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Research Article

MSC Transplantation in Eight Severe COVID-19 Patients: Can Cytokine Storm Be Reversed?

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ABSTRACT

Background: In this clinical case report, we evaluated the clinical and the efficacy outcomes of Mesenchymal Stem Cells (MSCs) transplantation in eight severe COVID-19 patients with pneumonia.

Methods: MSCs were administered to eight severe/critically severe patients, unresponsive to treatment algorithms advised by the Turkish COVID-19 Scientific Committee, between April 1st -May 4th , 2020. Three supplementary patients were also reviewed without MSC transplantation.

Results: Two severe patients were discharged from ICU on the 7th and 13th days and two critically severe patients were extubated and discharged on the 13th and 34th days after MSC transplantation respectively. The other four could not achieve clinical improvement and passed away. In all eight patients, compared to the baseline, there was a significant decrease in CRP (p=0.036), fibrinogen (p=0.012) and Hb (p=0.03) values on post treatment day 5. While there was an increase in lymphocyte count between baseline and post treatment, the change didn't reach statistical significance (p=0.06). There was no statistically significant change in ferritin, neutrophil count, respiratory rate, oxygen saturation, troponin and platelet count (p>0.05) between baseline and post-treatment day 5.

Conclusion: Four patients were discharged from the ICU after MSC transplantation. Although there was an immediate significant improvement in their prognostic markers, the other four patients remained in critically severe condition and passed away. In two severe patients, the recovery was faster after MSC transplantation than the other two critically severe recovered patients. This may indicate the potential benefit of MSC transplantation performed in an earlier clinical stage. Moreover, we can advise MSC transplantation when the poor prognostic markers (decrease in lymphocyte number, increase in fibrinogen and CRP) are observed in the severe COVID-19 patients, to overcome alveolar damage due to "cytokine storm." This observation may introduce an algorithm for a supportive treatment with MSC transplantation for COVID-19 patients, which needs to be confirmed by broader randomized controlled trials.

Keywords: MSCs; COVID-19; Medicinal signalling cells; Stem cell treatment; Immunomodulation; Cytokine storm; SARS-CoV-2; Mesenchymal stem cells

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Abbreviations: ARDS: Acute Respiratory Distress Syndrome; CRP: C-Reactive Protein; DC: Dendritic Cell; DM: Diabetes Mellitus; Hb: Hemoglobin; ICU: Intensive Care Unit; LC: Lymphocyte Count; MSCs: Mesenchymal Stem Cell; NC: Pericyte/MSC; RR: Respiratory Rate; HFNC: High Flow Nasal Cannula; CVD: Cardiovascular Disease; MI: Myocardial Infarction; TNF-α: Tumor Necrosis Factor-Alpha; IL-10: Interleukin 10; ACE2: Angiotensin-Converting Enzyme; TMPRSS2: Transmembrane Protease, Serine 2; IL-6: Interleukin 6; CK: Creatine Kinase

INTRODUCTION

The COVID-19 disease is a global pandemic, with the first case diagnosed in December 2019, as reported by World Health Organization (WHO). In 17% of patients, COVID-19 causes severe Acute Respiratory Distress Syndrome (ARDS) due to release of large amounts of pro-inflammatory cytokines and chemokines in the lungs [1]. In a retrospective observational study from Milan, 9% of the people, who tested positive for COVID-19, needed ICU care with respiratory support [2]. The demand for ICU beds and health care personnel brought significant overload to sustain the care of these patients. Search for effective therapies is underway. However, the result of severe infection of COVID-19 still leads to the inevitable fatalities with the current available therapies [3].

Mesenchymal Stem Cells (MSCs) have been isolated about 30 years ago (Supplementary Data 1). There are over 5,000 articles published on MSCs. Moreover, anti-inflammatory and immune-modulatory properties of MSCs have been well studied [4]. Exogenously administered MSCs are medicinal. They generate positive therapeutic outcomes by secreting bioactive factors that exhibit immunomodulatory, and regenerative effects by fabricating, and secreting antibiotic proteins, where they hone in on sites of injury or disease [5,6]. Hence, Arnold Caplan has proposed recently to change the name of MSCs to Medicinal Signaling Cells [7].

