Mesenchymal stem cells treatment in COVID-19 patient with multi-organ involvement

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ABSTRACT
The aim of this study is to evaluate the therapeutic effect of mesenchymal stem cells (MSCs) in a severe case of brain and multiple organ involvement in a patient with COVID-19. Here, a 51-year-old male patient with multi-organ involvement due to COVID-19 infection and developing cardiac arrest is presented. MSCs were transplanted to the patient four times systematically and once intrathecally. As a result, the application of MSCs has been found to have a healing effect on organs in this patient with severe COVID-19 infection. In addition, transplantation of MSCs both systematically and intrathecally is considered to be effective in the treatment of the central nervous system (Tab. 2, Fig. 2, Ref. 24).

KEY WORDS: mesenchymal stem cell, COVID-19, organ involvement.

Introduction
Clinical symptoms of COVID-19 infection include fever, cough, and myalgia or fatigue with pneumonia demonstrated on chest CT scan imaging (1). The clinical situation of patients varies from mild fever to acute respiratory distress syndrome (ARDS) and death (2, 3). Patients with severe disease were likely to display neurologic symptoms such as acute cerebrovascular diseases, impaired consciousness, loss of smell and taste, and skeletal muscle injury (2, 4).

Mesenchymal stem cells (MSCs) have been widely used in cell-based therapy, from basic research to clinical trials (5, 6, 7). Wharton’s jelly mesenchymal stem cells (WJ-MSCs) have distinct advantages of being abundant, easy to obtain with minimal invasiveness, and readily cultured in a sufficient number for transplantation without ethical issues of allografting (8). The aim of the case report is to determine WJ-MSCs treatment’s efficacy in a severe case of multiple organ failure and brain involvement in a patient with COVID-19 infection. In addition, another aim of the study is to get to understand the way in which MSCs are given, especially in patients with brain involvement.

Case report
This study was undertaken in the Bakirkoy Dr. Sadi Konuk Education and Training Hospital at Health Science University and Istinye University with the permission of the Ministry of Health. The study protocol was approved by the Ethical Committee. The informed consent was obtained from the patient’s relatives.

A 51-year-old male patient started to complain of cough, myalgia, high fever (39.5 °C), and diarrhea on March 11, 2020. Upon the progression of symptoms, the patient was hospitalized in the infection service on March 16. Thorax tomography was performed and throat swab was taken. Radiological involvement related to bilateral COVID-19 infection was observed in both lungs (Fig. 1A). The patient had no previous chronic illnesses or additional pathologies in his medical records. A swab was taken, and the diagnosis of COVID-19 was made using the PCR method. A supportive therapy was initiated. Upon progression of respiratory distress during his daily follow-up, the patient was transferred to the intensive care unit (ICU).

During the ICU follow-up, the patient was intubated oro-tracheally due to high fever; DSS: 33-40 breaths per minute (bpm), low O2 saturation, and gradually increasing respiratory distress. Due to hypoxemia in arterial blood gas (ABG) values and deterioration in P/F (Horowitz) values (< 150), the patient was placed in a prone position immediately after intubation. Methylprednisol-
lone was added to the treatment of the patient for 5 days. In the prone position, CPR was applied to the patient who developed sudden cardiac arrest for 10 minutes. Two hours after the arrest, a targeted temperature management was started. Body temperature was adjusted to 33 degrees. It was sedated to be between 20 and 40 according to patient state index. The antiviral treatment of the patient was changed to Avigan. The patient was thought to have a cytokine storm. On March 25, the patient was treated with tocilizumab (IL-6 antagonist) for 2 days.

Echocardiography performed after cardiac arrest revealed a global dyskinesia compatible with myocarditis, and ejection fraction (EF) of 25 % with severe apical akinesia. The patient deve-

Fig. 1. Radiological evaluation of patient lungs. A1–3: focal ground-glass opacity in the lower part of the lung observed on day 5 after first symptom onset. B 1–3: on day 10 day after first symptom onset, it is observed that lesions increase in both upper and lower lung lobes. It is seen that the lesions are converted into consolidation form and merge. C 1–3: on day 25 after first symptom onset, interlobular and intralobular septal thickening is observed in parenchyma of both lungs. In his previous film, the common consolidation areas and patched ground-glass densities are observed to be resorbed.
After MSC transplantation was performed systematically for the third time, as well 
formed both systemically and intrathecally. Here, after the transplantation of 
transplants had been given systematically, the patient was awake-
ded and extubated. Later, the patient who developed neurologi-
observered to be orientated and cooperative. After 5 days of fol-
low-up, the patient was taken to medical service. The patient 
was diagnosed with upper gastrointestinal bleeding. After the 
patient’s anti-thromboembolic treatment had been discontinued, 
his vital signs were stable, and it was decided to follow him up at 
the clinic. The patient was discharged from hospital on April 20, 
2020 as symptom-free and with no complaints. Information about 
the patient’s laboratory examination results are given in Table 1.

