Am J Stem Cells 2022;11(3):37-55
www.AJSC.us /ISSN:2160-4150/AJSC0143684

Original Article

Phase 1 clinical trial for intravenous administration of mesenchymal stem cells derived from umbilical cord and placenta in patients with moderate COVID-19 virus pneumonia: results of stage 1 of the study

Alok Sharma[1,2], Rohit Kulkarni[3], Hemangi Sane[4], Nilkanth Awad[5], Abhijit Bopardikar[3], Anagha Joshi[6], Sujata Baweja[7], Mohan Joshi[8], Chandra Vishwanathan[3], Nandini Gokulchandran[2], Prerna Badhe[9], Mazhar Khan[1], Amruta Paranjape[4], Pooja Kulkarni[4], Arjun K Methal[4]

[1]Department of Neurosurgery, LTMG Hospital and LTM Medical College, Mumbai, Maharashtra, India; [2]Department of Medical Services and Clinical Research, NeuroGen Brain & Spine Institute, Navi Mumbai, Maharashtra, India; [3]ReeLabs Pvt. Ltd, Mumbai, Maharashtra, India; [4]Department of Research & Development, NeuroGen Brain & Spine Institute, Navi Mumbai, Maharashtra, India; [5]Department of Pulmonary Medicine, LTMG Hospital and LTM Medical College, Sion, Mumbai, Maharashtra, India; [6]Department of Radiology, LTMG Hospital and LTM Medical College, Mumbai, Maharashtra, India; [7]Department of Microbiology, LTMG Hospital and LTM Medical College, Mumbai, Maharashtra, India; [8]Dean, LTMG Hospital and LTM Medical College, Mumbai, Maharashtra, India; [9]Department of Regenerative Laboratory, NeuroGen Brain and Spine Institute, Seawoods, Navi Maharashtra, India

Received April 22, 2022; Accepted June 10, 2022; Epub June 15, 2022; Published June 30, 2022

Abstract: Objective: Mesenchymal stem cells can serve as a therapeutic option for COVID-19. Their immunomodulatory and anti-inflammatory properties can regulate the exaggerated inflammatory response and promote recovery of lung damage. Method: Phase-1, single-centre open-label, prospective clinical trial was conducted to evaluate the safety and efficacy of intravenous administration of mesenchymal stem cells derived from umbilical cord and placenta in moderate COVID-19. The study was done in 2 stages with total 20 patients. Herein, the results of stage 1 including first 10 patients receiving 100 million cells on day 1 and 4 with a follow up of 6 months have been discussed. Results: No adverse events were recorded immediately after the administration of MSCs or on follow up. There was no deterioration observed in clinical, laboratory and radiological parameters. All symptoms of the study group resolved within 10 days. Levels of inflammatory biomarkers such as NLR, CRP, IL6, ferritin and D-dimer improved in all patients after intervention along with improved oxygenation demonstrated by improvement in the SpO2/FiO2 ratio and PaO2/FiO2 ratio. None of the patients progressed to severe stage. 9 out of 10 patients were discharged within 9 days of their admission. Improvements were noted in chest x-ray and chest CT scan scores at day 7 in most patients. No post-covid fibrosis was observed on chest CT 28 days after intervention and Chest X ray after 6 months of the intervention. Conclusion: Administration of 100 million mesenchymal stem cells in combination with standard treatment was found to be safe and resulted in prevention of the cytokine storm, halting of the disease progression and acceleration of recovery in moderate COVID-19. This clinical trial has been registered with the Clinical Trial Registry- India (CTRI) as CTRI/2020/08/027043. http://www.ctri.nic.in/Clinicaltrials/pmaindet2.php?trialid=43175.

Keywords: COVID-19, mesenchymal stem cells, umbilical cord, placenta, cytokine storm

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified in 2019. Due to its highly contagious nature, the World Health Organization (WHO) declared COVID-19 a global pandemic in January 2020 [1]. COVID-19 spread quickly around the world and has affected millions of people so far primarily affecting the respiratory tract and lungs. The clinical spectrum of COVID-19 ranges from asymptomatic infection to critical illness. Path-

ophysiological studies have reported cytokine dysregulation and hyperinflammation associated with viral infection in severe and critical illness [2]. Elevated levels of pro-inflammatory cytokines result in a cytokine storm causing lung tissue edema, air exchange dysfunction, acute respiratory distress (ARDS), lung fibrosis, secondary infection, multiorgan failure often leading to death. Pre-existing comorbidities such as diabetes, high blood pressure, and obesity result in escalated complications. Mortality rate is higher in these patients who demonstrate cytokine storm [3, 4].

Many treatment strategies have been recommended since the diagnosis of COVID-19 and lot of research is ongoing to establish a cure for the disease. Pharmacological management for treatment of COVID-19 includes hydroxychloroquine, azithromycin, remdesivir, favipiravir, to immunomodulators *e.g.* steroid and IL-6 antagonists (tocilizumab, itolizumab) [5]. Although, these agents showed promising results in in-vitro studies, the mortality rate remains high in moderate and severe COVID-19. Hence, other options are being evaluated either as drug therapy or cell therapy e.g. SARS-CoV-2 entry inhibitors, Fusion inhibitors, RdRp inhibitors, NSP-15 inhibitors, umbilical cord MSCs, etc. [6].

Due to its immunomodulatory properties, mesenchymal stem cells (MSC) have the potential to combat COVID-19 infection. In the past, MSC treatment was found to be safe and effective in various conditions such as pulmonary, orthopedic, neurologic, cardiac conditions, etc. It has also shown promise in other viral infections like Human immunodeficiency virus (HIV), hepatitis B virus, virus associated ARDS, influenza amongst others [7-10]. Patients with mild COVID-19 usually recover at home, with supportive care and isolation. Infection-hospitalization ratio is estimated to be 0.4% for individuals younger than 40 years and 9.2% for those older than 60 years. 10-20% of infected individuals progress to severe or critical stage due to cytokine storm and about 3.5% is the mortality rate [11, 12]. As cytokine storm is known to be one of the major reasons associated with mortality in COVID-19, immunoregulatory agents may result in a positive outcome [13]. Therefore, we need to evaluate whether MSCs can help preventing the progression in moderate stage patients, help in faster recovery or reduce mortality.

This study was conducted to evaluate the safety and efficacy of mesenchymal stem cells derived from umbilical cord and placenta in moderate COVID-19 pneumonia. The primary endpoint of the study was to evaluate safety of the MSCs administered while the secondary endpoint was to evaluate the efficacy of these MSCs in moderate COVID-19 patients.