As MSCs arise from pericytes, they can be isolated from a variety of vascularized tissues. Each separate tissue-specific stem cell interacts with its underlying vascular endothelial cells, and adjacent specific pericyte/MSC "Universal Stem Cell Niche" (pMSCs). Each specific pMSCs have both pMSCs common, and unique chemical, and functional features. Meanwhile, the major therapeutic role of pMSCs *in vivo* at various sites of disease or injury are very similar when comparing these different pMSCs. Over the past decade, the emphasis has shifted toward harnessing the pMSCs' ability to produce factors and cytokines that stimulate innate tissue repair, modulate inflammation, and immune responses [8,9]. MSCs express function on Toll-Like Receptors (TLRs). Triggering different TLRs, depending on exposure times promote either pro- or anti-inflammatory function in MSCs [10]. Pre-clinical studies demonstrated that the majority of infused MSCs initially distributed in the lungs [11]. Subsequent studies showed improved pulmonary functions beginning shortly after administration with no evidence of pulmonary safety risk. These studies indicated the local beneficial MSCs-mediated effect on pulmonary airways [12].

A recent pilot study from China explored the therapeutic outcomes of MSC transplantation in seven poor prognoses COVID-19 patients with pneumonia. The results revealed that MSC transplantation was safe and effective treatment option. The peripheral lymphocytes increased after the treatment, and the overactivated cytokine-secreting immune cells disappeared in 3-6 days. A group of regulatory DC cell population dramatically increased. Meanwhile, the level of TNF-α is significantly decreased, while IL-10 increased in the MSC transplantation group compared to the placebo control group. Furthermore, the gene expression profile showed MSCs were ACE2- and TMPRSS2-, which indicated MSCs were free from COVID-19 infection [13].

Here we report our clinical observations of eight cases, before and after MSC transplantation, to assess the clinical therapeutic effects of MSC transplantation on COVID-19 severe/critically severe patients. Though broader studies are needed, we advised a clinical application protocol and algorithm by evaluating the poor prognostic markers significantly related by MSC transplantation to prevent the overload in ICU clinics, as well as, to shorten hospitalization time.

METHODS

Intravenous MSC transplantation was performed on eight patients followed in ICU with COVID-19 pneumonia. These eight patients were clinically heterogeneous (Table 1) and unresponsive to unique COVID-19 medical treatment algorithms confirmed with Turkish Ministry of Health (advised clinical treatment). The patients were enrolled into the supportive treatment with each family's signed written consent form, in accordance with the Declaration of Helsinki.

Three supplementary patients with the same indication, under the advised clinical treatment were followed with placebo (Supplementary Data 2). The MSC transplantations were performed in the Department of Anesthesiology and Reanimation, Marmara University Medical School Hospital, Istanbul, Turkey and approved by the Ethics Committee of the University.

Patients

The patients (ages 18-95), were tested by the real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) (Bioexen RT-PCR kit, Light Cycler 96) assay of nasal and pharyngeal swabs and evaluated by thorax CT when they were first admitted to hospital (Table 1). In case one of them confirms the COVID-19 infection, then the patient started with the advised treatment, and a second PCR test was scheduled. All our eight and three supplemental patients were confirmed positive by a second PCR test, if the first one was negative. The patients were enrolled for the supportive MSC transplantation due to their

lack of response to advised treatments, between April 1st-8th 2020.

Patients were followed in ICU for primary safety and efficacy outcomes as introduced in previous COVID-19 studies in the literature, until they were discharged from the hospital or passed away [3,14]. Efficacy data was recorded in Table 2. Thorax CT findings (ground glass opacity) have been used as part of the diagnostic tool in COVID-19 pneumonia patients [3,14]. In the follow up, chest X-rays were used as screening tools during ICU stay. Clinical improvement in the patients was confirmed by efficacy outcomes, including chest X-rays in the discharged patients.

Primary safety data

Infusion related and allergic reactions

Life-threatening adverse events

Efficacy data

Table 1: Efficacy data.

C-reactive protein, (CRP),	0 mg/L-5 mg/L
Ferritin,	6 µg/L-323 µg/L
Oxygen saturation, (SaO2)	93%-100%
Lymphocyte count, (LC),	1.20-3.10 × 10^3 /µL
Neutrophil count, (NC),	1.40-6.20 × 10^3/µL
Fibrinogen,	200 mg/dL-400 mg/dL
Platelet count, (PC),	150-440 × 10^3/µL
Hemoglobin, (Hb),	12 g/dL-17 g/dL
Troponin,	0 ng/L-14 ng/L
D-dimer,	0.00 mg/L-0.5 mg/L
Fever,	36.1°C-37.2°C
Respiratory rate, (RR),	12-18 breaths/min
Diarrhea	0/1
Thorax CT scan	Normal/ground glass opacity

Cell preparation and transplantation

The clinical grade MSC were supplied with no cost by the Atigen-Cell Technology Center, Trabzon, Turkey licensed by Turkish Health Ministry, to manufacture clinical grade MSC. The total number of cell count was calculated as 1 × 10^6cells per kilogram. The cells were prepared for injection in 100 ml of normal saline. The MSCs were administered to the patients at a critical stage, when they did not show any improvement by the advised clinical treatment for COVID-19, as to whether the effectiveness of the cellular treatment on this severe stage of infection and inflammation could be observed. The cells were infused in 40 minutes with a rate of 2 ml/minute, as described in the literature [15].

