ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

DOI: 10.5336/medsci.2021-85395

Mesenchymal Stem Cell Therapy in COVID-19 Pneumonia: A Prospective, Randomized Clinical Research

COVID-19 Pnömonisinde Mezenkimal Kök Hücre Tedavisi: Prospektif, Randomize Klinik Araştırma

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ABSTRACT Objective: Deficiencies in immune-regulatory mechanisms such as immune activation and T-regulatory cells are classically referred to as cytokine storms. Mesenchymal stem cells (MSCs) act as living anti-inflammatory cells that can rebalance cytokine/immune responses to restore balance in patients with coronavirus disease-2019 (COVID-19) acute respiratory distress syndrome by reducing the activation of T and B cells, and dendritic and natural killer cells. The aim of this study is to provide immune modulation with stem cell transplantation by reducing the damage caused and COVID-19 infection to tissues and organs. **Material and Methods:** In this prospective randomized single-center clinical trial, patients were divided into 3 groups: intubated without comorbidity (n=7); intubated with comorbidity (n=7); not intubated (n=7). Dosage of MSCs transplantation for each group was 1 million cell/kg intravenous at days 0, 2, and 4. age, gender, APACHE II scores, procalcitonin, C-reactive protein (CRP) and leukocyte values, and cluster of difference 4 (CD4), CD8, interleukin 2 (IL-2), and IL-6 levels, morbidities, number of days in intensive care unit, mortality were recorded. Clinical results, changes in inflammatory and immune function levels, and side effects were evaluated. Each patient's improvement in oxygenation and symptoms were recorded in the days after MSC transplantation. After treatment, lymphocyte, CRP, tumor necrosis factor-α level, and IL-6 levels were recorded. **Results:** There was no statistically significant difference between the three groups in terms of stem cell therapy patients' age and exit/discharge days and mortality. Patients who were in the group of "intubated, no comorbidity" had significantly higher CD19 and CD19+HLA-DR+ values compared with patients in the groups of "intubated with comorbidity" and "not intubated." CRP, FiO2, IL-6 and SpO2 values were significantly different across periods. **Conclusion:** Our study may found a useful effect of MSCs on severe COVID-19 pneumonia and showed reversal of hypoxia and downregulation of cytokine storm in patients with severe COVID-19 following 3 intravenous doses with no adverse effects assignable to the treatment.

Keywords: Mesenchymal stem cell; COVID-19; pneumonia

ÖZET Amaç: Mezenkimal kök hücreler [mesenchymal stem cell (MSC)], T ve B hücrelerinin, dendritik ve doğal öldürücü hücrelerin aktivasyonunu azaltarak, koronavirüs hastalığı-2019 [coronavirus disease-2019 (COVID-19)] sitokin/bağışıklık tepkilerini yeniden dengeleyebilen canlı bir antiinflamatuar görevi görür. Bu çalışmanın amacı, kök hücre nakli ile immün modülasyonu sağlayarak, COVID-19 enfeksiyonun doku ve organlara verdiği zararı azaltmaktır. **Gereç ve Yöntemler:** Bu prospektif randomize tek merkezli klinik araştırmada hastalar 3 gruba ayrıldı: Komorbiditesi olmayan entübe (n=7); komorbidite ile entübe (n=7); entübe edilmemiş (n=7). Her grup için MSC transplantasyonunun dozu 0, 2 ve 4. günlerde 1 milyon hücre/kg intravenöz olarak verildi. Yaş, cinsiyet, APACHE II skoru, prokalsitonin, C-reaktif protein (CRP) ve lökosit değerleri, farklılaşma kümesi 4 [cluster of difference 4 (CD4)], CD8, interlökin 2 (IL-2), IL-6 seviyeleri, morbiditeler, yoğun bakımda geçen gün sayısı, mortalite kaydedildi. Klinik sonuçlar, inflamatuar ve immün fonksiyon düzeylerindeki değişiklikler ve yan etkiler değerlendirildi. MSC transplantasyonundan sonraki günlerde hastanın oksijenizasyonundaki iyileşme ve semptomları kaydedildi. Tedaviden sonra lenfosit, CRP, tümör nekrozis faktör-α düzeyi, IL-6 düzeyleri kaydedildi. **Bulgular:** Kök hücre tedavisi uygulanan hastaların yaşı, çıkış/taburcu olma günleri ve mortalite açısından 3 grup arasında istatistiksel olarak anlamlı fark yoktu. Sonuçlara göre entübe komorbidite yok grubunda yer alan hastaların CD19 ve CD19+HLA-DR+ değerleri, entübe ek hastalık eşlik eden ve entübe olmayan hastalara göre anlamlı olarak daha yüksek bulundu. CRP, FiO2, IL-6 ve SpO2 değerleri, zaman periyotları arasında önemli ölçüde farklılık gösterdi. **Sonuç:** Bu çalışma, MSC'lerin şiddetli COVID-19 pnömonisinde tedaviyle ilişkili hiçbir yan etki olmaksızın, şiddetli COVID-19'lu hastalarda 3 intravenöz dozun ardından hipoksinin tersine çevrildiğini ve sitokin fırtınasının olumsuz etkilerinin azaldığını gösterebilir.