Drug treatment of the patient was made on the basis of inter-
national literature and guideline prepared by the scientific com-
mitee related to this issue and affiliated to the ministry of health. 
The MSCs were slowly drawn into the syringe without pressure, 
suspended in 250 ml of 0.9 % NaCl, and then given intravenously 
over 1 hour (Tab. 2).

The levels of alanine transaminase, aspartate transaminase, 
total protein, albumin, total bilirubin, direct bilirubin, ferritin, 
lipidic acids, D-dimer, troponin I, myoglobin, procalcitonin, am-
monia, c-reactive protein, pro B-type natriuretic peptide, creatine 
kine, and alkaline phosphatase were determined in venous blood 
samples using Beckman Coulter AU5800 analyzer (Beckman 
Coulter, Brea, CA, USA). The complete blood count was analyzed 
with ADVIA 2120i autoanalyzer (Siemens Healthcare Diagnostics, 
Erlangen, Germany).

For coagulation assay, all analytical procedures were carried 
out on a random-access coagulation analyzer (Becker Succeeder 
Technology Inc. China).

Flow cytometric analyses were performed with Navios cyto-
mitter (BECLS)-Kalzu Software. Whole blood was stained with 
anti-human FITC-CD45, PE-CD4, ECD-CD8, PE-CY5.5-CD3 
(Beckman Coulter, Brea, California) antibodies.

All samples of WJ-MSCs as cell therapy medicinal products 
were isolated, expanded, and analyzed in cGMP-certified facility 
at Liv Hospital Center for Regenerative Medicine and Stem Cell 
Manufacturing (LivMedCell). Human WJ-MSCs were prepared 
and tested as described in our previous clinical trials (8, 9, 10).

Cryopreserved vials from each donor were thawed and mixed 
in the same tubes before seeded at a cell density of 4,000 cells/cm².

After harvest at the fourth passage, quality control tests were 
performed, such as flow cytometry analysis, endotoxin, rapid microbio-
logical and sterility tests. The final product was prepared as 3×10⁶ 
cells/kg/dose of allogeneic WJ-MSCs pooled from three donors.

Discussion

The central nervous system (CNS) is not immune to alterations 
that lead to neurological disease resulting from acute, persistent 
or latent viral infections (11). In some circumstances, opportunistic 
viral pathogens such as human corona viruses can avoid the 
immune response and cause more severe respiratory diseases or 
even spread to other tissues including the CNS (12). The detection 
of HCoV-RNA in human brain samples clearly demonstrates that 
these respiratory pathogens are naturally neuroinvasive in 
humans, and suggests that they establish a persistent infection in 
human CNS (13). On March 4, 2020, researchers from Beijing 
Ditan Hospital, China, first described a confirmed patient with 
2019-nCoV, whose cerebrospinal fluid (CSF) tested positive for 
2019-nCoV by gene sequencing, suggesting a need to consider 
direct infection when patients with 2019-nCoV present with neu-
rological disorders (14).

In this study, MSCs transplantation was successfully per-
formed both systemically and intrathecally. Here, after the first 2 
transplants had been given systematically, the patient was awake-
ness and extubated. Later, the patient who developed neurologi-
cal symptoms was reintubated. After that, the transplantation of 
MSCs was performed systematically for the third time, as well 
as an intrathecal stem cell transplantation. We thought the blood 
brain barrier (BBB) was an obstacle here. Neurologically, the dia-

Fig. 2. Radiological evaluation of the patient brain. A-D Focal inten-
sity increased in T2-weighted levels in the posterior side of the pons. 
The lesion is marked with an arrow.
Gnosis of brain involvement was demonstrated by MR and proved by COVID-19 antibodies in the spinal fluid. Upon examination of CSF samples, protein and IgG levels were elevated while albumin levels were found low. The CSF result is compatible with previous SARS-CoV-2 infection studies (15). The patient’s vital signs were improved, especially after intrathecal and systemic MSCs transplantations. After the patient had been extubated, his neurological symptoms regressed, consciousness restored, and he could speak. Most neurologic manifestations occurred early in the illness (the median time to hospital admission was 1-2 days) (16). In our patient, the time period between hospitalization and determining the neurological symptoms was approximately 11 days.

After transplanting MSCs through intravenous infusion, a part of the MSCs accumulate in the lung, which could potentially improve the pulmonary microenvironment, protect alveolar epithelial cells, prevent pulmonary fibrosis, and improve the lung function (2, 6, 14). They promote tissue and organ regeneration via secreting a variety of paracrine factors, conferring anti-inflammatory, immunomodulatory, angiogenic, anti-fibrotic, and structural reparative properties (17, 18). BM-MSCs significantly improved the efficiency of cardiomyogenesis and cardiac function (19, 20). When the treatment of patients with organ failure due to COVID-19 infection is examined, no treatment can be seen to improve the organ damage. Our patient had cardiac involvement due to COVID-19 infection. In our patient who underwent echocardiography after cardiac arrest, the ejection fraction was very low (25 %). In echocardiography performed after systematic transplantation of MSCs, this rate increased to the level of 60 %. It is our opinion that the transplantation of MSCs has a healing effect on the heart. After MSCs transplantation had been performed for the fourth time, the patient’s heart functions have returned to normal. Here, we think that MSCs have a positive and accelerating effect upon other healing mechanisms of the body.

