HUMAN CLINICAL ARTICLE

STEM CELLS
TRANSLATIONAL MEDICINE

Umbilical cord mesenchymal stromal cells as critical COVID-19 adjuvant therapy: A randomized controlled trial

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Abstract

One of the main causes of acute respiratory distress syndrome in coronavirus disease 2019 (COVID-19) is cytokine storm, although the exact cause is still unknown. Umbilical cord mesenchymal stromal cells (UC-MSCs) influence proinflammatory T-helper 2 (Th$_2$) cells to shift to an anti-inflammatory agent. To investigate efficacy of UC-MSC administration as adjuvant therapy in critically ill patients with COVID-19, we conducted a double-blind, multicentered, randomized controlled trial at four COVID-19 referral hospitals in Jakarta, Indonesia. This study included 40 randomly allocated critically ill patients with COVID-19; 20 patients received an intravenous infusion of 1×10^6/kg body weight UC-MSCs in 100 ml saline (0.9%) solution (SS) and 20 patients received 100 ml 0.9% SS as the control group. All patients received standard therapy. The primary outcome was measured by survival rate and/or length of ventilator usage. The secondary outcome was measured by clinical and laboratory improvement, with serious adverse events. Our study showed the survival rate in the UC-MSCs group was 2.5 times higher than that in the control group ($P = .047$), which is 10 patients and 4 patients in the UC-MSCs and control groups, respectively. In patients with comorbidities, UC-MSC administration increased the survival rate by 4.5 times compared with controls. The length of stay in the intensive care unit and ventilator usage were not statistically significant, and no adverse events were reported. The application of infusion UC-MSCs significantly decreased interleukin 6 in the recovered patients ($P = .023$). Therefore, application of intravenous UC-MSCs as adjuvant treatment for critically ill patients with COVID-19 increases the survival rate by modulating the immune system toward an anti-inflammatory state.

KEYWORDS

adjuvants, cord stem cell transplantation, COVID-19, cytokine release syndrome, immunology, mesenchymal stromal cells

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Lessons learned

Application of intravenous umbilical cord mesenchymal stromal cell infusion as an adjuvant treatment for critically ill patients with COVID-19 increases the survival rate by modulating the immune system toward an anti-inflammatory state.

Significance statement

There are some unfortunate patients with COVID-19 who are not responsive to all the combination treatment given. Therefore, this study exclusively involved intubated critically ill patients with COVID-19 in the intensive care unit. In contrast to existing studies, this study used regular umbilical cord mesenchymal stem cells without a special manipulation procedure that was directed at achieving angiotensin converting enzyme-2 (ACE 2)-negative mesenchymal stem cell. The results showed that the survival rate in those receiving umbilical cord mesenchymal stromal cells was 2.5 times higher than that in the control group.

1 | INTRODUCTION

Patients with COVID-19 are increasing around the world, with more than 61.8 million cumulative cases and 1.4 million deaths globally. In November 2020, a referral hospital's data in Jakarta showed that intensive care unit (ICU) occupancy had increased from 60% to 80%. Moreover, the mortality rate of critically ill patients with COVID-19-related pneumonia in the ICU was as high as 87% in a top referral COVID-19 hospital (Persahabatan Central Hospital, Jakarta, Indonesia); this situation necessitated that clinicians fashion a breakthrough therapy to increase the survival of patients in the ICU.

The current management of COVID-19 serves as an empiric and supportive therapy, as the curative regiment has yet to be found. Unfortunately, some patients are not responsive despite all the combination treatments given. There is still very limited research about the new potential therapeutic agents for treating critically ill patients with COVID-19.[1,2]

Acute respiratory distress syndrome (ARDS) is the leading cause of death in patients with COVID-19, and one of the main causes of ARDS in SARS-CoV-2 infection is cytokine storm, although this topic is still a matter of controversy.[3] In interactions with dendritic cells, mesenchymal stromal cells (MSCs) cause proinflammatory Th_2 cells to shift to anti-inflammatory Th_2 cells, including changes in cytokine profiles toward anti-inflammatory. Human umbilical cord mesenchymal stromal cells (hUC-MSCs) have a high proliferative capacity for sustaining paracrine effects and low risk of body rejection due to absent Human Leukocyte Antigen (HLA) expression.[4,5]

Clinical trials in China have shown that patients with COVID-19-related pneumonia treated with UC-MSCs were more likely to survive and have a faster recovery than patients without MSC therapy.[6-8]

In contrast to existing studies, our study exclusively involved intubated critically ill patients with COVID-19 in the ICU and used naïve umbilical cord mesenchymal stromal cells that were processed using the simple multiple harvest explant procedure methods without a special manipulation procedure that was directed at achieving ACE-2 negative MSCs. Therefore, this study aims to investigate whether administration of allogenic UC-MSCs as adjuvant therapy for critically ill patients with COVID-19 who are unresponsive to conventional supportive treatment can improve the survival rate of critically ill patients with COVID-19-related pneumonia in Indonesia.

2 | MATERIALS AND METHODS

2.1 | Study design

This is a multicentered, double-blind, randomized clinical trial, conducted between May 1 and October 10, 2020, at four COVID-19 referral hospitals in Jakarta (Sulianti Saroso Infection Disease Hospital, Persahabatan Central General Hospital, Cipto Mangunkusumo National Central General Hospital, and Universitas Indonesia Hospital).