Statistical analysis

Continuous variables were presented by medians and percentiles. Continuous variables for two repeated measures were compared by Wilcoxon test. A p-value below 0.05 is considered as the level of statistical significance.

RESULTS

Eight COVID-19 infected pneumonia patients in the Marmara University Hospital ICU were evaluated for this clinical cases report. They were followed with unique COVID-19 medical treatment algorithms confirmed with Turkish Ministry of Health (advised clinical treatment). Two patients were monitored as severe and six were as critically severe (Table 2). All eight patients were clinically heterogeneous considering their comorbidities and demographic characteristics (Table 3).

Table 2: Demographic and clinical characteristics of patients.

Patient No	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Supplementary Cases**		
									(Case 1)	(Case 2)	(Case 3)
Age	63	65	69	70	38	75	57	75	61	72	71
Gender	F	M	M	M	M	F	M	M	M	F	F
Weight	80 kg	70 kg	100 kg	70 kg	100 kg	70 kg	60 kg	65 kg			
Clinical Condition	Critically Severe	Critically Severe	Critically Severe	Severe	Critically Severe	Critically Severe	Critically Severe	Severe	Critically Severe	Critically Severe	Critically Severe
HT	+	-	+	+	-	+	-	-	-	-	+

DM	+	-	+	+	-	+	-	-	-	-	+
Intubated	+	+	+	-	+	+	+	-	+	+	+
PCR	-/+	+	+	+	+	+	+	+	+	+	+
Discharged Day	Died	Died	Died	7th Day	13th Day	Died	34th Day	13th Day	Died	Still in ICU (41 Days)	Died

HT: Hypertension; DM: Diabetes Mellitus

Table 3: Efficacy outcomes of the patients.

	CRP	Ferritin	Fever	RR	SaO2	Diarrhea	LC	NC	Hb	PC	Fibrinogen	D-Dimer	Troponin
Patient 1													
1st Day	7,34	657	36.7	24	99	0	0,5	12,8	10,5	292	711	>20	12,96
2nd Day	7,15	491	36,5	20	94	0	0,6	13,9	11,4	342	630	>20	31,95
5th Day	7,45	369	36,2	25	99	0	1	14,6	10,1	361	695	5,78	41,52
Discharge from ICU	7,37*	349,00*	38,3*	29*	95*	0*	0,5*	9,8*	9,8*	189*	638*	>20*	324,3*
Discharge from hospital	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Patient 2													
1st Day	175	1150	36,9	18	94	0	0,8	16,3	11,2	180	175	>20	20,35
2nd Day	112	787	37,3	17	94	0	0,7	24,2	11,1	227	150	10,59	20,54
5th Day	26,80	475	37,1	24	95	0	0,6	9,4	9,3	102	115	>20	27,26
Discharge from ICU	27,40*	413*	37*	25*	93*	0*	0,6*	2,5*	9*	82*	182*	5,3*	23,84*
Discharge from hospital	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Patient 3													
1st Day	7,39	657	36,5	24	93	0	0,5	12,8	10,5	292	711	1,36	30,03
2nd Day	7,15	491	36,7	20	96	0	0,6	13,9	11,4	342	630	1,75	34,88
5th Day	7,42	369	36,6	18	89	0	1	14,6	10,1	361	695	5	36,75