Material and methods

Study design and participants

This study was a Phase 1, single-centre open label, prospective clinical trial conducted at Lokmanya Tilak Municipal General (LTMG) Hospital and Medical College, Mumbai, India in collaboration with NeuroGen Brain and Spine Institute and ReeLabs Pvt. Ltd. The study was conducted in 2 stages with total 20 patients. Stage 1 of the study including first 10 patients receiving 100 million cells has been completed with 6 months follow up. The dose was escalated to 200 million cells on day 1 and day 4 for stage 2 including next set of 10 patients which is still ongoing. Herein, the results of stage 1 with first 10 patients have been discussed. The protocol of the study was approved by the Central Drugs Standard Control Organisation (CDSCO), Ministry of Health & Family Welfare, Government of India and the Institutional Ethics Committee of LTMG Hospital and Medical College. This clinical trial has been registered with the Clinical Trial Registry-India (CTRI) as CTRI/2020/08/027043. A written informed consent was obtained from all the patients and relatives before the intervention. An independent data safety and monitoring board (DSMB) was constituted to monitor any adverse events.

Inclusion criteria

• Patients admitted with RT-PCR confirmed COVID-19 illness.

• Age: 18-65 years without any uncontrolled co-morbidities.

• Has any of the two below criteria of moderate pneumonia:

(a) PaO2/FiO2: 200-300.

(b) Respiratory Rate > 24/min and SaO2 ≤ 93% on room air.

Exclusion criteria

- Pregnant women.
- Breastfeeding women.
- Critically ill patients:

(a) PaO2/FiO2 ratio < 200 (ARDS).

(b) Shock (Requiring Vasopressor to maintain a MAP ≥ 65 mmHg or MAP below 65).

- Patients with other severe co-morbidities like cancer, chronic renal, chronic liver failure and chronic cardiac failure. This will not include diabetes, hypertension, etc.
- Participating in any other clinical trial.

Pre-intervention assessment

In stage 1 (100 million cells), 10 patients who met the inclusion and exclusion criteria were recruited in the study. All these patients underwent a detailed assessment prior to the intervention. Their medical history was recorded alongwith clinical assessment. Serological, biochemical and hematological tests which included Complete blood count, Renal function test (BUN, creatinine and GFR), Liver function test, Serum Creatine Kinase (CK), Creatine Kinase Myocardial Band (CK-MB), Troponin and myoglobin test, Serum electrolytes test, Serum HbA1Ctest, Serum Fibrinogen test, D-dimer test, Serum C-reactive protein test, NT-pro BNP, HIV, HCV and HBV test was conducted. Arterial blood gas analysis (ABG), Electrocardiogram (ECG) was performed along with Chest X-ray and Chest CT scan.

Intervention

Cell transplantation

Cells used for this study were a mixture of MSCs derived from human umbilical cord blood and placenta. They were obtained from ReeLabs, Mumbai, a cGMP facility with a cord blood banking license and Form 29. The procurement and banking of these cells was done as per government guidelines. Cell culture medium used was StemProTM MSC SFM XenoFree for cell culture. All 10 patients received two doses of 100 million cells each on day 1 and day 4 along with standard treatment. Cells were suspended in 100 ml of normal saline and were administered intravenously over forty minutes with a speed of ~40 drops per minute.

Standard treatment

All patients received standard medication which included antibiotics (cephalosporin, ivermectin, doxycycline), one antiviral (Lopinavir/ritonavir or Favipiravir or Remdisivir), low molecular weight heparin, methylprednisolone, vitamin supplements (vitamin E, vitamin C, zinc, multivitamins) and antacid (pantoprazole). Other medicines were administered for symptomatic relief such as paracetamol for fever, antitussive for cough, etc. Supplemental oxygen for patients who had oxygen saturation (SpO2) below 95% on room air. None of the patients received Tocilizumab.

Data collection

Clinical parameters were measured daily by the clinical staff from Day 1 to discharge. Laboratory parameters, ABG and ECG were performed before the intervention (Day 1) followed by Day 2, 4, 6, 8 and 14. Chest X-Ray and Chest CT scan was performed on Day 1, 7, 14 and 28. Data was recorded in the case record form and electronically.

Outcome measures

Primary endpoint: safety evaluation

1. Avoidance of:

- Progression to severe ARDS (P/F ratio < 100).
- All-cause Mortality at 28 days.

2. Oxygenation Index: SpO2.

3. Multi Organ Function: CBC, LFT, Serum Creatinine, Electrolytes, troponin and myoglobin.

4. Clinical measures: Fever and Respiratory Rate.

5. Adverse Events occurring immediately after IV administration of MSCs (Hematoma, Local infection, Pain or Bleeding at the site of injection and Thrombophlebitis).

6. Other adverse events e.g. Pulmonary embolism, Stroke, Arrhythmias, Liver failure.

Secondary endpoint: efficacy evaluation

1. Time to symptom resolution:

• Fever.

• Cough.

2. Shortness of Breath.

3. Duration of Hospital stay after intervention.

4. Change in SOFA pre and post intervention.

5. Duration of respiratory support required:

• Duration of Invasive Mechanical Ventilation.

• Duration of Non-Invasive Ventillation.

6. Radiological improvement (Chest X Ray/ Chest CT scan) at Days 7, 14, 21 and 28 days after intervention.

7. Adverse events (AE) associated with intervention.

8. Duration of conversion of positive to negative Covid 19 from RT PCR at Days 7, 14 and 28 days after intervention.

9. Change in levels of biomarkers (CRP, IL6, Ferritin) at Days 2, 4, 6, 8, 14 days after intervention.

10. Time of Recovery on 8-point Ordinal Scale.

Safety and adverse event monitoring

Safety monitoring was performed everyday. Progression of symptoms, laboratory parameters, vital signs and clinical assessments were monitored closely.

The adverse events monitored included.

Major: Allergic reaction, anaphylactic reaction, secondary infection, life-threatening adverse events, acute respiratory failure requiring mechanical ventilation, myocardial infarct, heart failure, Pulmonary embolism, stroke, arrhythmias, liver failure and mortality.

Minor: Hematoma, local infection, pain or bleeding at the site of injection and thrombophlebitis.

Any adverse events if recorded were reported to the committee.

