RESEARCH ARTICLE

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Effectiveness of Mesenchymal Stem Cell Therapy for COVID-19 Patients in the Intensive Care Unit: A Case-Control Study

Yoğun Bakım Ünitesindeki COVID-19 Hastalarında Mezenkimal Kök Hücre Tedavisinin Etkinliği

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Abstract

Objective: Many methods are used in the treatment of coronavirus disease 2019 (COVID-19), which causes acute respiratory distress syndrome (ARDS), and there are conflicting reports in the literature regarding the results of mesenchymal stem cell (MSC) therapy, which is one of those methods. The aim of our study is to evaluate the effect of MSC treatment applied together with standard treatments on survival.

Materials and Methods: This retrospective case-control study evaluates the survival effect of MSC treatment administered to patients treated in intensive care after the development of ARDS due to COVID-19 between March 2020 and March 2021. The age, gender, comorbid disease status, APACHE II score, and overall and comorbidity-based survival rates were compared between patients who received standard medical treatment (SMT) and patients who received MSC treatment together with SMT.

Results: There were 62 patients in the group receiving only SMT and 81 patients in the group receiving SMT and MSC. No difference was observed between the groups in terms of age, gender, presence of comorbid diseases, or APACHE II scores. There were also no differences according to Kaplan-Maier analysis for the survival statuses of the groups. There was no serious adverse effect due to MSC treatment among these patients.

Conclusion: Our study presents the largest case series in the literature, and it was observed that MSC treatment may not significantly affect overall survival or comorbid disease-based survival, in contrast to many other studies in the literature.

Keywords: COVID-19, Mesenchymal stem cell, Emergency, Mortality, Survival

Öz

Amaç: Akut solunum sıkıntısı sendromuna (ARDS) neden olan koronavirüs hastalığı 2019 (COVID-19) tedavisinde birçok yöntem kullanılmakta olup, literatürde mezenkimal kök hücre (MSC) tedavisinin sonuçları ile ilgili çelişkili yayınlar bulunmaktadır. Çalışmamızın amacı standart tedavilerle birlikte uygulanan MSC tedavisinin sağkalım üzerine etkisini değerlendirmektir.

Gereç ve Yöntemler: Bu retrospektif olgu kontrol çalışması, Mart 2020 ile Mart 2021 arasında COVID-19'a bağlı ARDS gelişmesi sonrası yoğun bakımda tedavi edilen hastalara uygulanan MSC tedavisinin sağkalım etkisini değerlendirmektedir. Çalışmada, standart medikal tedavi (SMT) alan hastalar ile SMT ile birlikte MSC tedavisi alan hastalar arasında yaş, cinsiyet, komorbid hastalık durumu, APACHE II skoru, genel ve komorbiditeye dayalı sağkalım oranları karşılaştırıldı.

Bulgular: Sadece SMT kullanan grupta 62 hasta, SMT ve MSC kullanan grupta 81 hasta vardı. Gruplar arasında yaş, cinsiyet, eşlik eden hastalık varlığı, Apache II skorları açısından fark gözlenmedi. Ayrıca grupların hayatta kalma durumları için Kaplan-Maier analizine göre herhangi bir farklılık yoktu. Hastalar arasında MSC tedavisine bağlı ciddi bir yan etki görülmedi.

Sonuç: Çalışmamız literatürdeki en geniş olgu serisine sahip olup, literatürdeki birçok çalışmadan farklı olarak MSC tedavisinin hem genel sağkalıma hem de komorbid hastalık temelli sağkalıma anlamlı bir etkisi olmadığı görülmüştür.

Anahtar Sözcükler: COVID-19, Mezenkimal kök hücre, Acil servis, Mortalite, Hayatta kalma

Introduction

Coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, may lead to acute respiratory distress syndrome (ARDS) and even death [1,2,3]. Cytokine storms are responsible for ARDS in COVID-19 and these patients require respiratory support. It has been reported that 67% of critically ill COVID-19 patients develop ARDS and, among them, patients who are older than 65 years or have chronic diseases that cause immune dysfunction have higher mortality rates. An autopsy study stated that COVID-19 patients with severe pneumonia died as a result of severe infection with ARDS [1].

Although many drugs and methods of treatment have been used for COVID-19, there is currently no effective antiviral agent. The management of COVID-19 patients remains largely symptomatic and includes standard medical treatment (SMT) [4].