Discharge from ICU	118*	1298*	37*	28*	95*	0*	0,5*	14,4*	8,6*	156*	553*	7,88*	48,6*
Discharge from hospital	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Patient 4													
1st Day	134	238	36,6	30	89	0	0,9	6,2	12,8	364	738	1,25	16,97
2nd Day	181	222	36,8	28	94	0	0,6	7,2	13,6	416	841	1,98	13,82
5th Day	69	163	36	25	95	1	1,3	6,6	12,6	451	512	1,87	17,24
Discharge from ICU	7,09	128	36,5	20	95	0	2,3	7,1	12,2	407	671	1,39	18,46
Discharge from hospital	NA	NA	36,7	18	96	0	1,7	3,6	13,3	246	NA	0,59	17,89
Patient 5													
1st Day	198	1023	36,6	13	93	0	0,9	13,4	10,8	249	543	>20	3
2nd Day	110	973	36,7	12	96	0	1	9,2	11	203	366	14,69	3
5th Day	16,1	1103	36,5	14	96	0	2,9	12,1	11	171	222	16,96	3,92
Discharge from ICU	3,11	493	36,1	21	94	0	2,3	3,7	12,4	154	270	3,62	13,8
Discharge from hospital	3	199,3	36,3	16	97	0	2,2	2,9	12,7	181	NA	0,58	NA
Patient 6													
1st Day	230	4851	36,5	18	91	0	0,8	9,7	13,7	190	489	1,55	22,57
2nd Day	211	2977	36	20	98	0	0,5	8,7	12,7	149	421	2,48	38,77
5th Day	215	599	36,6	20	100	0	0,6	12,9	12,5	139	447	3,81	21,14
Discharge from ICU	50,1*	981*	36,8*	21*	100*	0*	1,4*	9,5*	11,3*	72*	296*	3,59*	76,64*
Discharge from hospital	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Patient 7													
1st Day	491	875,4	36	20	94	0	0,5	9,1	12,2	319	1015	5,19	14,5
2nd Day	187	897,2	36,7	27	92		0,5	9,2	12,4	336	767	3,97	12,98

5th Day	34,7	7975	36,4	13	100	0	2,5	39,4	9,4	238	279	7,3	9,17
Discharge from ICU	52	120	36.7	18	99	0	1,90	11,6	8,2	373	371	2,35	9,74
Discharge from hospital	45	132	36.5	20	98	0	2,3	13	8,6	363	401	2,52	10,5
Patient 8													
1st Day	181	375	36,8	28	89	0	0,7	5,4	12	119	662	1,54	16,65
2nd Day	121	280	37,3	25	92	0	0,5	5,3	11,7	151	620	1,48	23,18
5th Day	141	545	37	24	96	0	0,7	11,9	11,9	305	648	8,98	15,01
Discharge from ICU	35,7	323	36,6	25	99	0	1,2	5,7	11,5	277	580	3,23	13,75
Discharge from hospital	7	142	36,8	23	98	0	1,4	3,7	10,7	215	495	1,37	15,99
Supplementary Cases													
Case 1													
1st Day	178	926	36,3	25	91	0	0,3	5,5	13,4	167	698	1,56	24,41
2nd Day	256	813	37,8	22	93	0	0,3	6,1	13,8	183	783	1,57	19,24
5th Day	266	1670	38,2	24	90	0	0,4	3,9	12,8	175	1161	1,71	34,9
Discharge from ICU	266*	1670*	38,2*	24*	90*	0*	0,4*	3,9*	12,8*	175*	1161*	2,3*	113,1*
Discharge from hospital	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Case 2													
1st Day	160	318	37	28	92	0	0,5	7,9	9,1	340	620	6,01	21,9
2nd Day	121	294	37,1	27	93	0	0,5	8,2	9,2	319	601	10,43	26,53
5th Day	154	305	36,8	25	93		0,5	14,3	9,1	322	795	3,42	47,82
Discharge from ICU	still in ICU	still in ICU	still in ICU	still in ICU	still in ICU	0	still in ICU	still in ICU	still in ICU	still in ICU	still in ICU	still in ICU	still in ICU
Discharge from hospital	still in ICU	still in ICU	still in ICU	still in ICU	still in ICU	0	still in ICU	still in ICU	still in ICU	still in ICU	still in ICU	still in ICU	still in ICU
Case 3													

1st Day	237	358	36,3	24	93	0	0,5	7,6	8,3	179	798	12,37	56,46
2nd Day	189	335	36,5	28	94	0	0,5	20,8	8,7	266	798	12,11	53,33
5th Day	211	198	37	26	94		0,9	11,5	8	225	865	8,69	59,65
Discharge from ICU	189*	309,9*	36,6*	25*	95*	0*	0,7*	8,5*	7*	226*	894*	6,63*	116,7*
Discharge from hospital	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

(NA: not applicable due to discharge or exitus; *Last value before exitus)

Table 4: Demographic and clinical characteristics of patients.

Patient	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Supplementary Cases**		
									Case 1	**Case 2**	**Case 3**
Age	63	65	69	70	38	75	57	75	61	72	71
Gender	F	M	M	M	M	F	M	M	M	F	F
Weight	80 kg	70 kg	100 kg	70 kg	100 kg	70 kg	60 kg	65 kg			
Clinical Condition	Critical Severe	Critical Severe	Critical Severe	Severe	Critical Severe	Critical Severe	Critical Severe	Severe	Critical Severe	Critical Severe	Critical Severe
HT	+	-	+	+	-	+	-	-	-	-	+
DM	+	-	+	+	-	+	-	-	-	-	+
Intubated	+	+	+	-	+	+	+	-	+	+	+
PCR	-	+	+	+	+	+	+	+	+	+	+
Discharge day	Died	Died	Died	Day 7	Day 13	Died	Day 34	Day 13	Died	In ICU (41 Days)	Died

