=0.015). Lanzoni et al's study was conducted on mild-moderate and moderate-severe subjects, with an MSC dose of $100\pm20 \times 10^6$ cells intravenously in two doses, namely on the first day (H0) and the 3rd day of treatment (D3) which were observed up to the 30^{th} day. However, researchers only assessed the types of comorbidities, not accompanied by an assessment of the number of comorbid in each subject.[28] Shi et al conducted a study on 90 subjects with mild, moderate, to severe degrees of COVID-19 pneumonia, reporting no subject death in both groups until the 28th day of observation. The dose of UC-MSC given was $4x10^7$ cells intravenously.[29]

Differences in results between this study and other studies can be caused by factors of subject characteristics, treatment protocols and length of observation. Differences in the characteristics of the subjects in this study that could affect the outcome were fewer subjects aged 40-60 years in the treatment group, namely 7 subjects (52.8%) compared to the control group, namely 10 subjects (66.7%), the number of comorbidities ≥3 more in the treatment group, namely 5 subjects (38.5%) compared to the control group, namely 2 subjects (13.3%), comorbid CHF and CAD were only found in 6 subjects (46.1%) in the treatment group and not found in the control group. Controls and complications of AKI were more common in the treatment group with 9 subjects (69.2%) than 4 subjects (26.7%).

There are several weaknesses in this research. First, the number of subjects is limited caused of limited resources owned by researchers. Second, the assessment for evaluating myocardial injury and inflammatory markers can only be carried out at the level of biomarkers, namely Troponin I, NT-proBNP, and CRP, not yet up to the level of proinflammatory cytokines. This is also caused by the limited resources of the researcher. The

third, echocardiographic examination cannot be performed on all subjects and at the same observation time. This was due to the limited available resources and the limited field of view of the operator due to the use of level 3 PPE. Fourth, other adjuvant therapies were given to the subjects, namely tocilizumab and IVIG, which might have biased the results of this study.

CONCLUSION

There were no significant differences in troponin I, NT-proBNP, and CRP values, in COVID-19 pneumonia patients who received additional therapy for UC-MSC before and after implantation compared to controls, but there was a trend of decreasing values in the treatment group compared to controls. Besides that, there was no difference in heart pump function and the survival/death outcomes of COVID-19 pneumonia patients who received additional therapy for UC-MSC before and after implantation compared to controls. Therefore, it is necessary to carry out further research using a larger sample and administering a different treatment protocol. It is necessary to evaluate other cellular markers or proinflammatory cytokines that play a role in the pathogenesis of myocardial injury.

REFERENCES

1. Handayani D, Hadi DR, Isbaniah F, Burhan E, Agustin H. Corona Virus Disease 2019. J Respirologi Indones. 2020;40(2):119–29.
2. Kementrian Kesehatan Republik Indonesia. Infeksi Emerging Kementerian Kesehatan RI [Internet]. Kementrian Kesehatan Republik Indonesia. 2020. Available from: https://infeksiemerging.kemkes.go.id/
3. Zou F, Qian Z, Wang Y, Zhao Y, Bai J. Cardiac Injury and COVID-19: A Systematic Review and Meta-analysis. CJC open. 2020 Sep 1;2(5):386–94.
4. Bansal M. Cardiovascular disease and COVID-19. Diabetes Metab Syndr. 2020;14(3):247–50.
5. Atluri S, Manchikanti L, Hirsch JA. Expanded Umbilical Cord Mesenchymal Stem Cells (UC-MSCs) as a Therapeutic Strategy in Managing Critically Ill COVID-19 Patients: The Case for Compassionate Use. Pain Physician. 2020;23(2):E71–83.
6. Leng Z, Zhu R, Hou W, Feng Y, Yang Y, Han Q, et al. Transplantation of ACE2- Mesenchymal Stem Cells Improves the Outcome of Patients with COVID-19 Pneumonia. Aging Dis. 2020;11(2):216–28.
7. Moll G, Drzeniek N, Kamhieh-Milz J, Geissler S, Volk HD, Reinke P. MSC Therapies for COVID-19: Importance of Patient Coagulopathy, Thromboprophylaxis, Cell Product Quality and Mode of Delivery for Treatment Safety and Efficacy. Front Immunol. 2020;11:1091.
8. Weiss ARR, Dahlke MH. Immunomodulation by Mesenchymal Stem Cells (MSCs): Mechanisms of Action of Living, Apoptotic, and Dead MSCs. Front Immunol. 2019;10:1191.