Data analysis

A detailed analysis was performed to study the outcome of the intervention. Neutrophil-Lymphocyte Ratio, CRP, IL-6, Ferritin, D-Dimer, SpO2/FiO2 ratio and PaO2/FiO2 ratio were analysed. Median of their values was calculated and a line graph with standard error was plotted to study the overall trend in the study population. Resolution of clinical symptoms was evaluated by calculating frequency of symptoms present on Day 1 and average number of days taken for resolution of these symptoms. Chest X rays were scored subjectively based on visual assessment by an expert radiologist. X-ray findings before the treatment were noted and the days at which improvement was seen and complete resolution was seen was computed and used for analysis. Comparison of Chest CT scores on Day 1, 7, 14, 21 and 28 days was performed.

Results

Demographics

231 patients were screened, out of which 10 who met the inclusion criteria were included in the study. Mean age of the study group was 47.3 years (**Table 1**). The frequency of symptoms of COVID-19 observed in the recruited participants of the study has been tabulated in **Table 2**.

Primary endpoint: safety evaluation

No adverse reactions to the administration of MSCs were identified immediately after intervention or on follow up. Till day 14, there were no negative changes in laboratory parameters such as Total White cell count (WBCs), Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, Hemoglobin (g/dL), Red-cell count, Platelet count, Potassium, Sodium, Serum Creatinine, BUN, GFR, Total bilirubin, Direct bilirubin, Albumin, Aspartate amino transferase, SGOT, Alanine transaminase, SGPT, Serum Creatine Kinase-MB, Serum Creatine Kinase-Total, Troponin, Myoglobin, Serum fibrinogen, NT pro BNP. No deterioration in ABG parameters were observed: pH, Partial pressure of oxygen (PaO2) (mmHg), Partial pressure of carbon dioxide (PaCO2) (mmHg), Bicarbonate (HCO3-) (mEq/L), Oxygen saturation (O2 Sat), Oxygen (Litres) -ABG, Oxygen requirement, FiO2, SpO2/FiO2, PaO2/FiO2, and likewise in ECG.

Table 1. Demographic data of the study group

Demographics	Description	Numbers
Total Number of patients screened		231
Sample Size		10
Gender	Male	8
	Female	2
Age	Range	28 to 65 years
	Mean	47.3 years
Comorbidities	Diabetes	6
	Hypertension	4
	Vitilgo	1
	Past history of Tuberculosis	1
	None	3
Follow up duration		6 months

Table 2. Frequency of symptoms of COVID-19 observed in patients recruited in this study

Symptom	Frequency	Percentage
Cough	9	90
Sputum	5	50
Shortness of breath	10	100
Sore throat	8	80
Chest pain	3	30
Loss of appetite	9	90
Loss of Taste	6	60
Loss of Smell	4	40
Generalized weakness/Fatigue	9	90
Giddiness	2	20
Headache	2	20
Muscle aches and pains	1	10

At 6 months, follow up X-rays were performed for 7 patients which did not reveal any significant abnormality. One patient had expired due to cardiac arrest after 3.5 months post intervention (unrelated to cell therapy).

Secondary endpoint: efficacy evaluation

Efficacy of intervention was assessed by evaluating the time taken for COVID-19 symptoms to resolve, changes in levels of biomarkers, oxygenation and radiological findings.

Time to symptom resolution

Before completion of Day 14, all patients were clinically stable and discharged. Time to symptom resolution was noted for all the patients (**Table 3**).

Changes in levels of biomarkers

Neutrophil-Lymphocyte ratio (NLR): Neutrophil-Lymphocyte ratio is a cost-effective inflammatory biomarker used to study the progression of COVID-19. Increased NLR may be indicative of excessive inflammation and immune suppression caused due to SARS-CoV-2. Median of NLR was calculated for each patient and a line graph was plotted. In the study group, NLR increased on Day 2, decreased on Day 4 and showed an increase again on Day 6. However, Day 6 onwards median NLR reduced consistently. Overall, a decreasing trend was observed (**Figures 1**, **2**).

C-Reactive Protein (CRP): The rapid clinical improvement was accompanied by decrease in inflammatory biochemical markers level. The levels of CRP decreased sharply on the second day after the administration of MSCs (**Figures 3**, **4**) and were maintained within the normal range of 0-6 mg/L.

Interleukin 6 (IL6): The blood levels of IL-6 increased beyond the normal range of 0-7 pg/ml from Day 1 to Day 4. The values began to reduce from Day 4 onwards and were within normal limits on Day 14. Patient 7 showed extremely high levels of IL 6 on day 1 which further increased on day 2 and gradually reduced thereafter. This change may be attributed to the immunomodulatory effect of MSCs. (**Figures 5**, **6**) MSCs are able to increase the lymphocyte count and regulatory dendritic cells to raise their antiviral characteristic which results in the decreased level of CRP and pro-

Table 3. Duration of resolution of symptoms

Symptoms	Average no. of days for resolution
All symptoms	10
Cough	5
Sputum	4
Shortness of breath	9
Sore throat/Dryness	3
Chest pain	2
Loss of appetite	4
Loss of Taste	3
Loss of Smell	4
Generalized weakness/Fatigue	8
Giddiness	4
Headache	1
Muscle aches and Pain	11
Requirement of supplemental oxygen	9

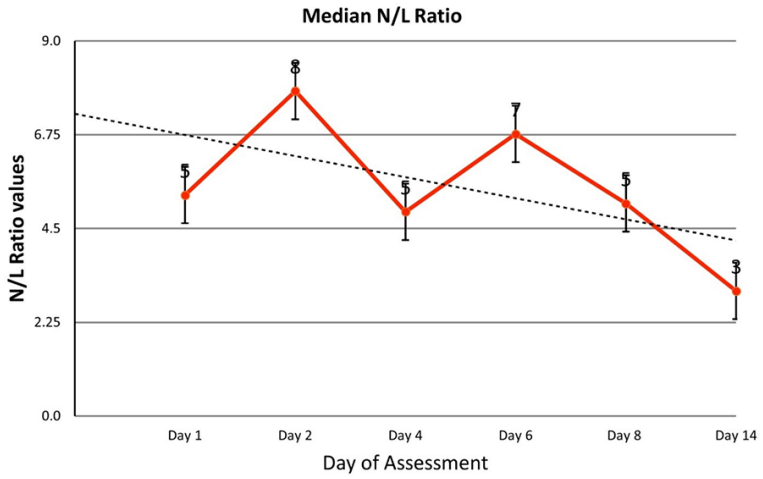

Figure 1. Changes in median Neutrophil-Lymphocyte Ratio after intervention on Day 1, 2, 4, 6, 8 and 14.