2.2 | Participants

Subjects in this study were selected using stratified random sampling and randomized using a computerized random number generator. A total of 40 subjects were included, with 20 in the control group and 20 in the experimental group. The number of subjects from this study was determined based on references to previous studies. This is a double-blinded study. Neither the subjects nor the care providers and those assessing the outcomes were aware of the treatment assignment. This is achieved by making the packaging of MSCs and the placebo in an identical way. All patient allocations were determined from a third party that was unrelated to clinical decision-making or data collection. Critically ill patients with COVID-19-related pneumonia were defined as those who were intubated with severe pneumonia clinically and radiologically, confirmed by a positive result in Reverse Transcription - Polymerase Chain Reaction (RT-PCR) swab from nasopharyngeal or bronchoalveolar lavage. The criteria of critical patients included the following: (a) respiratory failure that progressed into ARDS, defined as the partial pressure of oxygen in arterial blood (PaO_2)/the fraction of inspired oxygen (FiO_2) lower than 300 mmHg

and supported by mechanical ventilation; (b) shock, such as septic shock (persistent hypotension following fluid resuscitation and requiring vasopressor to maintain mean arterial pressure of ≥65 mmHg and serum lactate of >2 mmol/L); (c) multiple organ failure and monitored in the ICU.

2.3 | Inclusion criteria

In this study, inclusion criteria for the participant were (a) age 18-95 years, (b) critically ill patients with RT-PCR-confirmed COVID-19 obtained from a nasopharyngeal swab or bronchoalveolar lavage if intubated, (c) leukopenia and lymphopenia in peripheral blood and differential count, (d) presenting with pneumonia on chest x-ray and/or ground-glass opacity on thorax computed tomography (CT) scan, and (e) signed informed consent by subjects or family members.

2.4 | Exclusion criteria

Exclusion criteria in this study were (a) any history of malignancy, (b) pregnant or showed a positive pregnancy test, and (c) any history of or currently taking part in another clinical trial in the last 3 months.

2.5 | Standard protocol approvals, registrations, and patient consents

The study was approved by the ethics board of the Faculty of Medicine Universitas Indonesia (KET-A36/UN2.F1/ETIK/PPM.00.02/2020) (Supplemental Data S1). Written informed consent was obtained from family members because of the patients' poor general condition (Supplemental Data S2). The clinical trial was registered at ClinicalTrials. gov (NCT04457609, https://clinicaltrials.gov/ct2/show/NCT04457609). The timeline of the study can be observed in Figure 1.

2.6 | MSC collection, preparation, and administration

2.6.1 | Umbilical cord material collection and preparation

The MSCs were harvested from human umbilical cord produced by Stem Cells Medical Technology Integrated Service Installation, Cipto Mangunkusumo Central National General Hospital, Faculty of Medicine Universitas Indonesia. MSCs were cultured and harvested from passage 5 or 6 to ensure the best quality of the cells. Positive expression of CD90 and CD73 (>95%) and negative expression of CD34 (<2%) were found on cells acquired starting from passage 3, signifying the presence of MSCs. Our previous study showed that senescence is

observed after passage 10 and viability is observed until passage 18 for UC-MSCs.[9]

2.6.2 | Administration

After baseline assessments were done, subjects admitted to the study were given a single intravenous infusion of 1×10^6/kg body weight UC-MSCs in 100 ml saline (0.9%) solution for the experimental group or with placebo (100 ml saline [0.9%] solution) for the control group on day 8 (ranged from day 2-30) of treatment in the ICU.

2.7 | Study assessment

Baseline parameters including routine blood count, differential count, C-Reactive Protein (CRP), D-dimer, fibrinogen, and procalcitonin, and specific markers, including vascular endothelial growth factor (VEGF), ferritin, cytokine IL-6, IL-10, flow cytometry leukemia inhibitory factor (LIF), and lymphocyte subpopulation CX-CR3 CD4, CD8, and CD56, were assessed before the application was done. During 15 days of observation, laboratory evaluations were conducted on day 0 and day 1 and then once every 3 days for routine laboratory examination whereas specific markers were evaluated on day 0 and day 7 only. Adverse events (AEs) were closely observed in the MSCs group.

2.8 | Outcome measurements

The primary outcome is assessed by mortality rate and length of ventilator usage. We measured the onset of intubation, which was defined by the length of ventilator usage during the patient's care. The period of intubation until the application of allogeneic UC-MSCs was defined by the period of the patient still needing mechanical ventilation after being given intervention. Secondary outcomes were measured by (a) length of stay in the ICU; (b) improvement in the routine laboratory value, including routine blood count, differential count, CRP, D-dimer, fibrinogen, and procalcitonin; (c) improvement in biomarker laboratory value of cytokines and lymphocyte subpopulation (VEGF, ferritin, IL-6, LIF, CX-CR3 CD4, CD8, CD56 cell); and (d) AE or serious AE (SAE).