HT: Hypertension; DM: Diabetes Mellitus

Two patients were female, and six patients were male with mean ages of 69 and 62.33 respectively. Four patients presented with comorbidities, Diabetes Mellitus (DM), and hypertension (HT). Three of these patients, 63 years old, 69 years old, and 75 years old did not improve with MSC transplantation and passed away. One patient, 70 years old, with comorbidities, significantly improved, and was discharged from the ICU on the 7th day after the MSC transplantation. Two clinically severe patients did not need to be intubated during the ICU stay after the MSC transplantation and achieved better clinical conditions. They were discharged from ICU on the 7th and 13th days respectively. All critically severe patients were intubated before the MSC transplantation.

The patients were observed until they were discharged from ICU or passed away. Their laboratory and clinical data are recorded and evaluated in Table 4.

Primary safety outcome

No adverse effects were observed related to infusion or allergic reactions, secondary infection, or life-threatening adverse events in patients, who received MSC transplantation. The treatments were recorded within the predicted safety levels of MSC transplantation treatments mentioned in the previous studies [16].

Efficacy outcome

We evaluated the prognostic markers contributing to the efficacy outcome. We compared the 1st and the 5th day efficacy outcomes by Wilcoxon test (Table 5).

Table 5: Comparison of prognostic factors values before transplantation and after the 5th day MSC transplantation.

	Day 1 Median (25th-75th percentile)	Day 5 Median (25th-75th percentile)	p value
CRP	178.00 (39.94-222.00)	30.75 (9.69-13.25)	0.036
Ferritin	766.20 (445.50-766.20)	510.00 (369.00-977.00)	p>0.05
NC	11.25 (6.92-13.25)	12.50 (14.60-10.02)	p>0.05
LC	0.75 (0.50-0.87)	1.00 (0.62-2.20)	0.06
Fibrinogen	686.50 (731.25-502.50)	479.50 (236.25-683.25)	0.012
RR	22.00 (18.00-27.00)	22.00 (15.00-24.75)	p>0.05
O_2	93.00 (91.00-94.00)	96.00 (95.00-100.00)	p>0.05
Hb	11.60 (10.57-12.65)	10.55 (9.57-12.35)	0.03
Platelet	270.50 (182.50-312.25)	271.50 (147.00-361.00)	p>0.05
Troponin	16.81 (13.34-22.01)	19.19 (10.63-34.37)	p>0.05

CRP: C-Reactive Protein; NC: Neutrophil Count; LC: Lymphocyte Count

Baseline day 1 and post treatment day 5 values of the parameters are presented on Table 4. Compared to the baseline, there was a significant decrease in CRP (p=0.036), fibrinogen (p=0.012) and Hb (p=0.03) values on post treatment day 5. While there was an increase in lymphocyte count between baseline, and post treatment, the change did not reach statistical significance (p=0.06). There was no statistically significant change in ferritin, NC, RR, SaO_2 , troponin, and PC (p>0.05) between baseline, and post treatment day 5.

We observed significant improvement in prognostic values-CRP, LN and fibrinogen-in all MSC transplanted patients. Four out of the eight patients' improvement was also continued with positive clinical progression, and were discharged from the ICU on the 7th, 13th, 34th and 13th days respectively following MSC transplantation (Table 6).

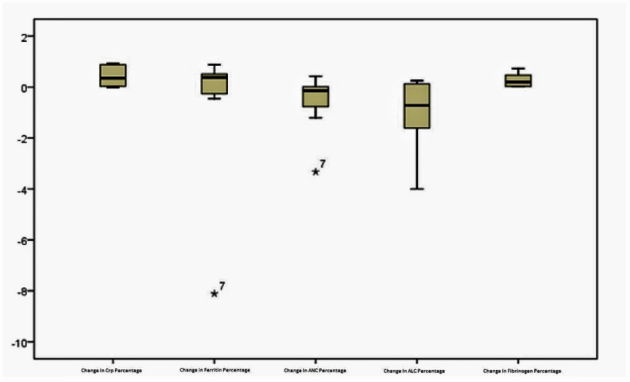

Figure 1: Poor prognostic markers pretransplant, and 5th day after transplantation with median values.

Table 6: Comparison of efficacy outcomes before transplantation and after the 5th day MSC transplantation.