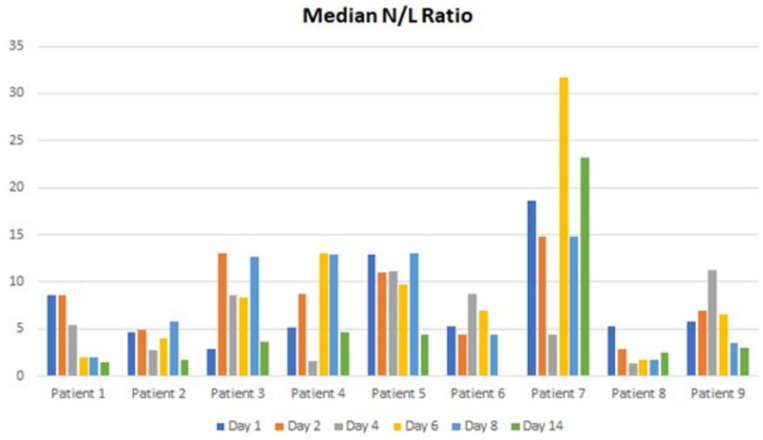

Figure 2. Changes in Neutrophil-Lymphocyte Ratio in each patient after intervention on Day 1, 2, 4, 6, 8 and 14.

inflammatory cytokines such as IL-6 to reduce the inflammation and oxidative stress.

Ferritin: The median ferritin levels were slightly higher than the normal range on Day 1 which further increased on Day 2. However, after administration of MSCs the levels of ferritin started to reduce and were found to be within normal range on Day 6 (**Figures 7**, **8**). In 4 out of 10 patients the levels showed a decreasing trend but did not normalize till Day 14. Ferritin is a key mediator of immune dysregulation, via direct immune-suppressive and pro-inflammatory effects, contributing to the cytokine storm which further results in a fatal outcome. However, as recorded in this study the median ferritin levels which were demonstrating an increasing trend started to reduce after intervention. This can be attributed to the immunomodulatory effects of MSCs.

D-Dimer: The levels of D-dimer in the blood increased from Day 1 to Day 6, after which it sharply decreased and did not differ from normal values. This suggests that MSCs promote the rapid elimination of intravascular hemocoagulation, which effectively prevents microthrombosis of the lung vessels (**Figures 9**, **10**).

Improvement in markers of oxygenation

SpO2/FiO2: The ratio of blood oxygen saturation to the fraction of inhaled oxygen (SpO2/FiO2) is a surrogate marker of the risk of developing acute respiratory distress syndrome (ARDS). A decrease in this index may indicate risk of acute

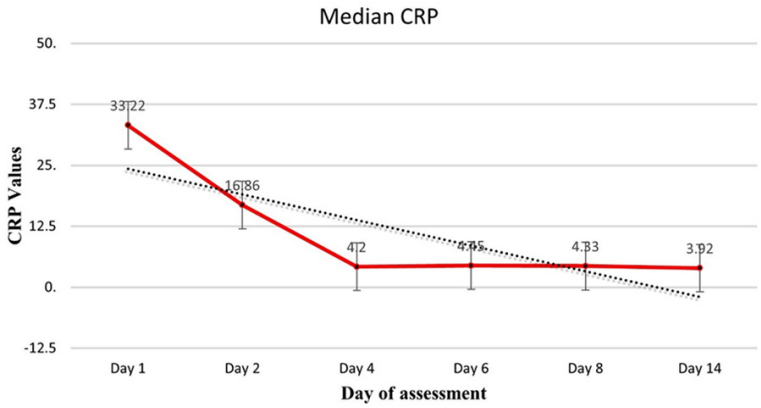

Figure 3. Changes in Median C-reactive protein after intervention on Day 1, 2, 4, 6, 8 and 14.

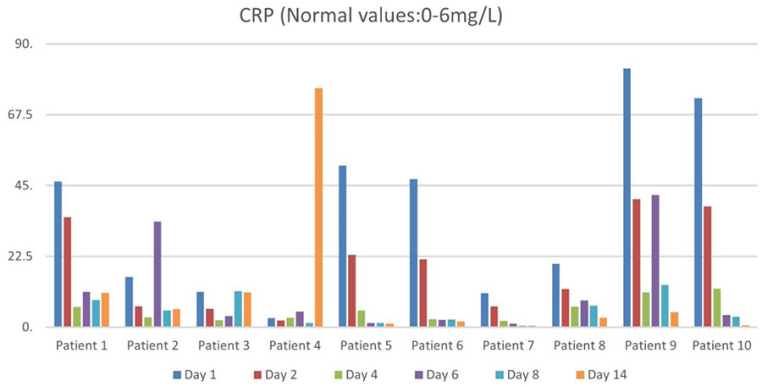

Figure 4. Changes in C-reactive protein levels in each patient after intervention on Day 1, 2, 4, 6, 8 and 14.

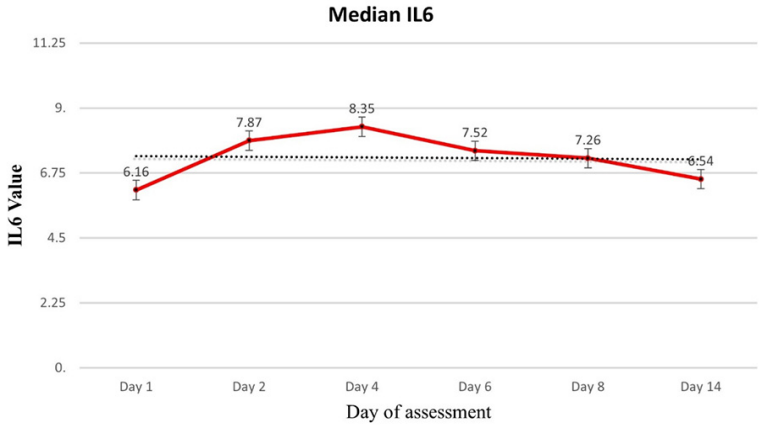

Figure 5. Changes in interleukin 6 levels after intervention on Day 1, 2, 4, 6, 8 and 14.

respiratory distress syndrome. In this study, administration of MSCs resulted in a rapid and effective increase in SpO2/FiO2 demonstrating the ability of MSCs to prevent the development of ARDS and hypoxemia in COVID-19 patients and further improve oxygenation (**Figures 11**, **12**).

PaO2/FiO2: PaO2/FiO2 is the oxygenation index used to assess the function of oxygen exchange in lungs. It is a ratio of partial oxygen tension in arterial blood to the oxygen fraction during inspiration. A decrease in this index is an accurate marker of the threat of ARDS.

On administration of MSCs, the PaO2/FiO2 increased rapidly and progressively, which indicates the ability of MSCs to significantly improve ventilation-perfusion mechanisms in patients with oxygen-dependent COVID-19 (**Figures 13**, **14**).