MSCs engraftment was observed in the injured lung and engraftment rates increased with the extent of tissue injury (6). In a study by Leng Z et al, 7 patients with COVID-19 infections were given MSCs systematically, which had a healing effect (20). When the thorax CTs of the patient were examined in our case study, it was observed that the upper and lower lobes of the lungs had healing effect (20).

<table>
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<td>10700</td>
<td>4500</td>
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<td>3380</td>
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<td>313</td>
<td>507</td>
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<td>1062</td>
<td>322</td>
<td>445</td>
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<td>3,585</td>
<td>967</td>
<td>876</td>
<td>633</td>
<td>483</td>
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<td>Fibrinogen (mg/dL)</td>
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<td>335</td>
<td>363</td>
<td>343</td>
<td>340</td>
<td>386</td>
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<td>Triglycerides (mg/dL)</td>
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<td>215</td>
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<td>Lymphocytes count (×10⁹/L)</td>
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<td>0.5</td>
<td>0.58</td>
<td>0.8</td>
<td>1.31</td>
<td>1.89</td>
<td>1.72</td>
<td>1.79</td>
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<td>2.3</td>
<td>1.69</td>
<td>22.49</td>
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<td>Neutrophils count (×10⁹/L)</td>
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<td>8.72</td>
<td>10.57</td>
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<td>14.3</td>
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<td>Basophils (×10⁹/L)</td>
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<td>0.04</td>
<td>0.05</td>
<td>0.04</td>
<td>0.09</td>
<td>0.11</td>
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<td>Monocytes (×10⁹/L)</td>
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<td>0.48</td>
<td>1.41</td>
<td>1.08</td>
<td>1.03</td>
<td>1.44</td>
<td>1.84</td>
<td>1.44</td>
<td>1.44</td>
<td>1.15</td>
<td>1.07</td>
<td>1.25</td>
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<tr>
<td>Ammonia (μg/dL)</td>
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<td>130</td>
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<td>114</td>
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</table>

AST – aspartate transaminase, ALT: alanine transaminase, LDH: lactate dehydrogenase, CK – creatine kinase, CRP – c-reactive protein, PCT – procalcitonin, BNP – B-type natriuretic peptide
Tab. 2. Drug and mesenchymal stem cell management.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Route of administration</th>
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<tr>
<td>Ritonavir + Lopinavir</td>
<td>Oral</td>
</tr>
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<td>Oseltamivir</td>
<td>Oral</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>Oral</td>
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<tr>
<td>Favipiravir</td>
<td>Oral</td>
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<tr>
<td>Hydroxychloroquine</td>
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<td>Methylprednisolone</td>
<td>Oral</td>
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<tr>
<td>Tocilizumab</td>
<td>Oral</td>
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<tr>
<td>MSC 1st application/ Day 1</td>
<td>3X10^6/kg</td>
</tr>
<tr>
<td>2nd application/ Day 3</td>
<td>3X10^6/kg</td>
</tr>
<tr>
<td>3rd application/ Day 6</td>
<td>3X10^6/kg</td>
</tr>
<tr>
<td>4th application/ Day 9</td>
<td>2X10^6/kg + 1X10^6/kg</td>
</tr>
</tbody>
</table>

of both lungs were commonly held, especially in the second thorax CT. In our patient, the lesions seen in both lungs had ground-glass appearance, and areas of consolidation were compatible with COVID-19 infection (21, 22). After the MSC transplantation for the last time in our patient, bilateral lung symptoms regressed on control thorax CT. After the patient had been discharged, there was no problem in his medical checkups.

After the first MSC transplantation in our patient, the values of AST, ALT, LDH, CK, pro-bnp, ferritin, triglyceride, fibrinogen, ammonia, and myoglobin began to decrease. The second time the MSCs had been given, CRP reached normal values (Tab. 1). We thought that MSCs were related to the immunomodulatory effect on cytokine storm. On day 2 after MSC transplantation, the lymphocyte count reached the normal level. What is important here, is that reaching the efficacy of lymphocyte count on day 2 was achieved by the administration of MSCs and tocilizumab at the same time. In the literature, it is seen that the normal level of the number of lymphocytes was reached on day 5 in the study of Xiaoling Xu et al (23). In our study, the increase in lymphocytes was thought to be related to MSCs transplantation. It was observed here, is that reaching the efficacy of lymphocyte count on day 2 was achieved by the administration of MSCs and tocilizumab at the same time. In the literature, it is seen that the normal level of the number of lymphocytes was reached on day 5 in the study of Xiaoling Xu et al (23). In our study, the increase in lymphocytes was thought to be related to MSCs transplantation. It was observed

**Learning points**

The transplantations of MSCs, both systematically and intrathecally, were effective in the treatment of the central nervous system. This activity is related to the fact that MSCs administered intrathecally can easily cross the blood brain barrier. Combining both ways of MSC treatment in multi-organ and brain involvement due to COVID-19 infection will increase the effectiveness of treatment.

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Received May 19, 2020.
Accepted May 27, 2020.