	Day 1 Median (25th-75th percentile)	Day 5 Median (25th -75th percentile)	p-value
CRP	178 (39.94-222.00)	30.75 (9.69-13.25)	0.036*
Ferritin	766.2 (445.50-766.20)	510.00 (369.00-977.00)	0.05
NC	11.25 (6.92-13.25)	12.50 (14.60- 10.02)	0.05
LC	0.75 (0.50-0.87)	1.00 (0.62-2.20)	0.06
Fibrinogen	686.5 (731.25-502.50)	479.50 (236.25-683.25)	0.012*
RR	22 (18.00-27.00)	(15.00- 24.75)	0.05
SaO_2	93 (91.00-94.00)	96 (95.00- 100.00)	0.05
Hb	11.6 (10.57-12.65)	(9.57-12.35)	0.03*
Platelet	270.5 (182.50-312.25)	271.50 (147.00-361.00)	0.05
Troponin	16.18 (13.34-22.01)	(10.63- 34.37)	0.05

*p-value ≤ 0.05

Baseline (day 1) and post treatment (day 5) values of selected parameters are presented. Compared to the baseline, there was a significant decrease in CRP (p=0.036), fibrinogen (p=0.012) and Hb (p=0.03) values on post treatment day 5. While there was an increase in lymphocyte count values between baseline and post treatment, the change didn't reach statistical significance (p=0.06). There was no statistically significant change in ferritin, NC, RR, O_2, troponin and platelet values (p>0.05) between pre and post treatment.

The significant improvement in the efficacy outcome was not correlated with the clinical progress in the other four out of eight patients. These patients could not establish good clinical progress, though we had seen the immediate significant

improvement in prognostic markers (CRP, fibrinogen, and lymphocyte count) on the 5th day (Figure 1). They remained in critically severe condition, progressed to multiorgan insufficiency, and passed away. The presence of comorbidities, gender or any other clinical factors did not seem to determine the clinical outcomes in the patients; while, our sample size was small.

Clinical case progress

We evaluated efficacy outcomes of the eight MSC transplanted patients as below:

1st case: 63 year old woman, she was admitted to hospital with fever, and dyspnea complaints. Her PCR result was negative, but COVID-19 was confirmed first by thorax CT scan. She had diabetes mellitus, and hypertension, and was transferred to the ICU as she progressed poorly. Her laboratory work, and clinical evaluation was confirmed as critically severe. She was intubated and followed by hydroxychloroquine, azitromycin and favipiravir treatments. MSC transplantation was pursued, since she did not respond to the treatment and her condition kept deteriorating. Her lymphocyte count improved from 500 (2.8%) to 1000 (4.1%) at pre-transplantation, and the 5th day after the MSC transplantation respectively. She developed secondary bacterial and candida infections due to her prolonged intubation. Subsequently, her symptoms kept deteriorating. The patient died because of bacterial infection and septic shock on the 25th day of the ICU stay.

2nd case: 65 year old man, admitted to hospital with no comorbidities. He had fever, cough, and dyspnea, and a diagnosis of COVID-19 was confirmed by PCR testing. He deteriorated rapidly and was transferred to ICU. He was evaluated as critically severe, and intubated. He received azithromycin, and hydroxychloroquine treatment. Once the urine culture showed staphylococcus haemolyticus, vancomycin was added to the treatment regimen. He was pursued with MSC transplantation with baseline lymphocyte count 800 (4.3%), fibrinogen 175. His clinical progress did not respond to the supportive treatments and died on the 17th day of the ICU stay.

3rd case: 69 year old man with diabetes and hypertension was admitted to hospital with dyspnea, fever complaints, and was confirmed with COVID-19 by PCR. He was transferred to ICU on the 2nd day of his hospital admission. He was intubated, and received a clinical treatment regimen with hydroxychloroquine, azithromycin, favipiravir, and piperacillin-tazobactam. MSC transplantation was pursued due to his guarded condition on the 3rd day in ICU with a lymphocyte count 500 (3.3%), D-dimer 1.36, and fibrinogen 711. His blood markers improved drastically in a short period of time with lymphocyte count to 1000 (7.6%), ferritin decreased to 391 on the 5th day following MSC transplantation. During the ICU stay, iatrogenic pneumothorax occurred with progressive clinical deterioration and he passed away.