Anahtar Kelimeler: Mezenkimal kök hücre; COVID-19; pnömoni

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Peer review under responsibility of Turkiye Klinikleri Journal of Medical Sciences.

Received: 08 Jul 2021 ***Received in revised form:*** 27 Oct 2021 ***Accepted:*** 13 Dec 2021 ***Available online:*** 21 Dec 2021

Mesenchymal stem cells (MSCs) have received special attention for their ability to prevent inflammation and cytokine storms, as demonstrated in various investigations.[1,2] MSC therapy in the early stages of acute respiratory distress syndrome (ARDS) has been studied and has correlated with improvements in the lung microenvironment in overactive inhibition of the immune system, tissue repair, protection of lung alveolar epithelial cells, pulmonary fibrosis, and the prevention of long-term preservation of respiratory function.

MSCs are immune evasive and immune modulatory and are known to function via several mechanisms relevant to acute lung injury. When administered intravenously, they sequester in the lung. MSCs' specific mechanisms of therapeutic action are fighting inflammation, inhibiting lung fibrosis, regenerating lung tissue, inhibiting the apoptosis of injured cells, clearing alveolar fluid, and producing extracellular vesicles. MSCs also secrete molecules that are antibacterial, antiviral, and analgesic.[3] After MSC injection, cytokine-secreting immune cells, including natural killer cells, disappeared and the level of proinflammatory cytokine tumor necrosis factor-α (TNF-α) decreased.[4]

MSCs can weaken cytokine storms through paracrine secretion of various anti-inflammatory cytokines, including transforming growth factor beta 1, interleukin 4 (IL-4), IL-6, IL-10, and IL-1, which reduce immunocompetent overactivation cells, thus regulating the inflammatory response. Intravenously transplanted MSCs are held by organs containing large capillary beds, including the lungs. MSCs deposited in the lung can improve the pulmonary microenvironment, protect alveolar epithelial cells, prevent the dysfunction of capillary endothelial cells, and help to improve lung function by preventing pulmonary fibrosis.[5,6] Due to the cytokine storm that develops as a result of coronavirus disease-2019 (COVID-19) infection, some patients are hospitalized in the intensive care unit for pneumonia, ARDS, and multiple organ failure. Mortality is higher in treatment-resistant cases.

The purpose of this study is to provide immune modulation by stem cell transplantation and to reduce the damage caused by cytokine storms to tissues and organs, and to correct immunosuppression and to fight against the COVID-19 virus by editing B and T cells. The purpose is also to accelerate the healing of organ damage by increasing growth factors through MSCs.

The patients were observed after MSC infusion, and clinical symptoms, laboratory tests, and radiological results were recorded and confirmed by experienced physicians. The primary clinical outcomes included the incidence of progression from severe to critical illness and the time to a clinical improvement of 2 points on a seven-category ordinal scale that has been used widely in clinical symptom assessment and discharge from hospitals. The secondary clinical outcomes included patient status at days 7 and 14 assessed with a seven-category ordinal scale, hospital stay, changes in oxygenation index, hematological inflammatory factors, and imaging.

MATERIAL AND METHODS

STUDY DESIGN AND PARTICIPANTS

This study was a single-center open-label, randomized trial conducted at Health Science University, Kanuni Sultan Suleyman Education and Training Hospital, Department of Anesthesiology and Reanimation in İstanbul from May to July 2020, and it was performed according to the Declaration of Helsinki and approved by the Kanuni Sultan Suleyman Hospital Ethical Committee (No: KAEK/2020.05.20) and Ministry of Health, Scientific Research Studies (No: 2020-05-07T15_39_24). Written informed consent was obtained from all patients or their representatives when data were collected prospectively (NCT04713878). All patients or their relatives signed the consent form for stem cell infusion.

Patients were divided into 3 groups (Figure 1):

Group 1: Intubated without comorbidity (n=7),

Group 2: Intubated with comorbidity (n=7),

Group 3: No intubated (n=7).

Dosage of MSCs infusion for each group was 1 million cell/kg intravenous at days 0, 2, and 4.