9. Abate BB, Kassie AM, Kassaw MW, Aragie TG, Masresha SA. Sex difference in coronavirus disease (COVID-19): a systematic review and meta-analysis. BMJ Open. 2020;10(10).
10. Karyono DR, Wicaksana AL. Current prevalence, characteristics, and comorbidities of patients with COVID-19 in Indonesia. J Community Empower Heal. 2020;3(2):77–84.
11. Kompaniyets L, Goodman AB, Belay B, Freedman DS, Sucosky MS, Lange SJ, et al. Body Mass Index and Risk for COVID-19–Related Hospitalization, Intensive Care Unit Admission, Invasive Mechanical Ventilation, and Death — United States, March–December 2020. MMWR Morb Mortal Wkly Rep. 2021;70(10):355–61.
12. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet (London, England). 2020;395(10229):1054–62.
13. Wei ZY, Geng YJ, Huang J, Qian HY. Pathogenesis and management of myocardial injury in coronavirus disease 2019. Eur J Heart Fail. 2020;22(11):1994–2006.
14. Fu L, Liu X, Su Y, Ma J, Hong K. Prevalence and impact of cardiac injury on COVID-19: A systematic review and meta-analysis. Clin Cardiol. 2021;44(2):276–83.
15. Prasitlumkum N, Chokesuwattanaskul R, Thongprayoon C, Bathini T, Vallabhajosyula S, Cheungpasitporn W. Incidence of Myocardial Injury in COVID-19-Infected Patients: A Systematic Review and Meta-Analysis. Diseases. 2020;8(4):40.
16. Efros O, Barda N, Meisel E, Leibowitz A, Fardman A, Rahav G, et al. Myocardial injury in hospitalized patients with COVID-19 infection-Risk factors and outcomes. PLoS One. 2021;16(2).
17. Santoso A, Pranata R, Wibowo A, Al-Farabi MJ, Huang I, Antariksa B. Cardiac injury is associated with mortality and critically ill pneumonia in COVID-19: A meta-analysis. Am J Emerg Med. 2021;44:352.
18. Yan W, Abu-El-Rub E, Saravanan S, Kirshenbaum LA, Arora RC, Dhingra S. Inflammation in myocardial injury: mesenchymal stem cells as potential immunomodulators. Am J Physiol Heart Circ Physiol. 2019;317(2):H213–25.
19. Hodges G, Pallisgaard J, Schjerning Olsen AM, McGettigan P, Andersen M, Krogager M, et al. Association between biomarkers and COVID-19 severity and mortality: A nationwide Danish cohortstudy. BMJ Open. 2020;10(12).
20. Shu L, Niu C, Li R, Huang T, Wang Y, Huang M, et al. Treatment of severe COVID-19 with human umbilical cord mesenchymal stem cells. Stem Cell Res Ther. 2020;11(1).
21. Guo Z, Chen Y, Luo X, He X, Zhang Y, Wang J. Administration of umbilical cord mesenchymal stem cells in patients with severe COVID-19

pneumonia. Crit Care. 2020;24(1).

22. Liang B, Chen J, Li T, Wu H, Yang W, Li Y, et al. Clinical remission of a critically ill COVID-19 patient treated by human umbilical cord mesenchymal stem cells: A case report. Medicine (Baltimore). 2020;99(31):E21429.
23. Bader F, Manla Y, Atallah B, Starling RC. Heart failure and COVID-19. Heart Fail Rev. 2021;26(1).
24. Cheng R, Liu C, Yang J, Yang Y, Chen R, Ding X, et al. Sex Differences in the Incidence and Risk Factors of Myocardial Injury in COVID-19 Patients: A Retrospective Cohort Study. Front Physiol. 2021;12.
25. Jeong H, Yim HW, Park HJ, Cho Y, Hong H, Kim NJ, et al. Mesenchymal Stem Cell Therapy for Ischemic Heart Disease: Systematic Review and Meta-analysis. Int J stem cells. 2018;11(1):1–12.
26. Biswas M, Rahaman S, Biswas TK, Haque Z, Ibrahim B. Association of Sex, Age, and Comorbidities with Mortality in COVID-19 Patients: A Systematic Review and Meta-Analysis. Intervirology. 2021;64(1):36–47.
27. Sánchez-Guijo F, García-Arranz M, López-Parra M, Monedero P, Mata-Martínez C, Santos A, et al. Adipose-derived mesenchymal stromal cells for the treatment of patients with severe SARS-CoV-2 pneumonia requiring mechanical ventilation. A proof of concept study. EClinicalMedicine. 2020;25:100454.
28. Lanzoni G, Linetsky E, Correa D, Messinger Cayetano S, Alvarez RA, Kouroupis D, et al. Umbilical cord mesenchymal stem cells for COVID-19 acute respiratory distress syndrome: A double-blind, phase 1/2a, randomized controlled trial. Stem Cells Transl Med. 2021;10(5):660–73.
29. Shi L, Huang H, Lu X, Yan X, Jiang X, Xu R, et al. Effect of human umbilical cord-derived mesenchymal stem cells on lung damage in severe COVID-19 patients: a randomized, double-blind, placebo-controlled phase 2 trial. Signal Transduct Target Ther. 2021;6(1):58.