Friedenstein was the first to describe mesenchymal stem cells (MSCs) from bone marrow in 1968. Caplan proposed that these cells would differentiate into mesodermal cells and he first used the term "mesenchymal stem cells" [5,6,7,8]. Today, it is known that MSCs are pluripotent stem cells that can differentiate into both ectodermal and endodermal cells [9,10,11,12].

MSCs are non-hematopoietic cells that play a role in immune modulation and have regeneration and differentiation abilities [13]. MSC treatment has been found to reduce pathological changes and prevent the cell-mediated immunoinflammatory response caused by influenza viruses in the lungs [14,15]. The safe application and efficacy of MSC treatments have been evaluated in COVID-19 patients with ARDS [16,17,18,19]. One study reported that intravenous administration of MSCs obtained from human umbilical cords may be a safe and well-tolerated treatment option for patients with moderate and severe COVID-19 [20]. However, there are also reports in the literature that there are limited benefits from MSC treatment and that results of long-term follow-up from larger numbers of individuals are still needed [16,19,20].

The aim of this study is to compare the survival and mortality results of patients who received MSC treatment with SMT and those who received only SMT.

Materials and Methods

This study was designed retrospectively and involved examining the hospital records of patients who had been treated in intensive care units (ICUs) from March 2020 to March 2021 after COVID-19 infection. The study was started after obtaining the approval of the Ethics Committee of the University of Health Sciences Turkey, Ankara City Hospital Clinical Research Center. This study was also approved by the Turkish Ministry of Health.

The patients were separated into two main groups according to the treatment methods they received: only SMT and SMT plus MSC treatment. The SMT group included patients who had been treated for COVID-19 pneumonia in the general ward of University of Health Sciences Turkey, Ankara City Hospital's ICU and had needed mechanical ventilation. The SMT plus MSC treatment group included patients who had been treated with SMT and MSC in any ICU of the university or training and research hospitals in Turkey.

The common inclusion criteria were that patients had been diagnosed with COVID-19 through reverse-transcription polymerase chain reaction, admitted to emergency medicine, and hospitalized in a third-level ICU with ARDS. Inclusion criteria for the patients who had received SMT plus MSC treatment were that they had received the standard treatment options for COVID-19 as determined by the Science Committee of the Turkish Ministry of Health and had received MSC treatment as well. After evaluating the patients in the SMT plus MSC group according to the inclusion and exclusion criteria, patients who could be matched in terms of age, gender, and comorbidity to the SMT plus MSC group were selected for the SMT group. The inclusion criterion for patients receiving SMT treatment in University of Health Sciences Turkey, Ankara City Hospital's ICU was that they had received only the standard treatment options for COVID-19 as determined by the Science Committee of the Turkish Ministry of Health. Common exclusion criteria were pregnancy, the presence of malignant tumors, a history of allergies, and having received additional treatments (i.e., intravenous immunoglobulin, plasmapheresis, or interleukin [IL]-6 receptor antagonists). The flow charts of the patients included in the study are presented in Figure 1. To demonstrate the effectiveness of MSC applications, random sampling was performed with regard to the control group to ensure that the comorbidities, ages, and gender ratios of this group and the treatment group were equal.