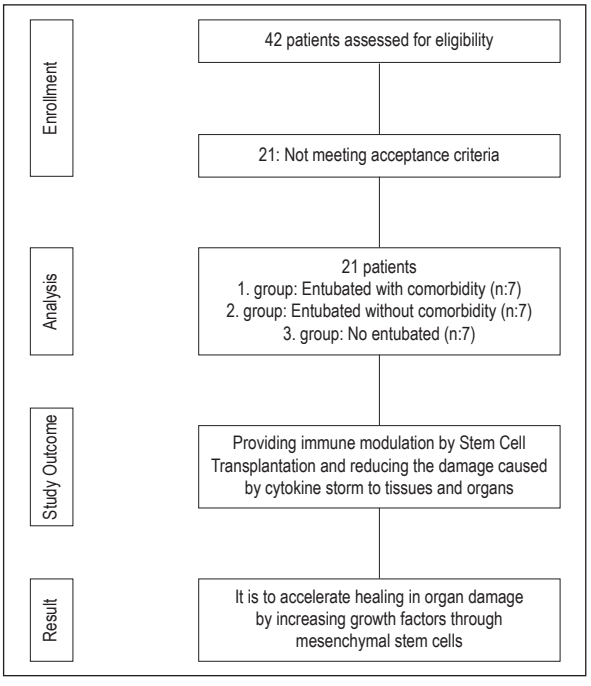

FIGURE 1: Flow diagram of the study.

Inclusion criteria included the following: aged 18-80, male or female, positive Real Time-Polymerase Chain Reaction (RT-PCR) test with 2019-nCoV infection, or pneumonia assessed by chest radiography or computed tomography, impairment in oxygenation criteria (respiratory rate ≥30 beats/min, oxygen saturation ≤93%, PaO_2/FiO_2 ≤300 mmHg, pulmonary imaging of focus within 24-48 hours >50% progression). Patients had received invasive or noninvasive mechanical ventilation therapy in intensive care unit. Exclusion criteria included pregnancy, any kind of cancer, severe liver disease, failure to provide informed consent or follow test requirements, known allergy, or hypersensitivity to MSCs.

Age, gender, mortality, APACHE II score, number of days in the intensive care unit, procalcitonin and C-reactive protein (CRP) values, morbidities (such as diabetes mellitus, hypertension, renal failure, asthma) and cluster of difference 4 (CD4), CD8, CD19, CD45, HLA-DR, IL-2, and IL-6 levels were recorded. Clinical results, changes in inflammatory and immune function levels, and side effects were evaluated. The patients' improvement in oxygenation and symptoms were recorded in the days after MSC transplantation (at days 0, 2, and 4). After treatment, lymphocyte, CRP, TNF-α level, and IL-6 levels were recorded.

STATISTICAL ANALYSIS

Cluster of Difference Values and Clinical Behavior

Patients who received stem cell therapy were divided into 3 groups (intubated without comorbidity, intubated with comorbidity, and not intubated) and had the descriptive characteristics in terms of CD values shown in Table 1 (mean±standard deviation) according to the dosage of MSCs. Four different time periods were handled: "Time 0" (before MSC infusion), "Time 1" (after 1st infusion), "Time 2" (after 2nd infusion), and "Time 3" (after 3rd infusion). A linear mixed-effects model was used to determine whether group membership and time affected blood laboratory values differently. Comparisons of demographic profiles between the groups of 21 stem cell therapy patients were made using the Kruskal-Wallis test for age and number of days before exit/discharge. In addition, comparisons between the groups for gender and discharge status were made using a χ^2 test with a significance level set at 0.05.

The clinical behaviors of the patients who received stem cell therapy were divided into three groups (intubated without comorbidity, intubated with comorbidity, and not intubated), and the laboratory values were recorded for 6 different time periods: "Time 0" (before MSC infusion), "Time 1" (after 1st infusion), "Time 2" (after 2nd infusion), "Time 3" (after 3rd infusion), "Time 4" (extubation time), and "Time 5" (discharge). A linear mixed-effects model was used to determine whether group membership and time affected blood laboratory values differently.

RESULTS

Table 1 shows the descriptive statistics in terms of age, gender, discharge status, and number of days before exit/discharge of the 3 groups of patients (intubated without comorbidity, intubated with comorbidity, and not intubated). The results showed that there is no statistically significant difference between the 3 groups in terms of stem cell therapy patients' age (p=0.083), patients' exit/discharge days (p=0.070), and patients' discharge status (χ^2=5.571,

TABLE 1: Demographic characteristics of the stem cell therapy patients.

	Groups			
Characteristics	**Intubated/no comorbity**	**Intubated/with comorbity**	**No intubated**	**p-value**
Age (mean±std.dev.)	(48±10.8781)	(62.2857±8.2606)	(56.8571±13.2718)	0.083
Days (mean±std.dev.)	(16.7143±7.1813)	(27.2857±7.4546)	(20±8.3066)	0.070
Gender N(%)				
Female	1(33.3%)	1(33.3%)	1(33.3%)	<0.00*
Male	6(33.3%)	6(33.3%)	6(33.3%)	
Discharge status N(%)				
Discharge	3 (42.9%)	4(57.1%)	7(100%)	0.062
Dead	4 (57.1%)	3(42.9%)	0(0.0%)	

*p<0.05.

df=2, p=0.062). However, it was shown that the majority of the patients were males in all 3 groups of stem cell therapy patients (p<0.00).