2.9 | Statistical analysis

Descriptive statistics were used to describe demographic and parameter characteristics, including mean, SD, frequencies, and percentages. The rate of change in laboratory value was calculated for each parameter and compared between the control and experiment groups using the Mann-Whitney-U test. Internal analysis of the experiment group was done in patients treated with MSCs, comparing the value before and after treatment using the Wilcoxon signed-rank test. P values <.05 were considered statistically significant. The survival analysis
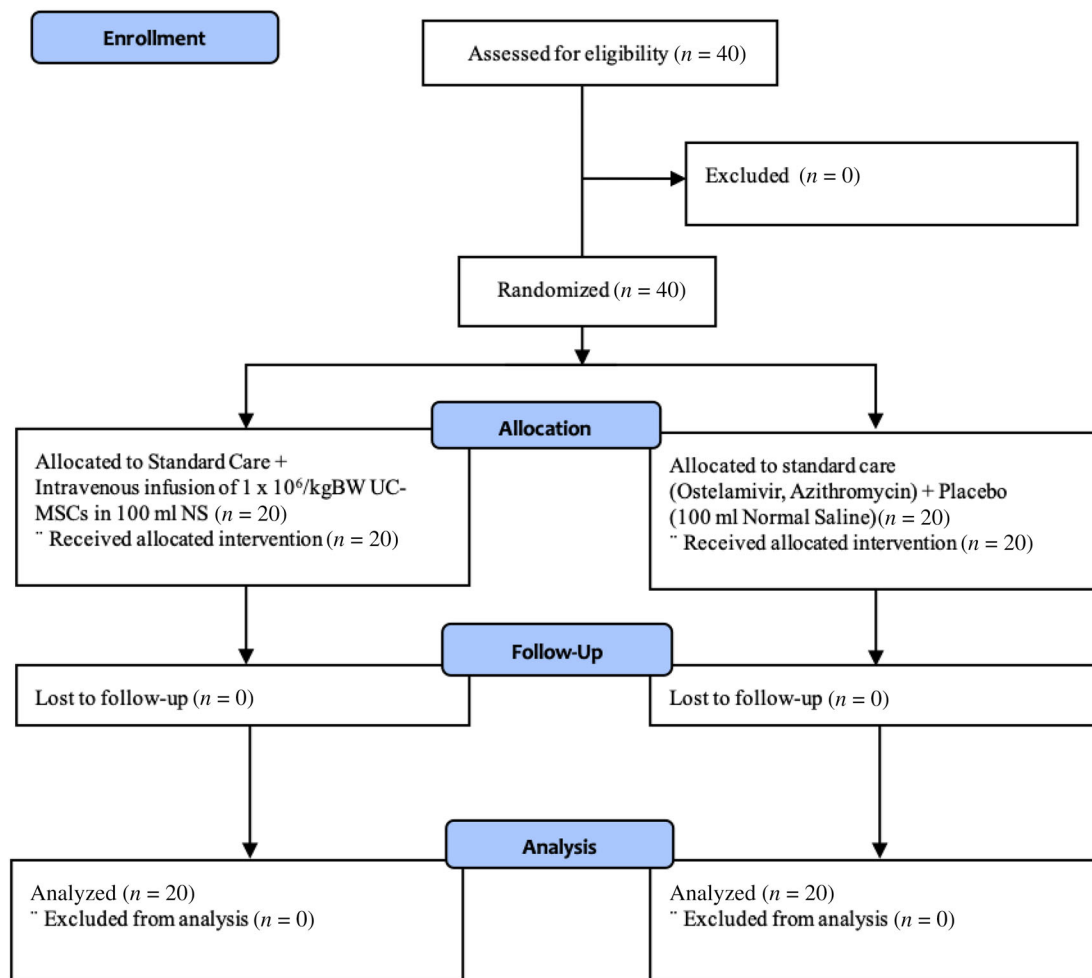
FIGURE 1 Timeline of the study. Standard of care consists of therapy given in accordance to the guidelines, namely, azithromycin 500 mg and oseltamivir 75 mg, which is given tailored to the patient's need and the attending's discretion and clinical judgment. BW, body weight; NS, normal saline; UC-MSCs, umbilical cord mesenchymal stromal cells

was assessed using Cox regression. The analysis was performed using SPSS ver. 25 (IBM, Armonk, NY).

3 | RESULTS

A total of 40 critically ill patients with COVID-19-related pneumonia were included. No patients were excluded or dropped out of the trial. The baseline characteristics of the 40 patients are summarized in Table 1.

3.1 | Primary outcome

Of the 40 subjects, males (75%) were significantly affected compared with females ($P = .049$). The mortality rate was 65% ($n = 26$) and the survival rate was 35% ($n = 14$), in which 71.4% ($n = 10$) of the recovered group were from the MSCs group and 28.6% ($n = 4$) were from the control group. Thus, the survival rate in the MSCs group was 2.5

times higher than that in the control group ($P = .047$). When only analyzing patients with comorbidities, our study showed that UC-MSC administration increased the survival rate by 4.5 times compared with controls, which is nine patients and two patients in the MSCs and control group, respectively. The outcome of the subjects is described in Table 2.