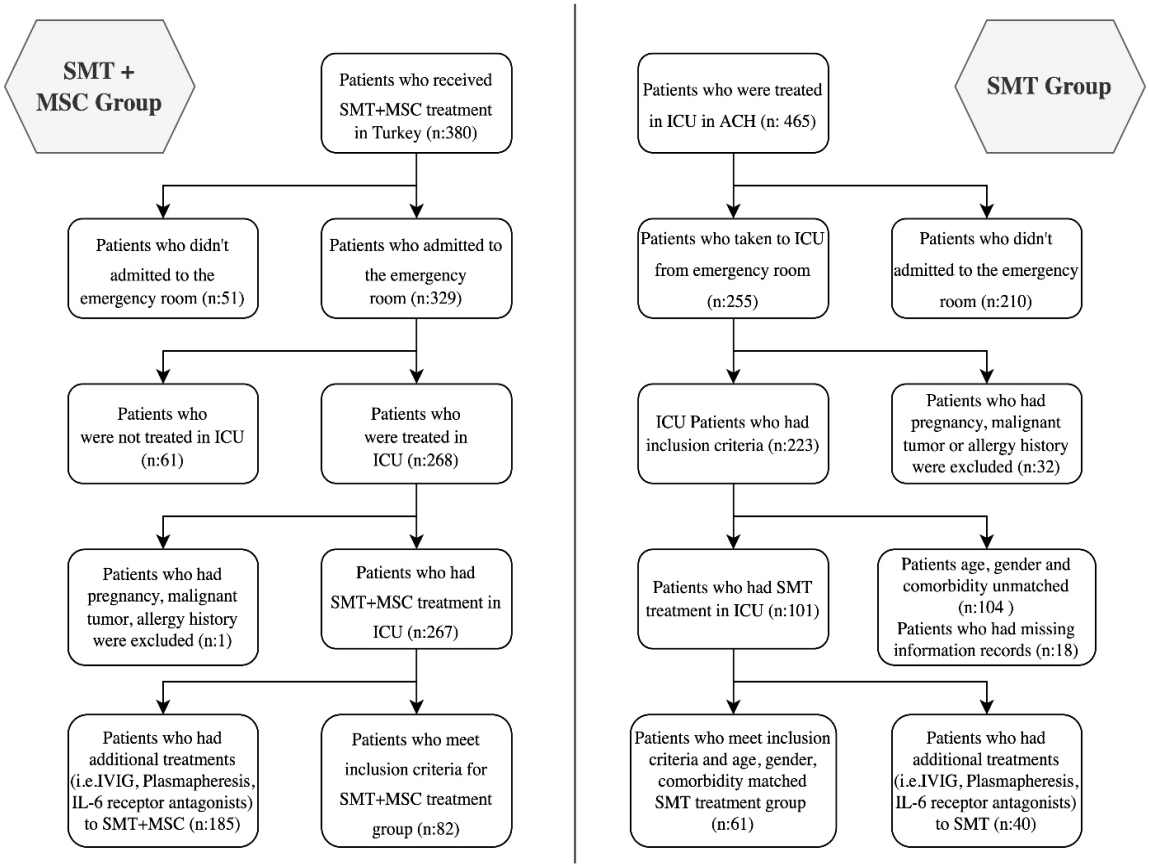

Figure 1. Inclusion of the patients in the study.

SMT: Standard medical treatment, MSC: mesenchymal stem cell treatment, ICU: intensive care unit, IVIG: intravenous immunoglobulin, ACH: Ankara City Hospital.

The standard treatment for COVID-19 was administered according to the Science Committee of the Turkish Ministry of Health. The recommended hydroxychloroquine regimen for all hospitalized patients was a loading dose of 400 mg twice on day 1, followed by 400 mg daily for 4 additional days. Favipiravir was initiated at a loading dose of 1600 mg twice on day 1, followed by 600 mg twice daily for a further 4 days for patients with severe pneumonia or persistent fever in spite of treatment with hydroxychloroquine. Unless there was a contraindication, doses of 1x40 mg/day of enoxaparin were started as prophylaxis for all patients subcutaneously. In cases of severe pneumonia, D-dimer levels of 1000 ng/mL, body mass indexes of 40 kg/m^2, and acute venous thromboembolism, subcutaneous enoxaparin was administered at a therapeutic dose of 2x40 mg/day.

MSCs were prepared from umbilical cords or adipose tissues, and the MSC treatments were applied with 1x10^6 or 10x10^6 cells/kg each time, administered intravenously or intratracheally once or twice throughout the treatment, based on the decisions of ICU directors. The patients received 10 mL of MSCs at doses of 0.5 million/kg (55x10^6 cells) administered into an endotracheal tube by interrupting the mechanical ventilator application twice every 5-7 days, given only to intubated patients as intratracheal MSC administration. The ages, genders, comorbid diseases, and 75-day follow-up results for mortality were noted for all patients.

Statistical Analysis

The Jamovi program (version 1.6.18) was used in the statistical analysis of the data that were obtained. The distribution of the data was evaluated using the Shapiro-Wilk test. Continuous variables with nonparametric and parametric distributions were compared with the Mann-Whitney U test and t-test, respectively. The chi-square test was used in the analysis of qualitative independent data. Survival analyses were performed using Kaplan-Meier survival analysis regarding receiving MSC treatment, and values of $p<0.05$ were considered to be significant.

Results

The mean age of the 143 patients who were included in this study was 62.81 (27-92) years and the female/male ratio was 41/102. There were 61 patients in the group that received only SMT and 82 patients in the group that received SMT and MSC therapy.