CLUSTER OF DIFFERENCE RESULTS

Table 2 shows the descriptive characteristics (mean±standard deviation) of CD results according to the dosage of MSCs. The estimation results showed that CD3, CD3+CD4+CD8+, CD3+CD4, CD3+CD8+, CD3+CD4-CD8-, CD3-16/56, CD4/CD8, CD19-HLA-DR+ and natural killer T values have no group or time effects (Table 2). The changes between groups and time periods were not statistically significant at 95% confidence intervals.

Table 3 shows that CD19 and CD19+HLA-DR+ values were significantly different across groups. According to the estimation results, patients who were in the "intubated without comorbidity" group had significantly higher CD19 and CD19+HLA-DR+ values compared with patients in the groups "intubated with comorbidity" and "not intubated" groups.

CLINICAL INFECTION OF BLOOD LABORATORY VALUES

Table 4 shows the estimation results, and it was observed that D-dimer and procalcitonin values had no group or time effects, indicating that the changes in these blood laboratory values remained the same across time periods and groups.

Table 5 indicates that CRP, FiO_2, IL-6, and SpO_2 values were significantly different across time periods. The changes in SpO_2 were reflected clinically positively on the patients.

The majority of stem cell therapy patients were males (χ^2=5.459, df=1, p=0.027). The discharge status of patients who received stem cell therapy was the same as that of those who did not (χ^2=1.556, df=1, p=0.212). In nontherapy patients, 10 (47.6%) were discharged and 11 (52.4%) died. In stem cell therapy patients, 14 (66.7%) were discharged, and 7 (33.3%; p=0.212) died.

A reduction of more than 50% opacity in radiological imaging was accepted as improvement. After MSCs infusion, clinically significant improvement was observed in chest X-rays and tomography.

DISCUSSION

This prospective study demonstrated that the human umbilical cord-derived MSC product can be administered safely through intravenous infusion. When the literature on the subject was investigated, it was seen that all applications were done 3 times on consecutive days and the present study was planned in that way.[7]

In the present study, there was no statistically significant difference between mortality but no mortality was observed in non-intubated group. The sample was small in order to obtain meaningful results

TABLE 2: Blood values (cluster of differences) with no group and time effects.

Value					Value				
Predictors	**Estimates**	**CI**	**p value**	**ANOVA (p-value)**	**Predictors**	**Estimates**	**CI**	**p value**	**ANOVA (p-value)**
CD3					**CD3+CD4-CD8-**				
(Intercept)	69.98	62.59-77.37	<0.001		(Intercept)	1.56	-0.14-3.26	0.072	
Time 1	-1.42	-4.05-1.21	0.284	0.7337	Time 1	0.26	-0.64-1.16	0.569	0.4325
Time 2	-1.09	-3.71-1.54	0.412		Time 2	0.24	-0.66-1.14	0.598	
Time 3	-0.74	-3.37-1.88	0.574		Time 3	0.73	-0.17-1.63	0.109	
Group 2	3.67	-7.05-14.38	0.481	0.2121	Group 2	1.93	-0.46-4.32	0.108	0.2571
Group 3	9.31	-1.40-20.02	0.084		Group 3	0.71	-1.68-3.10	0.541	
CD3+CD4					**CD3+CD8+**				
(Intercept)	46.35	38.31-54.38	<0.001		(Intercept)	21.43	15.75-27.10	<0.001	
Time 1	1.50	-1.84-4.84	0.374	0.5093	Time 1	0.26	-2.14-2.66	0.831	0.7337
Time 2	2.48	-0.86-5.82	0.143		Time 2	-1.12	-3.52-1.28	0.355	
Time 3	0.89	-2.45-4.23	0.598		Time 3	0.22	-2.18-2.62	0.853	
Group 2	-6.26	-17.81-5.28	0.269	0.1932	Group 2	8.30	0.15-16.44	0.05	0.2121
Group 3	4.10	-7.45-15.65	0.465		Group 3	4.72	-3.42-12.87	0.239	
CD3+CD4+CD8+					**CD3-16/56**				
(Intercept)	2.19	0.25-4.13	0.028		(Intercept)	10.35	4.22-16.48	0.001	
Time 1	0.38	-0.11-0.87	0.127	0.4931	Time 1	1.56	-0.51-3.63	0.137	0.4102
Time 2	0.23	-0.26-0.72	0.357		Time 2	0.09	-1.98-2.16	0.931	
Time 3	0.20	-0.29-0.69	0.425		Time 3	0.33	-1.74-2.40	0.752	
Group 2	-0.54	-3.38-2.31	0.696	0.6966	Group 2	3.29	-5.62-12.19	0.448	0.7213
Group 3	-1.16	-4.01-1.68	0.402		Group 3	0.71	-8.19-9.61	0.869	
CD4/CD8					**CD19-HLA-DR+**				
(Intercept)	2.51	0.83-4.19	0.004	<0.0001	(Intercept)	2.51	0.83-4.19	0.004	
Time 1	0.19	-0.78-1.16	0.695	0.9137	Time 1	0.19	-0.78-1.16	0.695	0.9137
Time 2	-0.00	-0.97-0.96	0.992		Time 2	-0.00	-0.97-0.96	0.992	
Time 3	-0.16	-1.12-0.81	0.746		Time 3	-0.16	-1.12-0.81	0.746	
Group 2	1.80	-0.54-4.13	0.124	0.2777	Group 2	1.80	-0.54-4.13	0.124	0.2777
Group 3	1.27	-1.07-3.61	0.269		Group 3	1.27	-1.07-3.61	0.269	
NKT									
(Intercept)	1.62	0.41-2.83	0.010						
Time 1	0.50	-0.13-1.13	0.118	0.2397					
Time 2	-0.10	-0.73-0.53	0.752						
Time 3	0.03	-0.60-0.66	0.928						
Group 2	0.26	-1.44-1.96	0.751	0.6740					
Group 3	-0.46	-2.16-1.24	0.579						