4th case: 70 year old man with a history of coronary arterial disease, diabetes mellitus, and hypertension presented with fatigue, dry cough and shortness of breath. His nasopharyngeal swab was obtained for PCR test and turned out positive for COVID-19. Thorax CT scan revealed bilateral ground-glass opacities. He was tachypneic with 38 breaths per minute and desaturated (sPO_2:89%) under 6 L/min O_2 via face mask. After ICU admission, he was put on a non-breathing mask 6 L/min. His respiratory rate decreased down to 28 breaths/min and peripheral oxygen saturation increased to 93%. He refused prone positioning. On the 3rd day of the ICU stay, he had severe hypoxemia (PaO_2/FiO_2:100) and high flow nasal cannula (HFNC) was advanced with FiO_2:0.80, 60 L/min. He received MSC transplantation on the 4th day of the ICU stay. The laboratory tests before and after the MSC transplantation are presented in Table 2. He was able to be weaned off the HFNC, after day 4 of receiving MSC transplantation. His sPO_2 remained constant and above 95% under 5 L/min O_2 via face mask, his respiratory rate was 18-20/min. He was discharged to the ward under 2 L/min O_2 via nasal cannula on day 7.

5th case: 38 year old man with no comorbidities came to emergency room with continuing fever, and headache complaints for 10 days. He was diagnosed with COVID-19 due to the ground-glass opacities on his thorax CT scan and transferred to the infectious disease clinics. He started a regimen with hydroxychloroquine and azithromycin. He was transferred to ICU, when he developed shortness of breath and required more oxygen. He was intubated on the 2nd day in ICU and connected to ECMO support due to a deep hypoxia. As his clinical condition was worsening, he was administered MSC transplantation. He had lymphocyte count 900 (6%), D-dimer >20, fibrinogen 543 before the transplantation. In the first 24 hours after the MSC transplantation, lymphocyte count increased to 1000 (9.5%) with an immediate response to therapy as a decrease in fibrinogen to 366. With an improving clinical progress, he was extubated on 10th day, and transferred to the ward on 13th day after MSC transplantation.

6th case: 75 year old woman with known diabetes mellitus, hypertension, and coronary heart disease came to emergency room with fever, cough, and shortness of breath. She was followed in the internal medicine service, with positive PCR test. Subsequently, she was intubated and transferred to the ICU. She was put on advised clinical treatment, including hydroxychloroquine, azithromycin, and favipiravir. As she poorly progressed, she was administered MSC transplantation with a d-dimer 1.55, lymphocyte count 800 (7.4%), and fibrinogen 489. On the 5th day of the MSC transplantation, we recorded an increase in lymphocytes to 1400 (11.3%) and a significant decrease in fibrinogen. Unfortunately, she died on the 9th day of the ICU stay due to septic shock.

7th case: 57 year old man with a known Gout disease was admitted with fever and diagnosed with COVID-19 due to a thorax CT scan consistent with bilateral ground-glass opacities, and a positive PCR test. He was placed on the medical regimen of hydroxychloroquine, azithromycin, and favipiravir. He was transferred to the ICU, when he developed dyspnea on the 9th day of hospital admission. He was intubated and required ECMO secondary to deep hypoxia. He was classified as critically severe and MSC transplantation was administered as a support therapy. He had an increase in his lymphocyte count from 500 to 2500 on the 5th day of the MSC transplantation compared to

pre transplantation. He was extubated when his clinical condition improved. He was discharged from the ICU on the 34th day after MSC transplantation.

8th case: 75 year old man without a coexisting disease, presented with high fever, fatigue, cough producing sputum and shortness of breath. His son was positive for COVID-19. His nasopharyngeal swab was obtained for PCR test and was positive. Thorax CT scan supported viral pneumonia. He was tachypneic (44 breaths per minute) and desaturated (sPO_2:88%) under 6 L/min. via face mask. After ICU admission, he was put on high flow nasal canula (HFNC; FiO_2:0.70, 60 L/min). His respiratory rate decreased to 33 breaths/min and peripheral oxygen saturation increased to 91%. Cyclic prone position was provided under HFNC. Despite of this intensive support with HFNC and prone position, his high oxygen demand did not diminish on the 4th day of ICU admission. He received MSC transplantation on the 5th day of the ICU stay. The laboratory tests before and after the MSC transplantation are presented in Table 2. He was weaned off HFNC on the 10th day, after five days of the MSC transplantation. His sPO_2 remained constant above 95% under 5 L/min O_2 via face mask and his respiratory rate was 18-23/min. He was discharged to the ward under 2 L/min O_2 via nasal cannula on day 13th after MSC transplantation.

DISCUSSION

In this report, eight clinically heterogeneous, unresponsive COVID-19 pneumonia patients received MSC transplantation and clinical improvement was achieved in four out of the eight patients. Thus, MSC transplantation could be introduced as a supportive treatment for severe/critically severe COVID-19 patients to improve their clinical outcomes by decreasing the need for mechanical respiratory support and shortens the hospitalization time in the ICU. Our results suggest a beneficial effect of MSC transplantation on systemic collapse due to the cytokine storm in COVID-19 patients. We demonstrated this positive effect by the significant improvement in the efficacy outcomes on the 5th day of the MSC transplantation.