No differences were observed between the groups in terms of the demographic parameters that were examined, including age, gender, and the presence of comorbid diseases, as shown in Table 1. The distributions of comorbid diseases in Group 1 and Group 2 were as follows: diabetes mellitus (28 and 16 patients, respectively), hypertension (37 and 24 patients, respectively), chronic obstructive pulmonary disease (8 and 8 patients, respectively), and coronary artery disease (8 and 6 patients, respectively). No differences were observed between the groups in terms of the APACHE II scores of the patients (Table 1). Additionally, no patients had serious adverse effects caused by MSC treatment.

Kaplan-Meier analysis showed no differences in survival between patients in the group that received SMT plus MSC and those in the group that received only SMT ($p>0.05$) (Figure 2). In addition, no differences in survival were found between the two groups in Kaplan-Meier analyses performed for patients with comorbid diseases ($p>0.05$) (Figure 3) and for those with coronary artery disease ($p>0.05$) (Figure 4).

Discussion

During the COVID-19 pandemic, which has had high mortality rates due to pneumonia and thromboembolic events in the lungs, many treatment modalities such as immunosuppressive drugs and anti-IL-6 treatments have been used in the acute phase for symptomatic patients and for post-COVID pulmonary sequelae [21].

Table 1. The demographic parameters and comorbid diseases of the patients.

	SMT + MSC group (n: 82)	SMT group (n: 61)	p
Age, mean (SD)	61.2 (12.5)	65 (14.8)	0.098[a]
Gender (female/male)	22/60	19/42	0.572[b]
Presence of comorbid diseases (+/-)	52/30	39/22	0.949[b]
Hypertension (n) (+/-)	37/45	24/37	0.490[b]
Diabetes mellitus (n) (+/-)	28/54	16/45	0.310[b]
Chronic obstructive pulmonary disease (n) (+/-)	8/74	8/53	0.529[b]
Coronary artery disease (n) (+/-)	8/74	6/55	0.987[b]
APACHE II scores, median (IQR)	19 (19)	17 (17)	0.085[c]

[a]: t-test, [b]: chi-square test, [c]: Mann-Whitney U test, IQR: interquartile range, SD: standard deviation, APACHE: Acute Physiology and Chronic Health Evaluation, SMT: standard medical treatment, MSC: mesenchymal stem cell.

Before the COVID-19 pandemic, MSC treatments had been used for ARDS in many clinical studies. However, most of these studies have not yet been completed [22]. We found only three phase 1 and 2 studies that have been published about MSC treatments for ARDS [16,18,19].

In one preclinical study, MSCs were used in the treatment of gram-negative pneumonias in mice, and MSC treatment was shown to increase the survival of the mice and the amounts of lipocalin-2, which plays a role in bacterial growth [23]. MSC treatment for lung infections caused by influenza has been shown to decrease proinflammatory cytokine release, decrease inflammatory cells in the lungs, and reduce lung damage by increasing alveolar macrophages [14,24,25]. One study found that MSC treatment significantly reduced the mortality among a group of patients with ARDS resulting from H7N9 influenza compared to a control group (17.6% and 54.5%, respectively) [15].

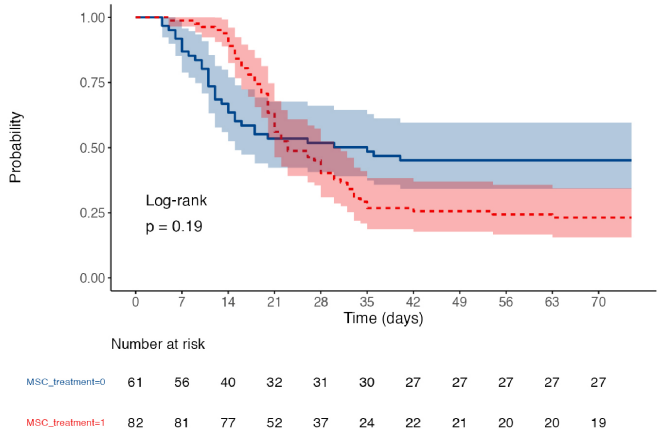

Figure 2. Kaplan-Meier survival analysis of the groups for all patients. Blue line and red dotted line represent SMT and SMT plus MSC treatment. Number at risk corresponds to the days given on the x-axis, showing surviving patients. Faded colored areas represent 95% confidence interval.

SMT: Standard medical treatment, MSC: mesenchymal stem cell treatment.