The changes between groups and time periods were not statistically significant at 95% confidence intervals.

CD: Cluster of differences; CI: Confidence interval; ANOVA: Analysis of variance; NKT: Natural killer T.

for nonparametric statistics. No statistically significant difference could be obtained due to the small sample size. We can only express it numerically. Mortality rates were estimated as high in patients requiring invasive oxygen support. Our findings suggest that MSCs may be a preventative measure against progress to invasive oxygen support and mechanical ventilation.[8] In our study, we added patients with severe pneumonia to our study group to show the effect in intubated and non-intubated patients. Xiao et al. found similar results with mortality but they demonstrate that MSCs have obvious therapeutic effects, such as promoting the repair of epithelial and endothelial tissues, clearance of alveolar fluid and microorganisms, and anti-inflammatory and antiapoptotic effects.[4] In that clinical study, which included elderly patients, MSC transplantation apparently improved the outcomes of all patients after

TABLE 3: Blood values (cluster of differences) with group effects.

Value					
CD19					
Predictors	**Estimates**	**CI**	**p value**		
(Intercept)	22.60	17.74-27.46	**<0.001**		
Time 1	-1.57	-3.34-0.20	0.081		
Time 2	-0.29	-2.06-1.47	0.741		
Time 3	0.78	-0.99-2.54	0.382		
Group 2	-9.09	(-15.90)-(-2.28)	**0.012** *		
Group 3	-14.99	(-21.65)-(-8.33)	**<0.001****		
σ^2	3.60				
Marginal R^2	0.916				
				CD19 Changes in Groups	CD19 Changes in Time
Post hoc (Tukey)	Significant difference between "Intubated-with comorbity" and "Intubated-no comorbity" (p=0.0139'). Significant difference between "No intubated" and "Intubated-no comorbity" (p<0.0001***).				
CD19+HLADR+					
Predictors	**Estimates**	**CI**	**p value**		
(Intercept)	20.46	15.15-25.76	**<0.001**		
Time 1	-0.60	-3.88-2.68	0.716		
Time 2	1.58	-1.70-4.86	0.339		
Time 3	2.00	-1.28-5.28	0.228		
Group 2	-8.95	(-16.24)-(-1.66)	**0.019** *		
Group 3	-14.21	(-21.50)-(-6.92)	**0.001****		
σ^2	28.27				
Marginal R^2	35.10				
				CD19+HLADR+ Changes in Groups	CD19+HLADR+ Changes in Time
Post hoc (Tukey)	Significant difference between "Intubated-with comorbity" and "Intubated-no comorbity" (p=0.0269'). Significant difference between "No intubated" and "Intubated-no comorbity" (p<0.001***).				

Table 3 shows that CD19 and CD19+HLA-DR+ values were significantly different across groups.
CD: Cluster of differences; CI: Confidence interval.

TABLE 4: Blood values (clinical infection) with no group and time effects.