Radiological findings

Chest X-ray: On chest X-ray, 8 out of 10 patients showed significant bilateral lung infiltrates. 7 (87.5%) out of these 8 patients resolved completely in average 19 days (**Figure 15**). 1 of these 8 had significantly improved by day 21 but did not completely resolve. No new abnormality or adverse effect of MSCs transplantation was noted in Chest radiograph 6 months post the treatment.

Chest CT scan scores: Average CT severity score of all of the patients was 14 on Day 1 and reduced to 8 on Day 28 showing that there was significant reduction in the severity of the disease. 9/10 patients showed reduction in the CT score on Day 7. All of the patients showed significant reduction in CT scores by

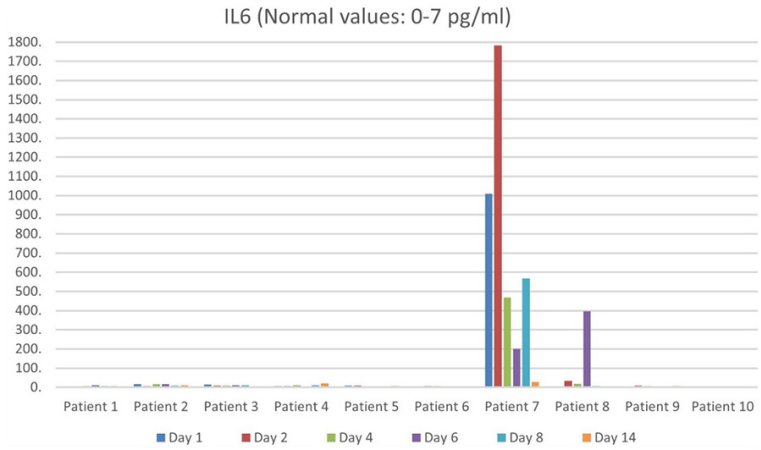

Figure 6. Changes in IL 6 levels of each patient after intervention on Day 1, 2, 4, 6, 8 and 14.

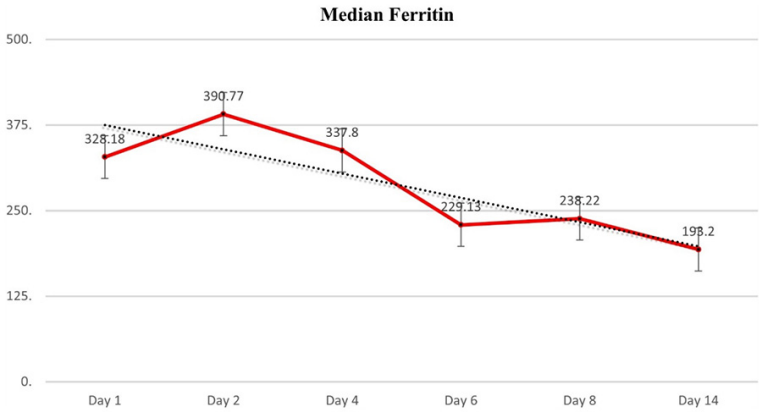

Figure 7. Changes in Levels of blood ferritin after intervention on Day 1, 2, 4, 6, 8 and 14.

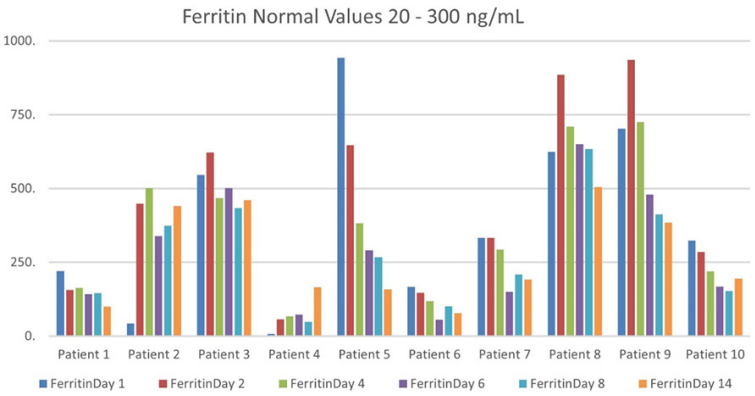

Figure 8. Changes in levels of blood ferritin in each patient after intervention on Day 1, 2, 4, 6, 8 and 14.

Day 28 (**Table 4**). **Figure 16A-C** show changes in serial CT scans.

Discussion

Pathophysiology of SARS-CoV-2

SARS-CoV-2 binds to ACE2 receptors to enter the target cell, where the virus replicates and subsequently infects other cells. The virus connects to ACE2 via a glycoprotein called peplomer. The lungs are the most affected as the enzyme ACE2 is abundantly present in the type II alveolar cells of the lungs. But these receptors are also present on heart, liver, kidney, intestines etc. When the virus enters the lungs, it infects the upper respiratory tract and then alveolar tissue, which may eventually progress to respiratory failure leading to death. As it enters blood circulation the virus spreads and may cause damage to other distant organs such as kidneys, heart and brain, gastrointestinal organs, liver etc. [14, 15]. Cytokine storm and pneumonia-associated hypoxia might also contribute to liver injury or even develop into liver failure in patients with COVID-19 who are critically ill [16].

Cytokine storm in COVID-19

It has been reported that, dysregulated cytokine/chemokine responses and higher virus titers cause an inflammatory cytokine storm with lung immunopathological injury in COVID-19. Such Inflammation associated with the cytokine storm may begin at one local site and further spread throughout the body via the systemic circulation [17]. Similarly, infected patients have shown increased plasma concentrations of inflammatory cytokines, including interleukins (IL) 2, 6, 7, and 10, granulocyte-colony

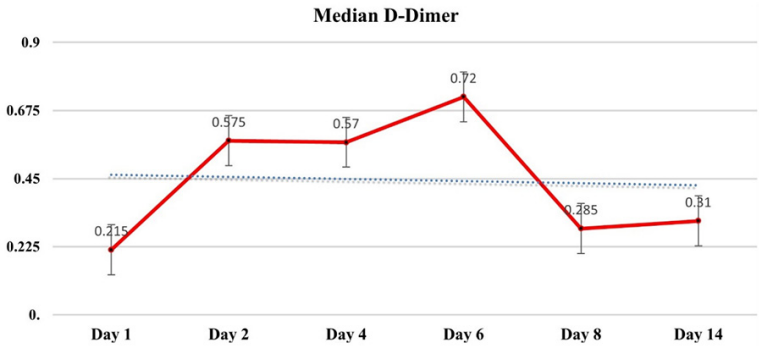

Figure 9. Changes in levels of D-dimer after intervention on Day 1, 2, 4, 6, 8 and 14.