There were 19 subjects (47.5%) who had >2 comorbidities. These subjects had a higher mortality rate than those with <2 comorbidities (79.17% died). The majority of those with >2 comorbidities who recovered came from the MSCs group, with a 4:1 ratio to the control group. Furthermore, there was a significant difference between the subject outcome and the number of comorbidities ($P = .023$). In the distribution of comorbidities among subjects who died, 65% of subjects had diabetes mellitus, 46.15% had hypertension, 26.93% had chronic kidney disease, 15.38% had coronary artery disease, 7.69% had congestive heart failure, and 7.69% had tuberculosis. Three (21.4%) of the 14 patients who survived and 17 (65.3%) of the 26 patients who died had diabetes as a comorbidity. Further experiments to diagnose endothelitis were not performed in this study.

There is no significant difference between both groups regarding the period of intubation and the period from intubation until application. The mean period of intubation is 15.69 ± 10.37 days for the MSCs group and 16.63 ± 5.4 days for the control group, whereas the mean period from intubation until the application is 9.23 ± 6.89 days for the MSCs group and 8.63 ± 2.44 days for the control group.

TABLE 1 Demographic background of subjects

	Control group (n)	MSCs group (n)	P value
Subjects	20	20	
Age, yr			>.05
<40	3	4	
40-60	7	8	
>60	10	8	
Sex			.642
Male	15	15	
Female	5	5	
Comorbidities, n			.122
0–1	7	9	
≥2	13	11	

Abbreviation: MSCs, mesenchymal stromal cells.

TABLE 2 The outcome of the subjects

	Recovered (n)		Died (n)		
Characteristics	MSC	Control	MSC	Control	P value
Subjects	10	4	10	16	.047
Age, yr					.062
<40	4	1	0	1	
40–60	3	2	4	8	
>60	3	1	6	7	
Sex					.492
Male	7	4	8	16	
Female	3	1	2	4	
Comorbidities, n					.023
0–1	6	3	3	4	
≥2	4	1	7	12	
Types of comorbidities					
Diabetes mellitus	1	2	7	10	
Hypertension	3	1	3	9	
Chronic kidney disease	0	0	2	5	
Coronary arterial disease	0	1	2	2	
Congestive heart failure	0	0	1	1	
Tuberculosis	0	0	1	1	
Others[a]	7	0	3	6	

Abbreviations: MSC, mesenchymal stromal cell.
[a]Other comorbidities include gastric perforation, pleural effusion, multiple rib fractures, obesity, hypercoagulation, and lung contusion in the Recovered group and icterus, stroke infarction, Disseminated Intravascular Coagulation (DIC), atrial fibrillation, obesity, acute kidney injury, myocardial infarction, and hypertensive heart disease in the Died group.

The illustration of the day of survival using Kaplan-Meier is shown in Figure 2, and it shows a longer survival time for the MSCs group. Further analysis was performed to see the contribution of age, treatment group, and the number of comorbidities to the time of death. Although none of these factors affected death significantly, it shows that MSCs contribute to the improvement of the subjects compared with placebo. Both age and comorbidities positively contribute to early time of death for the subjects.

3.2 | Secondary outcomes

3.2.1 | Length of stay

The length of stay in the ICU was longer for the MSCs group (12.23 + 8.86 days) compared to the control group (10.44 + 7.37 days) with no statistically significant difference.

3.2.2 | Improvement in biomarker laboratory values of organ functions

After comparing the change of every laboratory parameter of the MSCs group and the control group, statistical analysis showed that
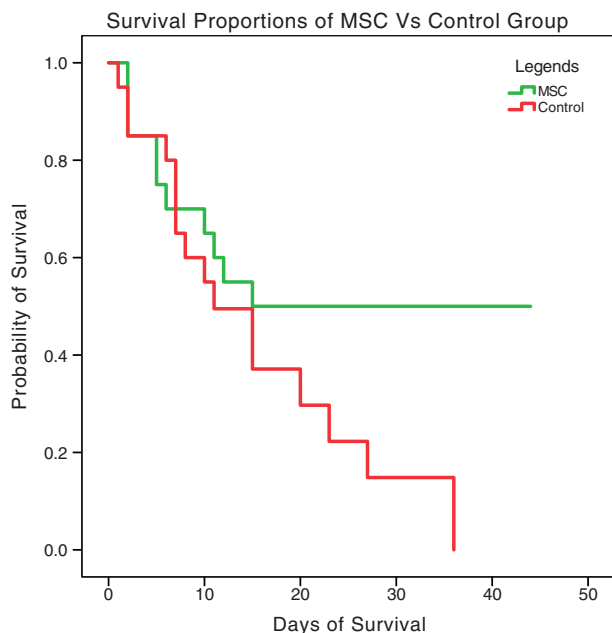
FIGURE 2 Kaplan-Meier diagram of the time from intervention (administration of umbilical cord MSCs or placebo) to death or improvement. MSC, mesenchymal stromal cell

there were no significant differences in the complete blood count (hemoglobin, hematocrit, leukocyte, lymphocyte, and thrombocyte between the MSCs group and the control group with P value, respectively: .630; .782; .423; .414; .295).

There was no significant difference between D-dimer and fibrinogen in the MSCs group vs the control group (P value, respectively: .233; .979). Inflammatory markers, namely, procalcitonin, and CRP were not significantly different between the MSCs group and the control group (P value, respectively: .212; .827; .707).