Value					Value				
D-Dimer					Procalcitonin				
Predictors	**Estimates**	**CI**	**p value**	**ANOVA (p value)**	**Predictors**	**Estimates**	**CI**	**p value**	**ANOVA (p value)**
(Intercept)	1.90	-9.47-13.27	0.741		(Intercept)	21.02	-0.27-42.31	0.053	
Time 1	8.39	-5.45-22.23	0.232	0.3484	Time 1	-22.49	-48.49-3.50	0.089	0.3995
Time 2	4.04	-9.80-17.88	0.564		Time 2	-22.98	-48.98-3.01	0.082	
Time 3	1.22	-12.62-15.06	0.862		Time 3	-23.24	-49.23-2.76	0.079	
Time 4	0.14	-13.70-13.98	0.984		Time 4	-23.47	-49.46-2.52	0.076	
Time 5	13.00	-0.84-26.84	0.065		Time 5	-23.36	-49.35-2.64	0.078	
Group 2	-0.76	-11.29-9.77	0.881	0.8868	Group 2	-1.02	-20.65-18.62	0.915	0.4622
Group 3	1.65	-8.88-12.18	0.746		Group 3	9.73	-9.91-29.36	0.312	

Table 4 shows the estimation results, that indicating that the changes in these blood laboratory values remained the same across time periods and groups.
CI: Confidence interval; ANOVA: Analysis of variance.

MSC injection without adverse effects; patient pulmonary function and symptoms improved within 2 days after MSC injections, and cytokine-secreting immune cells disappeared within 1 week in patients with severe ARDS.[4]

There is an urgent need for reliable and effective treatment for COVID-19 patients, especially critically ill patients. Considering that drug development studies will take a long time to conclude, methods that can be effective and reliable in the treatment of the disease gain importance. Therefore, MSCs are recommended as a safe and effective therapeutic approach for COVID-19 patients, especially for critically ill patients. All patients were administered

TABLE 5: Blood values (clinical behavior) with time effects.

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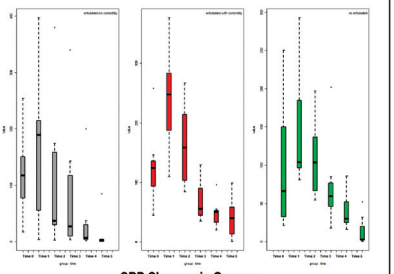
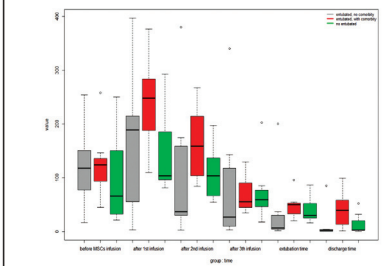
CRP

Predictors	Estimates	CI	p value
(Intercept)	112.60	64.99-160.21	**<0.001**
Time 1	67.26	17.24-117.27	**0.009** **
Time 2	12.92	-27.97-53.80	0.532
Time 3	-37.72	(-75.06)-(-0.38)	**0.048** *
Time 4	-72.90	(-105.54)-(-40.25)	**<0.001** ***
Time 5	-92.95	(-128.36)-(-57.54)	**<0.001** *
Group 2	8.56	-43.34-60.46	0.733
Group 3	1.15	-50.75-53.05	0.963
σ^2	5686.91		
Marginal R^2	0.342		



CRP Changes in Groups

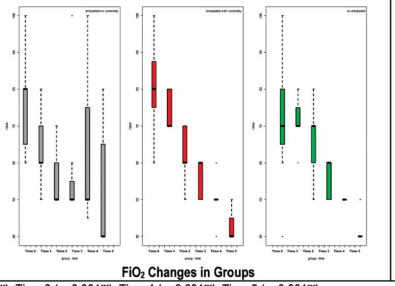


CRP Changes in Time

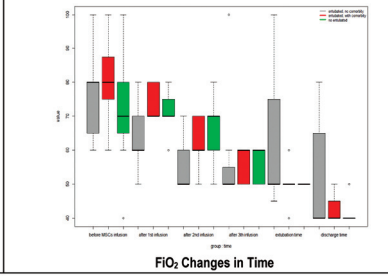
Post hoc (Tukey): Significant difference between Time 0 and; Time 4 (p<0.001***), Time 5 (p<0.001***).
Significant difference between Time 1 and; Time 3 (p<0.001***), Time 4 (p<0.001***), Time 5 (p<0.001***).
Significant difference between Time 2 and; Time 3 (p=0.01073') Time 4 (p<0.001***), Time 5 (p<0.001***).
Significant difference between Time 3 and; Time 4 (p=0.00141**), Time 5 (p<0.001***).
Significant difference between Time 4 and Time 5 (p=0.03572').