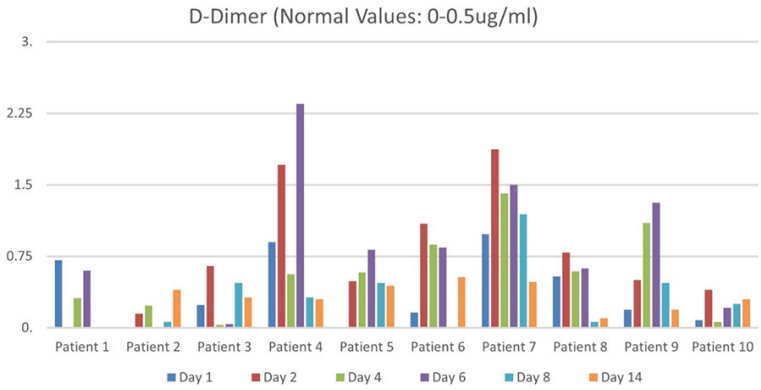

Figure 10. Changes in levels of D-dimer in each patient after intervention on Day 1, 2, 4, 6, 8 and 14.

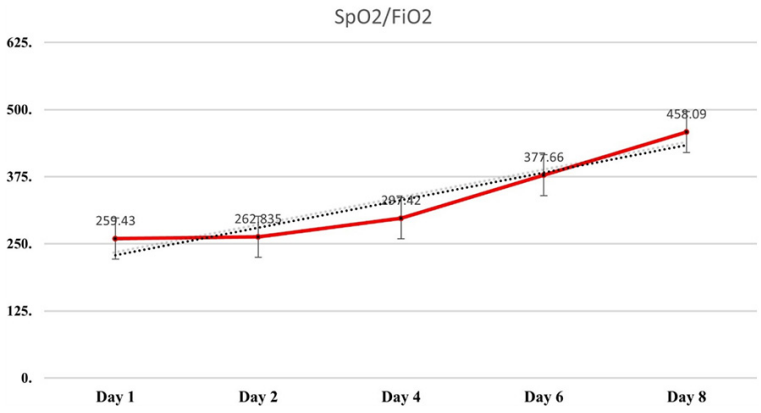

Figure 11. Changes in the SpO2/FiO2 index after intervention on Day 1, 2, 4, 6, 8 and 14.

stimulating factor (G-CSF), monocyte chemoattractant protein 1 (MCP1), macrophage inflammatory protein 1 alpha (MIP1A), interferon-ɣ-inducible protein 10 (IP10) and TNF-α [18].

Large amount of inflammatory cell infiltrations have been observed in lungs from severe COVID-19 patients, these aberrant pathogenic Th1 cells and inflammatory monocytes may enter the pulmonary circulation in huge numbers and play an immune damaging role to cause lung functional disability and quick mortality. In a study including 41 patients with COVID-19, Huang, et al. demonstrated a cytokine profile that was like that of secondary hemophagocytic lymphohistiocytosis (sHLH), a hyper inflammatory condition triggered by viral infection [19]. The patients admitted in the ICU showed higher levels of granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma-induced protein 10 (IP10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1 alpha (MIP1A), and tumor necrosis factor alpha (TNFα). The role of a hyperinflammatory response underlying development of severe and critical illness was confirmed in another study by higher serum levels of C-reactive protein (CRP), interleukin-6 (IL-6) and Ferritin in patients that had died [20]. The dysregulated immune response results in endothelial injury and formation of micro and macro blood clots which together with pulmonary inflammation led to a diffuse alveolar damage, fibrin exudates and fibrotic healing in the lungs which further compromises the oxygen absorption causing oxygenation failure and acute respiratory distress syndrome (ARDS). This may also result in secondary pulmonary infections such as bacterial pneumonia [21]. Prevention or attenuation of cytokine storm is therefore crucial to lower COVID-19-induced mortality.

There are no approved treatments for COVID-19 but some medications including antiviral,

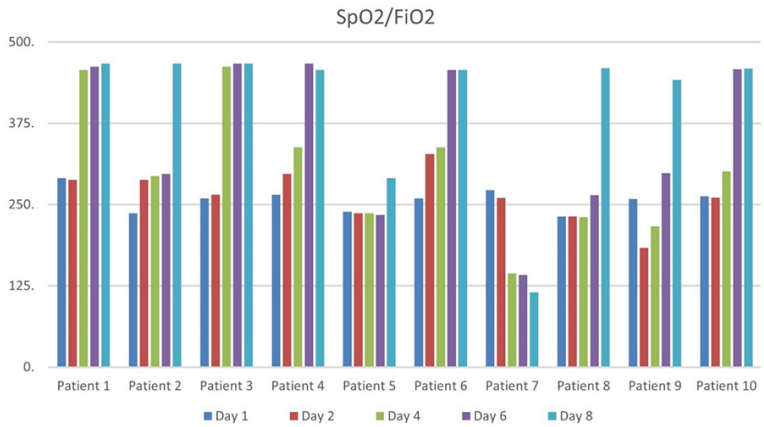

Figure 12. Changes in the SpO2/FiO2 index in each patient after intervention on Day 1, 2, 4, 6, 8 and 14.

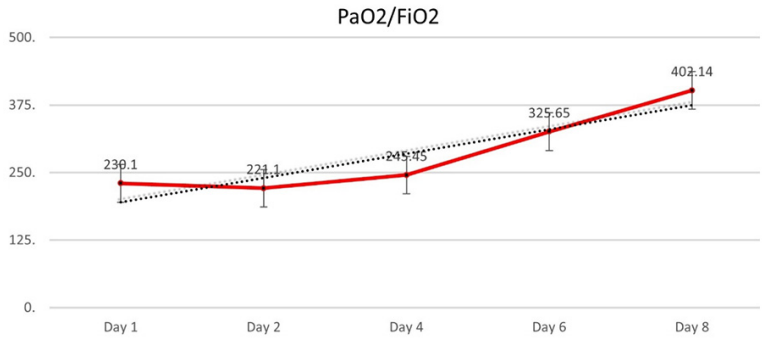

Figure 13. Changes in the PaO2/FiO2 index after intervention on Day 1, 2, 4, 6, 8 and 14.