Ten subjects (62.5%) in the control group had a reduced value of IL-10, whereas most of the subjects in the MSCs group showed an increased IL-10 value on the seventh day after MSC application with no significant difference between groups (P = .661). However, an increased value from day 0 to day 7 was observed, from an average 3.26 pg/ml to 4.70 pg/ml in the MSCs group. There was a decreased trend in IL-6 after MSC application; in contrast, an increasing trend was observed in the control group. The application of infusion UC-MSCs significantly decreased IL-6 in the recovered patients (P = .023). The trend of cytokine analysis is shown in Figure 3.

VEGF also showed an increasing trend in the MSCs group, compared with a decreased trend in the control group on day 7, but there was no significant difference (P = .826). A significant increase in LIF value was also observed in subjects receiving MSC application (P = .002). Ferritin was also reduced in the MSCs group but increased in the control group, however, the difference was not statistically significant (P = .861). Details of the cytokine analysis are presented in Table 3.

3.2.3 | Flow cytometry analysis of lymphocyte subpopulations

We examined lymphocyte subpopulations (Supplemental Figure 1), namely, CD4-CXCR3, CD8-CXCR, and CD56-CXCR3 (Supplemental Figure 2). We found that MSCs were better in suppressing the population of CD8-CXCR3 and CD56-CXCR3 among critically ill subjects, but no significant difference between groups was detected (P = .061). CD4-CXCR3 analysis revealed that there was also no significant difference between the MSCs and control group during the baseline and day 7 evaluation (P value, respectively: .064; .745) (Supplemental Table 1).

3.2.4 | AE or SAE within treatment care period

The intravenous infusion of MSCs was found to be safe and well tolerated with no life-threatening complications or acute allergic reactions during the administration. The critically ill patients with severe COVID-19 showed no immediate deaths or acute anaphylactic shock after MSC application.

4 | DISCUSSION

This study showed that the combined application of mesenchymal stromal cells and standard regimen improved survival rates for the critically ill patients with COVID-19. Of the recovered subjects, 10 (71.4%) came from the MSCs group and 4 (28.6%) from the control group. For instance, 50% of subjects with COVID-19 from the MSCs group showed recovery, whereas only 20% of subjects in the control group recovered. A previous pilot study of modified ACE-2-negative MSC application by Leng et al. showed improvement in seven patients with COVID-19 with varying grades from moderate to a critical degree.[6] In contrast, our study specified the UC-MSC application for critically ill patients with COVID-19, and we used a naïve umbilical cordmesenchymal stromal cells without special manipulation procedure that was directed at achieving ACE-2-negative MSC. Using a similar dose and route of administration, our results are consistent with existing reports that administering UC-MSCs as adjuvant therapy is efficient in treating critically ill patients with COVID-19.