FiO_2

Predictors	Estimates	CI	p value
(Intercept)	74.35	67.03-81.66	**<0.001** ***
Time 1	-5.95	-13.12-1.22	0.103
Time 2	-14.52	(-21.74)-(-7.31)	**<0.001** ***
Time 3	-19.29	(-27.14)-(-11.43)	**<0.001** ***
Time 4	-21.43	(-30.77)-(-12.09)	**<0.001** ***
Time 5	-30.24	(-38.50)-(-21.97)	**<0.001** ***
Group 2	2.88	-3.12-8.89	0.326
Group 3	1.94	-4.07-7.94	0.507
σ^2	217.08		
Marginal R^2	0.320		



FiO_2 Changes in Groups

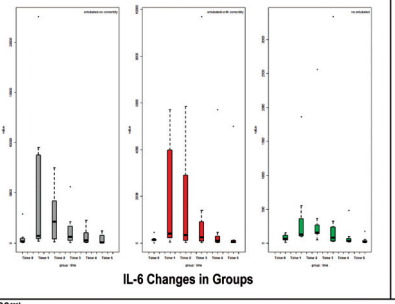


FiO_2 Changes in Time

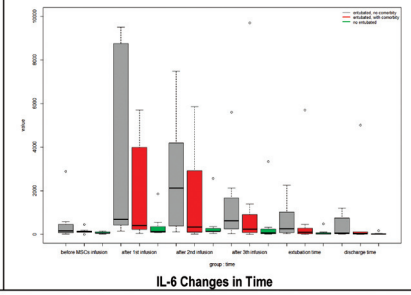
Post hoc (Tukey): Significant difference between Time 0 and; Time 2 (p<0.001***), Time 3 (p<0.001***), Time 4 (p<0.001***), Time 5 (p<0.001***).
Significant difference between Time 1 and; Time 2 (p=0.0037'), Time 3 (p<0.001***), Time 4 (p<0.001***), Time 5 (p<0.001***).
Significant difference between Time 2 and Time 5 (p<0.001***).
Significant difference between Time 5 and Time 3 (p=0.0214').

IL-6

Predictors	Estimates	CI	p value
(Intercept)	1079.81	-466.01-2625.62	0.169
Time 1	2547.10	1235.26-3858.94	**<0.001** ***
Time 2	1373.71	61.88-2685.55	**0.040** *
Time 3	961.76	-350.08-2273.59	0.149
Time 4	289.87	-1021.97-1601.70	0.662
Time 5	124.91	-1186.92-1436.75	0.851
Group 2	-733.05	-2669.79-1203.68	0.437
Group 3	-1673.59	-3610.33-263.14	0.086
σ^2	1507554.52		
Marginal R^2	0.157		



IL-6 Changes in Groups

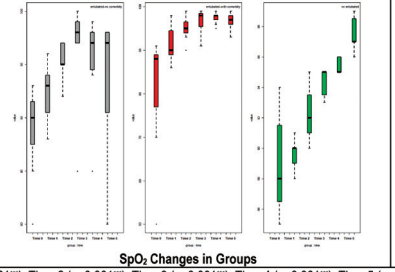


IL-6 Changes in Time

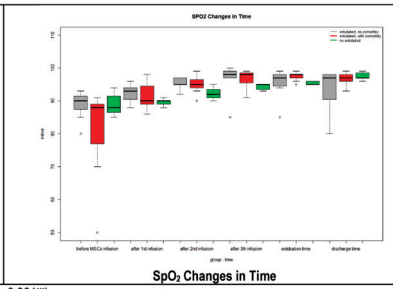
Post hoc (Tukey): Significant difference between Time 0 and; Time 1 (p=0.00163**).
Significant difference between Time 1 and; Time 4 (p=0.00831**), Time 5 (p=0.00343**).

SpO_2

Predictors	Estimates	CI	p value
(Intercept)	86.33	83.59-89.06	**<0.001** ***
Time 1	5.19	2.26-8.12	**0.001** **
Time 2	8.29	5.36-11.21	**<0.001** ***
Time 3	9.81	6.88-12.74	**<0.001** ***
Time 4	10.05	7.12-12.98	**<0.001** ***
Time 5	9.86	6.93-12.79	**<0.001** ***
Group 2	-0.62	-3.57-2.33	0.665
Group 3	-0.50	-3.45-2.45	0.726
σ^2	22.87		
Marginal R^2	0.369		



SpO_2 Changes in Groups



SpO_2 Changes in Time

Post hoc (Tukey): Significant difference between Time 0 and; Time 1 (p=0.00581**), Time 2 (p<0.001***), Time 3 (p<0.001***), Time 4 (p<0.001***), Time 5 (p<0.001***).
Significant difference between Time 1 and; Time 3 (p=0.02156*), Time 4 (p=0.0129**), Time 5 (p=0.01957**).

Table 5 indicates that CRP, FiO2, IL-6, and SpO2 values were significantly different across time periods.
CRP: C-reactive protein; CI: Confidence interval; IL: Interleukin.