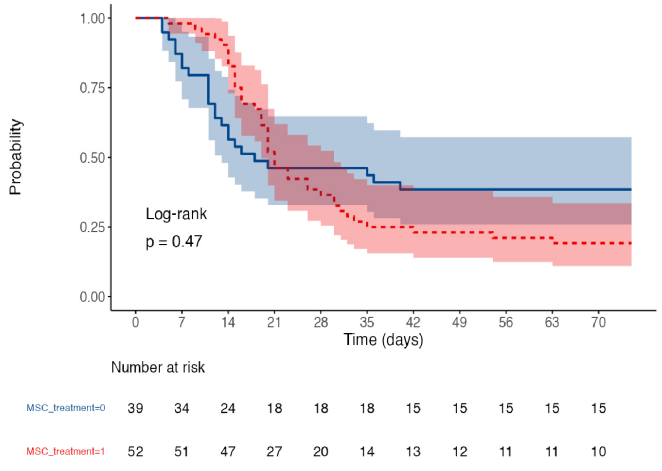

Figure 3. Kaplan-Meier survival analysis of the patients who had comorbid diseases. Blue line and red dotted line represent SMT and SMT plus MSC treatment. Number at risk corresponds to the days given on the x-axis, showing surviving patients. Faded colored areas represent 95% confidence interval.

SMT: Standard medical treatment, MSC: mesenchymal stem cell treatment.

Numerous studies have reported reductions in the main inflammatory biomarkers (C-reactive protein [CRP], IL-6, IL-8, and tumor necrosis factor-α) due to MSC treatment [26]. Although many clinical trials involving MSC treatment have reported regulation of the inflammatory response in COVID-19 patients, it is unclear how MSCs achieve this effect [27,28]. However, some published studies have shown that MSCs are capable of reducing the release of proinflammatory cytokines from cells in the immune system [29]. In preclinical studies, MSCs were shown to release the cytokines (transforming growth factor-β, IL-10, IL-4, and prostaglandin E2) that play anti-inflammatory roles in ARDS and sepsis [29,30]. MSCs also protect endothelial cells from inflammation and oxidative stress, restore the epithelial permeability for tissue repair, and reduce pulmonary edema in the event of intratracheal administration [31,32,33].

In the first case report of MSC treatment used for COVID-19, a 65-year-old female patient who had been diagnosed with the disease was transferred to the ICU in spite of having

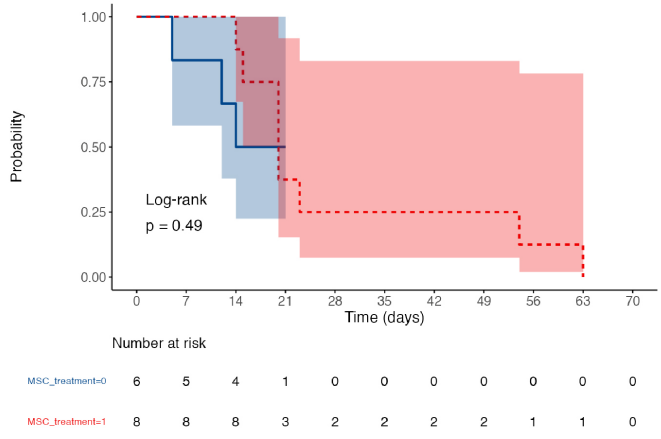

Figure 4. Kaplan-Meier survival analysis of the patients who had coronary artery disease. Blue line and red dotted line represent SMT and SMT plus MSC treatment. Number at risk corresponds to the days given on the x-axis, showing surviving patients. Faded colored areas represent 95% confidence interval.

SMT: Standard medical treatment, MSC: mesenchymal stem cell treatment.

received SMT because she needed mechanical ventilation. After intravenous administration of umbilical cord MSCs at a dose of $50x10^6$ cells, the general condition of the patient improved [34].

Most of the initial case reports of MSC administration due to COVID-19 involved male patients over 50 years of age and were reported from China. The source of the MSCs that were administered was reported as being Wharton's jelly, umbilical cord blood, and bone marrow, and the intravenous route was preferred for the administration of MSCs in all cases except one. Because intravenously administered MSCs usually attach to the pulmonary vessels, and the lungs are the most frequently affected organs in COVID-19, the intravenous administration of the infusion is the most logical option. In these cases, all of the patients also received other treatment methods, such as antivirals, antibiotics, and/or corticosteroids, in addition to MSC treatment. In all cases, no side effects were observed as a result of the MSC treatment and the symptoms of COVID-19 disappeared completely [35].