Endothelial dysfunction has been identified as an important factor in the manifestation of vascular abnormalities in critical COVID-19 cases with thrombosis and coagulation manifestation.[10] Besides the intimate proximity between alveolar epithelial cells and endothelial cells, which facilitate efficient oxygen exchange, endothelial cells also express ACE-2. SARS-CoV-2 can be found in endothelial cells not only in the lungs but also in various other organs. Of 26 patients who died in this study, 17 (65.3%) of them had diabetes as a comorbidity. These findings are in line with several meta-analyses that support that diabetes mellitus increases the mortality rate in COVID-19 cases.[11,12] Endothelial dysfunction occurs in patients with diabetes because of oxidative stress processes and chronic inflammation. With endothelial conditions that are already compromised by this process, it cannot be denied that endothelial dysfunction will worsen with COVID-19, particularly in older patients.[13]
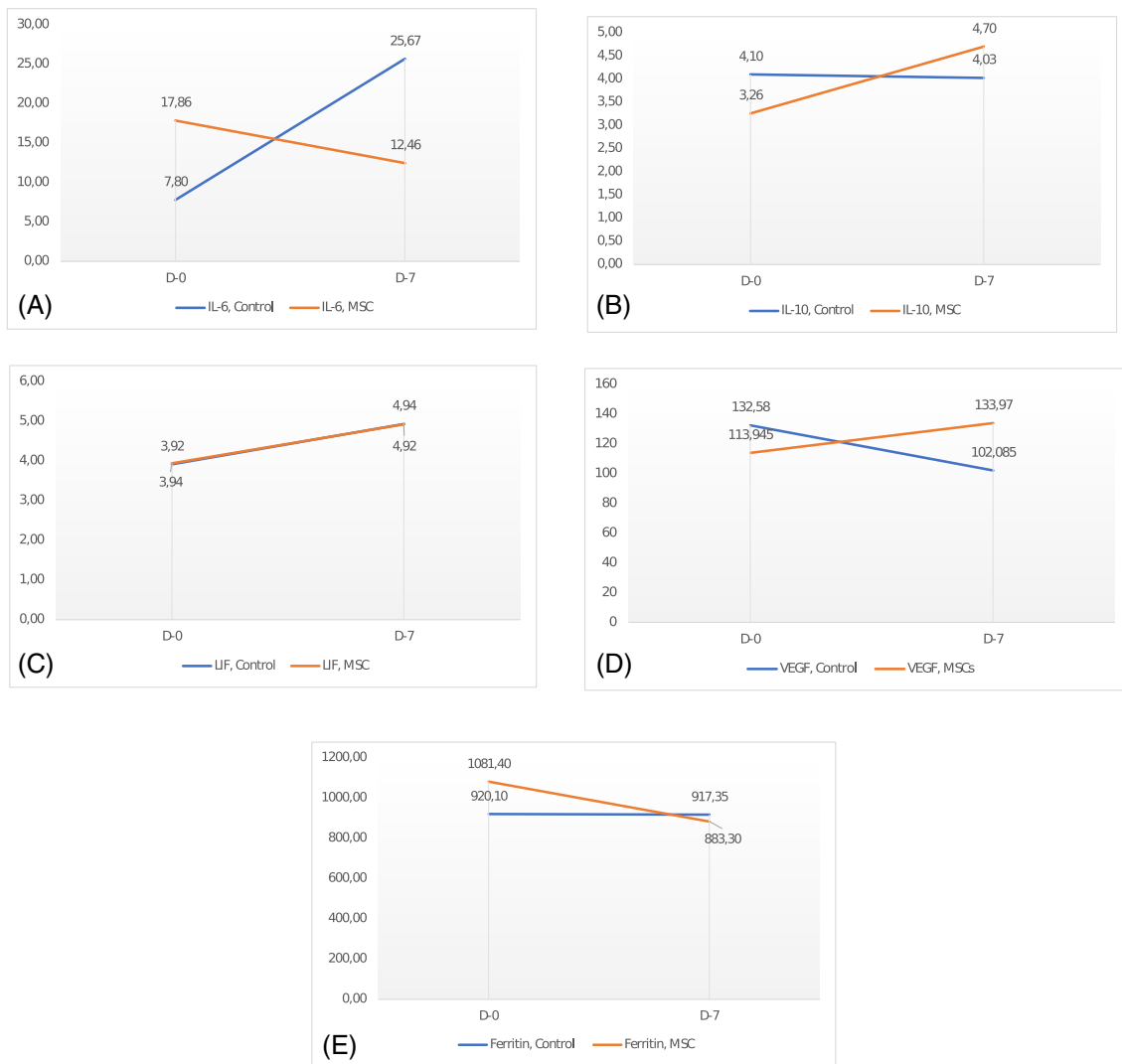
FIGURE 3 Trend of IL-6 (A), IL-10 (B), LIF (C), VEGF (D), and ferritin (E) in the MSCs group vs the control group with the interval of 7 days. D-0: Day 0 (before application), D-7: Day 7 (7 days following the application); IL, interleukin; LIF, Leukemia Inhibitory Factor; MSC, mesenchymal stromal cell; VEGF, vascular endothelial growth factor

TABLE 3 Analysis of cytokine before and after UC-MSC application

Cytokine	P value (before application in total subjects)	P value (7 days after application in total subjects)	P value (7 days after application in subjects who recovered)	P value (7 days after application in subjects who died)
IL-6	.11	.469	.023	.312
VEGF	.646	.826	.888	.665
Ferritin	.372	.861	.48	.47
IL-10	.285	.826	.229	.348
LIF	.524	.843	.620	.613

Notes: The analysis was carried out to find the difference between the MSCs and control groups using bivariate analysis via a Mann-Whitney U test. Values were obtained using an Analysis of Covariance (ANCOVA) test with preapplication value as a covariate.
Abbreviations: IL, interleukin; LIF, Leukemia inhibitory factor; UC-MSC, umbilical cord mesenchymal stromal cell; VEGF, vascular endothelial growth factor.

Critically ill patients with COVID-19 often have high systemic procoagulants and are at risk of Disseminated Intravascular Coagulation (DIC) and thromboembolism. Tang et al. stated that 71% of patients who died of critical-grade COVID-19 met the diagnostic criteria for DIC.[14] Administration of MSCs via the intravenous route is controversial because MSCs with high levels of tissue factor (TF)/CD142 have a high risk of causing a patient to fall into a hypercoagulation state.[15]

Adipose tissue-derived MSCs are the MSC source that expresses TF/CD142 the most, and Perlee et al. showed an increase in

thrombin-antithrombin-complex and D-dimer after administration of 4×10^6 cells/kg body weight AT-MSCs; thus, an application dose less than 4×10^6 cells/kg body weight is recommended.[16] Not analyzing the TF/CD142, we used UC-MSCs with 1×10^6/kg body weight dose. Supported with no significant differences in the D-dimer between two groups at baseline and after application, the use of MSCs in this study is not associated with hypercoagulation.[17]

Our study revealed that there was a decreased trend of IL-6 in the MSCs group and an increasing trend of IL-10 with no significant difference. Zheng et al. and Meng et al. also showed a decreasing trend of IL-6 after MSC application for moderate to severe COVID-19 with no statistically significant value.[18,19] Consistently, Leng et al. reported an increase of IL-10 in the MSCs group compared with the placebo group with a significant value ($P < .05$).