Anti-inflammatory and immunomodulatory activities of MSCs have been well documented in more than 300 clinical trials registered for diseases like Multiple Sclerosis (MS), Type 1 Diabetes Mellitus (T1DM), Graft Versus Host Disease (GVHD), Osteoarthritis (OA), and Inflammatory Bowel Disease (IBD) [17]. In previous studies the immunomodulatory effects of MSCs are shown to be triggered further by the activation of TLR receptor in MSCs, which is stimulated by pathogen-associated molecules such as Lipopolysaccharide (LPS) or double-stranded RNA from virus, which is also presumed by HCoV-19 [4,18]. C-reactive protein is a sensitive biomarker in inflammatory processes, and reflects patient's response to infection including the production of cytokines, particularly TNF α , and IL-6 [19]. C-reactive protein is also a biomarker of myocardial damage [20]. Patients with higher CRP levels had a greater risk of MI and CVD. The most common pattern of coagulopathy found in severe COVID-19 patients is characterized by elevations in fibrinogen, and D-dimer levels [21].

We demonstrated that there is a significant relation between poor prognostic factors (CRP, NL, fibrinogen) and MSC transplantation before and the 5th day after the MSC transplantation. We have observed an immediate significant increase in lymphocyte count, with a decrease in CRP, and fibrinogen levels after the 5th day of the MSC transplantation in all patients (Table 4). This acute improvement in prognostic factors was especially striking in all patients, who received MSC transplantation. However, these laboratory improvements were not sufficient for four critically severe patients to reverse their clinical outcome and they passed away. These results may indicate proven characteristics of MSC transplantation to modulate immune and anti-inflammatory effects on severe COVID-19 cases. Additionally, none of the 3 supplementary COVID-19 patients demonstrated recovery in their prognostic laboratory markers.

Furthermore, two severe patients presented with poor prognostic markers showed a rapid recovery following the MSC transplantation and were discharged from the ICU on the 7th and 13th days after MSC transplantation without intubation. They exhibited a rapid improvement than the other critically severe recovered patients, who were already intubated, and subsequently extubated, and were discharged on the 13th and 34th days from the ICU. This clinical observation might suggest a potential benefit of earlier MSC transplantation that requires broader randomized studies. The COVID-19 syndrome has exhibited many unusual systemic involvements, including microthrombus and Disseminated Intravascular Coagulation (DIC) [22]. As earlier described, the systemic effect of MSCs is a paracrine regulatory mode in vivo rather than a cellular regenerative engraftment, suggesting the beneficial systemic paracrine support of MSCs for cardiac, neurologic, and other organ dysfunctions exhibited in COVID-19 pathogenesis [6]. Additionally, MSCs were identified as effective in reducing coagulation by suppressing fibrin microthrombi formation that subsequently reduced coagulation and alleviated liver, heart, lung, and renal injuries in LPS induced DIC rat model [23]. In literature, MSCs have been demonstrated by upregulation in the expression of genes linked to cell proliferation, survival, glycolysis, and angiogenesis under hypoxic pre-conditioning [6,24-26]. We postulate the clinical improvement in coagulopathy parameters like fibrinogen levels, and further recovery in our poor prognosis COVID-19 patients might be due to the combination of all these immunomodulatory and anti-inflammatory effects of MSC transplantation. More studies are required to establish the potential beneficial therapeutic effects of MSC transplantation on COVID-19 patients.

CONCLUSION

MSC transplantation offers a supportive treatment option in non responsive COVID-19 patients. We advise performing the MSC transplantation at an earlier stage of the disease to enable the positive immune regulatory effect of MSCs on the destructive cytokine storm. Accordingly, our findings could be a road map for larger randomized controlled studies to investigate biotechnological tools like MSCs, to enlighten the physio pathological pathway of action mechanisms and the clinical

application algorithms to achieve more accurate treatment options for COVID-19.

Our observations and the recent studies on COVID-19 suggest that COVID-19 without a doubt, has a new generation acting mechanism, which we have not recognized before with any other infectious agent in literature; reminding us that it may not be the last one. These consequences could be considered as a strong warning to scientists that we should focus on investigating de nova biotechnology products to diagnose, treat, and prevent the new infectious agents by keeping the safety methodology and guidelines. The new products should aim to be effective on the multidimensionally systemic-likewise artificially intelligent-infectious/pathological agents like COVID-19, and possible others.

CONFLICT OF INTEREST

The authors report no conflicts of interest concerning the materials or methods used in this review or the findings specified in this paper. The authors have no competing financial interests related to this study.

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