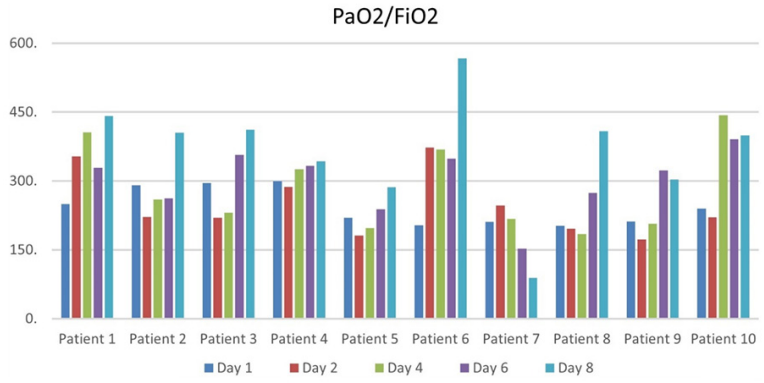

Figure 14. Changes in the PaO2/FiO2 index in each patient after intervention on Day 1, 2, 4, 6, 8 and 14.

anti-inflammatory have shown to be beneficial. There is an urgent need to explore treatment strategies that can prevent patients from worsening clinically and progressing to severe or critical stage. Due to its immunomodulatory properties, cell therapy has a potential to halt the progression of the disease and accelerate the recovery process.

To evaluate the safety and efficacy of cell therapy in COVID-19, we administered 10 patients with moderate illness with a mixture of 100 million MSCs derived from umbilical cord and placenta.

Rationale for use of MSCs derived from umbilical cord and placenta

Cellular therapy has been studied widely for treating various conditions, including pulmonary, immunological, haematological, cardiac, neurological, hepatic, endocrine, musculoskeletal, skin, and ophthalmological diseases [22-28]. Various clinical studies have demonstrated the safety and efficacy of umbilical cord and placental MSCs individually [29-34]. For this study, a mixture of umbilical cord and placenta derived MSCs was used since, a mixture results in an enhanced outcome combining the benefits of both types of cells. Invitro studies have shown that this mixture of cells secretes increased concentration of paracrine molecules such as IGF-1, KGF, VEGF, SDF-1α as compared to individual cell types which are responsible for stimulating lung repair, angiogenesis, stem cell recruitment, etc. Acute toxicity, chronic toxicity, genotoxicity, immunotoxicity, tumoregenic potential studies conducted for this mixture of cells showed that it was non-toxic, non- mutagenic, non-tumorogenic and had no immunotoxic effect on the organs of the immune system, and the cell parameters of peripheral blood and bone marrow. These cells are safe,

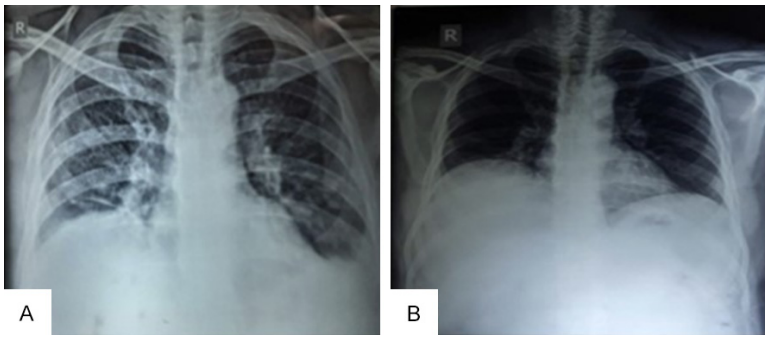

Figure 15. Comparitive Chest Radiographs done on Day 1 and Day 14 showing complete resolution of opacities in bilateral mid & lower zones.

Table 4. Radiological improvement as observed on chest CT scan scores (Patients 4 and 6 did not come to do the CT scan on Day 28)

Patient name	Pre-Intervention CT	Day 7 CT	Day 14 CT	Day 21 CT	Day 28 CT
Patient 1	11	11	9	6	4
Patient 2	15	13	12	10	7
Patient 3	14	12	10	9	7
Patient 4	23	19	19	19	Not done
Patient 5	10	9	7	6	6
Patient 6	11	7	6	5	Not done
Patient 7	15	17	16	14	13
Patient 8	17	15	12	12	12
Patient 9	10	8	6	5	4
Patient 10	17	15	12	10	10

effective and are easily available without any ethical barriers.

Immunomodulatory (paracrine) effects of MSCs counteracting the cytokine storm

Umbilical cord cells also attenuate lung inflammation by a multitude of paracrine functions, including enhanced interleukin (IL)-10 expression and modulation via prostaglandin-E2 (PGE2), GM-CSF, IL-6 and IL-13 [35]. Both umbilical cord and placenta derived stem cells secrete paracrine factors, including human angiopoietin-1 (Ang-1), Hepatocyte Growth Factor (HGF), insulin-like growth factor I (IGF-I), prostaglandin E2 (PGE2), transforming growth factor beta 1 (TGF-β1), vascular cell adhesion protein 1 (VCAM-1) and Vascular Endothelial Growth Factor (VEGF), in varying levels. Although they have different growth dynamics, stem derived cells show the highest secretion of Ang-1 and VEGF and the lowest secretion of TGF-β1, while umbilical cord derived cells show the highest secretion of IGF-I, PGE2 and TGF-β1, HGF and VCAM-1 [36].

Leng et al. 2020 have also demonstrated that anti-inflammatory and trophic factors like Transforming Growth Factor (TGF)-β, HGF, VEGF, Leukaemia Inhibiting Factor (LIF), Galanin (GAL), Nitric Oxide Associated protein 1 (NOA1), Fibroblast Growth Factor (FGF), Epidermal Growth Factor (EGF), Brain-derived Neurotrophic Factor (BDNF), and Nerve Growth Factor (NGF) are highly expressed in mesenchymal stem cells, further bolstering their immunomodulatory role [37].

Differentiation of MSCs into AT2 cells and their immunity towards COVID-19

The COVID-19 causing novel coronavirus 2019-nCoV uses the angiotensin converting enzyme II (ACE2) and Transmembrane Serine Protease 2 (TMPRSS2) receptor to enter host cells [38]. ACE2 is enriched in human alveolar cells [39] and TMPRSS2 is a known human airway and alveolar protease [40]. This may explain the rapid infection rate observed worldwide. It is shown that mesenchymal stem cells are ACE2 and TMPRSS negative, which indicates that these cells may not be susceptible to COVID-19 [37].

Surface Protein (SP) A and C are highly expressed in umbilical mesenchymal stem cells, indicating that these might differentiate to alveolar epithelial (AT2) cells [37]. These cells have also been shown to differentiate into a variety of alveolar cells and integrate into target tissue in rodent models [35, 41]. The human placenta also enriches mesenchymal stem cells, which are multipotent progenitors [42].