Subsequent case report series have generally included a limited number of patients while evaluating the applicability and effectiveness of MSC treatment. Leng et al. [27] used ACE-2-negative MSCs obtained from an unknown source to treat COVID-19 pneumonia and reported that all seven patients included in their study recovered. However, all the patients in that study had over 90% oxygen saturation levels when breathing room air. Sánchez-Guijo et al. [36] reported that there were two deaths among 13 patients (15%) in 16 days of median follow-up after patients received MSCs obtained from allogenic adipose tissue. Another study reported that the mortality rate was 45% in the 60-day follow-up of 11 COVID-19 patients who were monitored in the ICU [26].

According to the results of non-randomized phase 1 clinical research involving 18 patients with moderate and severe COVID-19, transient flushing and fever developed in two patients and transient hypoxia was seen in one patient who underwent umbilical cord MSC treatment, but no serious adverse events were reported [20]. MSC infusions from umbilical cords are safe and have significantly improved the survival and shortened the recovery time of patients according to the results of a double-blind, phase 1/2a study that included 24 patients with COVID-19 [37]. In another study by Shu et al. [38], umbilical cord MSCs administered to a group of 12 patients resulted in significantly shorter recovery times, rapid disappearance of symptoms, and rapid reductions in lung inflammation compared to a control group. MSCs derived from adipose tissue were given to 13 patients who had severe COVID-19 and needed mechanical ventilation, and improved clinical statuses and radiological healing were observed. The need for mechanical ventilation was also reduced [36].

Although many publications in the literature show that MSCs are beneficial in the treatment of ARDS and COVID-19, there are also studies that state that MSCs do not have significant effects on mortality. Zheng et al. [19] evaluated the efficacy of MSCs obtained from adipose tissue in patients with ARDS and stated that although MSC treatment can be applied safely, they did not observe any superiority with regard to this treatment in terms of duration of hospitalization, length of ICU stay, need for mechanical ventilators, or improvement in blood parameters on the 28^{th} day of hospitalization. Matthay et al. [16] compared MSCs derived from bone marrow, administered at $10x10^6$ cells/kg, to a placebo in ARDS patients in a phase 2 study and determined that MSC treatment had no effect on 28-day mortality. In a phase 1 study, Meng et al. [20] evaluated the effectiveness of MSCs prepared from umbilical cords in the treatment of COVID-19 patients in two groups, with nine patients in each group, with four severe and five moderate cases, who either received or did not receive MSC treatment. MSC treatment was administered intravenously in three doses at $3x10^7$ cells per dose, no serious adverse events were observed in any patients, and there was no mortality among the patients. However, these authors emphasized that although MSCs can be applied safely, phase 2/3 studies should be performed to determine their contributions to treatment [20].

Our study is the largest case series report to date in the literature of the use of MSCs in COVID-19. We observed that MSC treatment did not improve overall survival rates or comorbid disease-based survival in the 75-day follow-up of critically ill COVID-19 patients who were treated in ICUs. This may be because the MSC administrations in this study were prepared at different doses and with different methods.

The main limitation of our study is that it does not involve a standardized MSC treatment method. The absence of immunological tests before and after treatment is another important limitation. Missing data on the sources, doses, and administration routes of MSCs limited further inferences about different treatment modalities' effects.

Conclusion

Many methods are used in the treatment of COVID-19 and there are conflicting reports in the literature about MSC treatment. A common feature among these publications is that the studies were performed with small case groups. To our knowledge, the present study has the largest case series in the literature to date. Although increasing evidence has indicated the therapeutic potential of MSC treatment, according to the present study's limited findings MSC treatment might not contribute to the improvement of overall or comorbid disease-based survival. Further studies with more comprehensive data may clarify the effects of different MSC treatment modalities on discrete subgroups of COVID-19 patients.

Ethics

Ethics Committee Approval: The study was started after obtaining the approval of the Ethics Committee of the University of Health Sciences Turkey, Ankara City Hospital Clinical Research Center.

Informed Consent: Retrospective study.

Authorship Contributions

Concept: H.C., F.K., S.T., H.C.D., M.G., U.A., A.Ç.İ; Design: H.C., F.K., S.T., H.C.Ç., H.E.A., E.K.H.A.; Data Collection or Processing: H.C., S.T., H.C.D., M.G., İ.T.P., A.L.; Analysis or Interpretation: H.C., U.O.İ., H.C.Ç., İ.T.P., M.K., İ.C.H.; Literature Search: H.C., U.O.İ., A.B., M.G., İ.R., M.K.M.C.; Writing: H.C., U.O.İ., H.C.Ç.

Conflict of Interest: No conflict of interest was declared by the authors.

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