MSCs without any infusion reaction in the present study. There were no adverse effects. Similarly, Sengupta et al. did not find any side effects in their patients who were given stem cells. They showed that profound reversal of hypoxia, immune reconstitution, and downregulation of cytokine storm in patients with severe COVID-19 following a single intravenous dose, with no adverse effects.[9]

There were no significant differences in age and hospitalized days between groups in this study. There was a significant difference between FiO_2 and SpO_2 across time periods and between groups. This matches the findings of a previous study, which found that treatment with allogeneic bone marrow MSCs was associated with an 83% survival rate and a significant improvement in oxygenation, as well as reducing oxygen support requirements within 48-72 h; an improved PaO_2/FiO_2 ratio greater than 200 mmHg by day 3 after treatment was strongly predictive of eventual hospital discharge and recovery.[9]

The underlying mechanisms of recovery after MSC infusion appear to be a result of strong anti-inflammatory activity. Such processes include an increased number of peripheral lymphocytes, a decrease in CRP, and a decrease in overactive cytokine-releasing immune cells (CD4+ T cells, CD8+ T cells, and natural killer cells).[3] In our study, when CD values were compared between the groups, an increase was observed in the group without comorbidity in CD19 and CD+HLA-DR+. It may be that an underlying but undetectable immune deficiency is what causes patients with comorbidities to be intubated.[10] Memory T cells can shape susceptibility and clinical severity to subsequent infections. In a study, CD4 and CD8 T cells, which recognize multiple regions of the N protein, were found in all individuals recovering from COVID-19.[11] In another study, Luo et al. showed that, IL-6 and CD8+ T cell counts are 2 reliable prognostic indicators that exactly stratify patients into risk categories and predict COVID-19 mortality.[12] Dong and colleagues report that convalescent patients with COVID-19 harbor functional memory CD4+ and CD8+ T cells that recognize multiple epitopes that span the viral proteome. CD4+ T cells predominated the memory response in patients with severe disease, whereas higher proportions of CD8+ T cells were found in patients with mild disease.[13] COVID-19 falls within the scope of septic syndromes in which immunosuppression is a major death factor. Sepsis-induced immunosuppression is most often identified and monitored by measuring decreased expression of HLA-DR molecules in circulating monocytes. In critically ill patients, results homogeneously indicate a decreased expression of HLA-DR, indicative of an immunosuppression state with profound lymphopenia and other functional changes.[14]

MSCs can regulate inflammation through a lot of mechanisms, including promoting the recruitment of CD4+CD25+T lymphocytes and CD8+CD28- T lymphocytes, inhibiting excessive proliferation and differentiation of B lymphocytes, the maturation of dendritic cells and promoting macrophages to anti-inflammatory phenotypic polarization. Therefore, the immunomodulation function of MSCs may be the key to reduce the occurrence of cytokine storm syndrome in COVID-19 patients. In addition, MSCs transplanted intravenously can quickly reach the lungs.[15] We observed the benefit of pre- and post-treatment MSC infusion in our patients in the lung X-ray films that given after 3 times.

In the present study, patients had no secondary infections, and when we compared D-dimer to procalcitonin, there was no difference across time periods between groups. This lack of difference shows the homogenous nature of the patients in the study. CRP differed significantly from IL-6 across time periods and between groups.

CONCLUSION

Many studies and reviews showed that, MSCs are safe agents that can be used without side effects.[16] The present study may have found a positively useful effect of MSCs on severe COVID-19 pneumonia patients. MSC therapy brings hope as a noninvasive treatment that is a very effective and easy (intravenous infusion) method for clinical practice; it is becoming more popular at the current critical moment due to the lack of effective approaches to the treatment of severe COVID-19. This study can demonstrate reversal of hypoxia and downregulation of cytokine storm in patients with severe COVID-19 following three intravenous doses with no adverse effects assignable to the treatment.

This single-center study is limited by the small size of the sample, which did not allow subgroups to be stratified. Also, because this is a preliminary comparative clinical study, the relevant mechanism needs further clarification.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Ayça Sultan Şahin, Ebru Kaya;* ***Design:*** *Ayça Sultan Şahin, Ebru Kaya;* ***Control/Supervision:*** *Ali Kocataş, Kemal Dolay;* ***Data Collection and/or Processing:*** *Ayça Sultan Şahin, Ebru Kaya;* ***Analysis and/or Interpretation:*** *Ayça Sultan Şahin, Ebru Kaya;* ***Literature Review:*** *Ali Kocataş, Kemal Dolay;* ***Writing the Article:*** *Ayça Sultan Şahin, Ebru Kaya;* ***Critical Review:*** *Ayça Sultan Şahin, Ebru Kaya;* ***References and Fundings:*** *Gürsel Turgut;* ***Materials:*** *Gürsel Turgut.*

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