Having the ability to secrete paracrine factors that suppress IL-6 serum level, MSCs evoked the tolerance state by which proinflammatory cytokine subsides.[20] Indeed, our result showed IL-10 increment on day 7 after MSC application in comparison to baseline. Released by MSCs, IL-10 activated T-lymphocyte suppressor or regulator and also play roles in the healing of the organ tissue.[21-24]

Increased expression of LIF in our study was shown in 80% of recovered patients who were treated with MSCs. Similarly, Leng et al. also reported $10\times$ RNA analysis of transplanted MSCs, which was highly expressed in LIF. As one of the cytokine family that is still less explored for use in lung injury in COVID-19, LIF possesses the capability to repair and regenerate through stem cell niches of type II alveolar epithelial cells.[6,25] LIF also plays a role in controlling excessive inflammatory cascade, in which the increase has an inhibitory effect on overactive T-lymphocyte populations CXCR3+CD4+, CX-CR3 +CD8+, and CX-CR+CD56 cells that play roles in cytokine storms.[26,27]

We found that there was a suppression of the population of CD8-CXCR3 and CD56-CXCR3 after the MSC application. A decrease in the population of these immune cells in the peripheral blood circulation indicates a subsidence of cytokine storms and progression toward clinical improvement.[28,29] However, our analysis did not show a suppression of the population of CD4-CXCR3, but the increment in the MSCs group on day 7 is showing a flatter trend when compared with the control group. The increment pattern of CD4-CXCR3 after MSC application indicates the proliferation of these Th1 populations. MSCs have been indicated to regulate the balance between proinflammatory (Th1) and anti-inflammatory(Th$_2$), and a shift toward Th$_2$ phenotype is the target of MSC application.[30-32]

We also studied VEGF, an angiogenic factor that is essential in the recovery of the damaged lung. Whereas VEGF tended to go down in the control group, MSCs gave a boost to increase VEGF in the circulation so that regeneration of capillary at the lung could occur.[23] Similarly, Leng et al. also demonstrated the same result where VEGF is increased significantly after the application of MSCs ($P = .0556$). MSCs are well known to secrete keratinocyte growth factor, VEGF, and hepatocyte growth factor, which play a role in regenerating lung type II alveolar cells, preventing apoptosis of pulmonary capillary endothelial cells, and improving air–alveolar barrier repair in ARDS.[33] Thus, MSCs act not only as an immunomodulator but also to regenerate and to repair the damaged lung tissue in COVID-19 pneumonia.[5] As evidenced by Shu et al. in their study, it was proved that administering MSCs to patients with critical-grade COVID-19 gave better results in terms of Computed Tomography (CT) scores, the number of lobes involved, and the ground-glass opacity image, which represented a reduced lung inflammation.[7]

This study has several limitations. First, the limited subject in this study hold up to acquire a statistically significant result. Second, we did not perform further supplementary tests to check for endotheliitis. Another limitation is that we did not apply exact criteria about how long the critically ill patient with COVID-19 had been treated in the ICU.

5 | CONCLUSION

Application of intravenous infusion MSCs as an adjuvant treatment for critically ill patients with COVID-19 increases the survival rate by modulating the immune system toward an anti-inflammatory state.

CONFLICT OF INTEREST

D.A. declared institutional funding from Ministry of Research, Technology and Higher Education, Indonesia. The other authors declared no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

I.H.D.: conception and design, provision of study material, data analysis and interpretation, manuscript writing, final approval of manuscript; D.A., A.S., E.B., T.D., P.A.S.: conception and design, provision of patients, collection of data, manuscript writing; N.M.: conception and design, administrative support, collection of data; R.D.A. and I.K.L.: provision of study material, data analysis and interpretation, manuscript writing, final approval of manuscript; T. Kispa, F.M., N.N., E.L.: provision of study material, data interpretation; T. Kurniawati and A.M.T.L.: conception and design, administrative support; D.R.: conception and design, administrative support, assembly of data, data analysis, manuscript writing.

DATA AVAILABILITY STATEMENT

The data contained in this study will be accessible with the publication of this article. Data can be accessed by other researchers who is doing

a related research has received permission to request data. Data can be requested by directly emailing the corrseponding author.

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REFERENCES

1. Kaye RJ. Overview of stem cell therapy for acute respiratory distress syndrome with focus on COVID 19. *Pain Physician*. 2020;23(4S): 421-432.
2. Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med*. 2020;8:420-422.
3. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395: 497-506.
4. Yessayan L, Szamosfalvi B, Napolitano L, et al. Treatment of cytokine storm in COVID-19 patients with immunomodulatory therapy. *ASAIO J*. 2020;66:1079-1083.
5. Esquivel D, Mishra R, Soni P, Seetharaman R, Mahmood A, Srivastava A. Stem cells therapy as a possible therapeutic option in treating COVID-19 patients. *Stem Cell Rev Rep*. 2021;17:144-152.
6. Leng Z, Zhu R, Hou W, et al. Transplantation of ACE2-mesenchymal stem cells improves the outcome of patients with COVID-19 pneumonia. *Aging Dis*. 2020;11:216-228.
7. Shu L, Niu C, Li R, et al. Treatment of severe COVID-19 with human umbilical cord mesenchymal stem cells. *Stem Cell Res Ther*. 2020;11: 361-371.
8. Liang S, Jiao HL, Chi LK, et al. Human umbilical cord mesenchymal stem cells inhibit glioma growth. *Chinese J Tissue Eng Res*. 2013;16: 9179-9185.
9. Mediana D, Liem IK, Pawitan JA, Goei N. Passage effect on aging of human umbilical cord derived mesenchymal stem cell. *Online J Biol Sci*. 2015;15:170-177.
10. Gavriilaki E, Anyfanti P, Gavriilaki M, Lazaridis A, Douma S, Gkaliagkousi E. Endothelial dysfunction in COVID-19: lessons learned from coronaviruses. *Curr Hypertens Rep*. 2020;22:63.
11. Kumar A, Arora A, Sharma P, et al. Is diabetes mellitus associated with mortality and severity of COVID-19? A meta-analysis. *Diabetes Metab Syndr Clin Res Rev*. 2020;14:535-545.
12. De Almeida-Pititto B, Dualib PM, Zajdenverg L, et al. Severity and mortality of COVID 19 in patients with diabetes, hypertension and cardiovascular disease: a meta-analysis. *Diabetol Metab Syndr*. 2020;12:1-12.
13. Roberts J, Pritchard AL, Treweeke AT, et al. Why is COVID-19 more severe in patients with diabetes? The role of angiotensin-converting enzyme 2, endothelial dysfunction and the immunoinflammatory system. *Front Cardiovasc Med*. 2021;7:629933.
14. Tang N, Bai H, Chen X, Gong J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J Thromb Haemost*. 2020;18: 1094-1099.
15. 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.
16. Perlee D, de Vos AF, Scicluna BP, et al. Human adipose-derived mesenchymal stem cells modify lung immunity and improve antibacterial defense in pneumosepsis caused by *Klebsiella pneumoniae*. *Stem Cells Translational Medicine*. 2019;8:785-796.
17. 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;24:420.
18. Zheng G, Huang L, Tong H, et al. Treatment of acute respiratory distress syndrome with allogeneic adipose-derived mesenchymal stem cells: a randomized, placebo-controlled pilot study. *Respir Res*. 2014;15:39.
19. Meng F, Xu R, Wang S, et al. Human umbilical cord-derived mesenchymal stem cell therapy in patients with COVID-19: a phase 1 clinical trial. *Signal Transduct Target Ther*. 2020;5:172.
20. Rajarshi K, Chatterjee A, Ray S. Combating COVID-19 with mesenchymal stem cell therapy. *Biotechnol Rep (Amst)*. 2020;26:e00467.
21. Ng THS, Britton GJ, Hill EV, et al. Regulation of adaptive immunity; the role of interleukin-10. *Front Immunol*. 2013;4:129.
22. Rojas JM, Avia M, Martín V, et al. IL-10: a multifunctional cytokine in viral infections. *J Immunol Res*. 2017;2017:6104054.
23. Costela-Ruiz VJ, Illescas-Montes R, Puerta-Puerta JM, Ruiz C, Melguizo-Rodríguez L. SARS-CoV-2 infection: The role of cytokines in COVID-19 disease. *Cytokine & Growth Factor Reviews*. 2020;54:62-75. https://doi.org/10.1016/j.cytogfr.2020.06.001.
24. Dienz O, Rud JG, Eaton SM, et al. Essential role of IL-6 in protection against H1N1 influenza virus by promoting neutrophil survival in the lung. *Mucosal Immunol*. 2012;5:258-266.
25. Metcalfe SM. COVID-19 lockdown: de-risking exit by protecting the lung with leukaemia inhibitory factor (LIF). *Med Drug Discov*. 2020;6:100043.
26. Weiss ARR, Dahlke MH. Immunomodulation by mesenchymal stem cells (MSCs): mechanisms of action of living, apoptotic, and dead MSCs. *Front Immunol*. 2019;10:1191.
27. Kavianpour M, Saleh M, Verdi J. The role of mesenchymal stromal cells in immune modulation of COVID-19: focus on cytokine storm. *Stem Cell Res Ther*. 2020;11:404.
28. Bhardwaj S, Ahmad F, Wedemeyer H, et al. Increased CD56bright NK cells in HIV-HCV co-infection and HCV mono-infection are associated with distinctive alterations of their phenotype. *Virol J*. 2016;13:67.
29. Sengupta V, Sengupta S, Lazo A, Woods P, Nolan A, Bremer N. Exosomes derived from bone marrow mesenchymal stem cells as treatment for severe COVID-19. *Stem Cells Dev*. 2020;29:747-754.
30. Gustine JN, Jones D. Immunopathology of hyperinflammation in COVID-19. *Am J Pathol*. 2021;191:4-17.
31. Pontes Ferreira C, Moro Cariste L, Henrique Noronha I, et al. CXCR3 chemokine receptor contributes to specific CD8+ T cell activation by pDC during infection with intracellular pathogens. *PLoS Negl Trop Dis*. 2020;14:e0008414.
32. Yu X, Wang M, Cao Z. Reduced CD4+T cell CXCR3 expression in patients with allergic rhinitis. *Front Immunol*. 2020;11:581180.
33. Durand N, Mallea J, Zubair AC. Insights into the use of mesenchymal stem cells in COVID-19 mediated acute respiratory failure. *NPJ Regen Med*. 2020;5:17.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.