Antiviral effects of MSCs

MSCs transplantation has shown significantly reduced mortality in H7N9 induced ARDS [43].

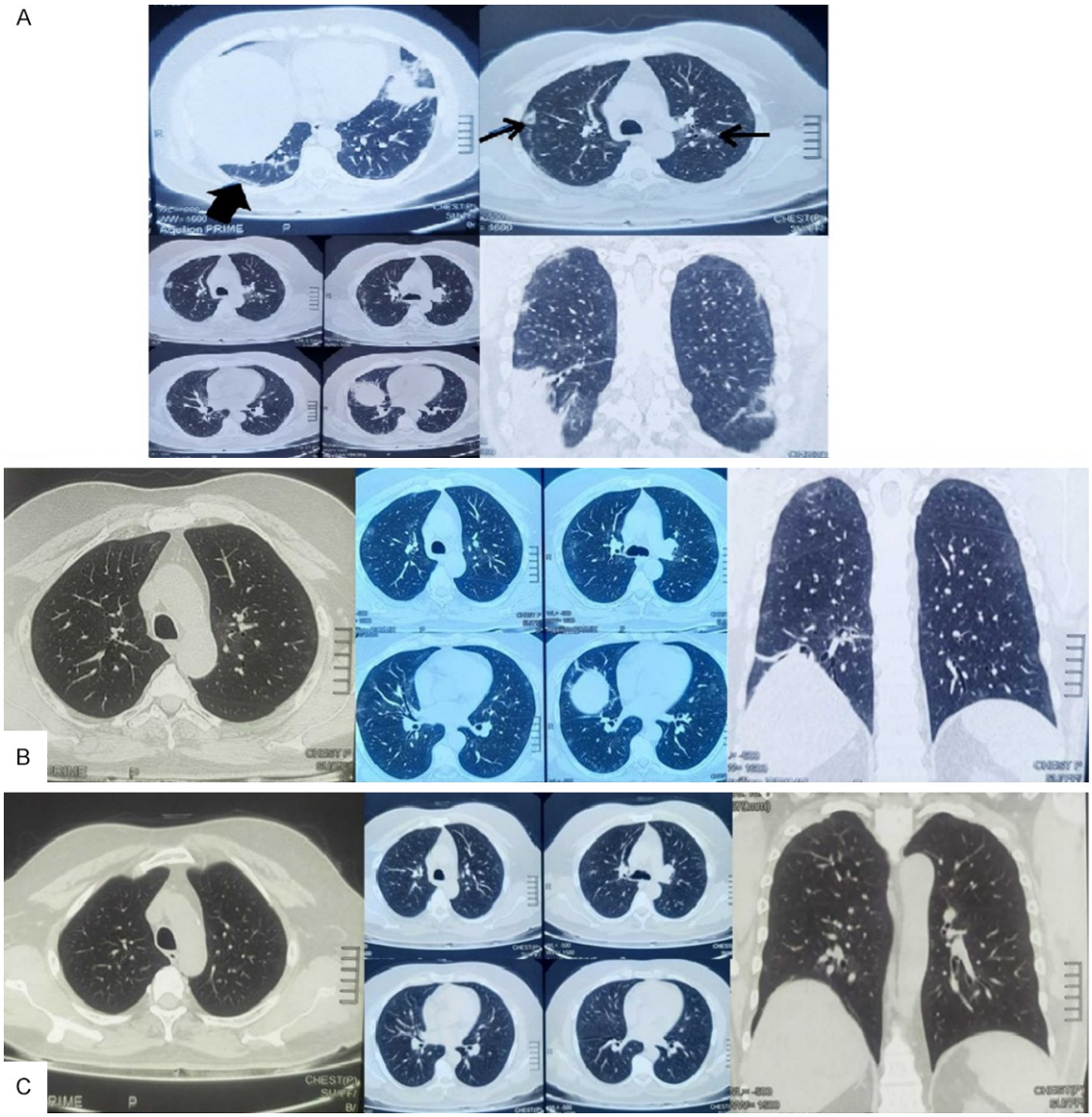

Figure 16. Comparative HRCT scan images of Day 1, Day 7 and Day 28 showing significant reduction in the ground glass opacities and atelectatic changes on Day 7 and complete resolution of ground glass opacities, subpleural lines and atelectatic bands on day 28.

In severe influenza, MSCs' can tackle inflammatory cytokine excess that leads to acute lung injury induced due to H5N1 infection [44]. MSCs also regulate inflammatory responses, improve alveolar fluid clearance, and maintain lung epithelial and endothelial integrity [45, 46]. Moreover, KEGG pathway analysis has shown that these cells are closely involved in the antiviral pathways, specifically those related to Epstein-Barr, Hepatitis B, viral carcinogenesis and human T-cell leukaemia virus 1 infection [47]. They have also been implicated in several other pathways, namely herpes viral infections via the cGAS-STING pathway [48], Japanese encephalitis via up-regulation multiple pro-survival pathways [49], and a non-canonical PI3K NFκB pathway against latent HIV-1 [50].

Anti-inflammatory and immune regulatory ability

MSCs have shown to induce immunomodulation primarily through paracrine signaling, stim-

ulation of secretion of anti-inflammatory molecules such as Interleukin (IL)-10. Also, they increase the lymphocyte count thereby increasing their antiviral characteristic which in turn results in decreased C-reactive protein and pro-inflammatory cytokines including IL-6, TNFα, IL-8, etc. Studies have shown that the anti-inflammatory ability of MSCs can attenuate virus-induced lung injury and death in mice [51, 52]. These cells also have a known safety and efficacy profile in ARDS, chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), obstructive bronchiolitis (OB) and bronchopulmonary dysplasia. Cell therapy using MSCs has therefore been proposed as a suitable treatment approach and several clinical trials have begun.

The immune modulating action of stem cells is mediated by TLRs (TLR, mainly TLR3 and TLR4) present on the surface of MSCs. RNA viruses [act as Pathogen-associated molecular pattern (PAMP)] activate these TLRs, which leads to secretion of certain chemokines like MIP-1α and MIP-1β, RANTES, CXCL9, CXCL10, and CXCL11 etc, which leads to an anti-inflammatory response [53]. This response may be useful against the hyperimmune response/cytokine storm observed in COVID-19. There has been hypersecretion of pro-inflammatory cytokines which includes IL-2, IL-6, IL-7, G-CSF, IP10, MCP1, MIP1A and TNFα which influences MSCs to release anti-inflammatory molecules (IL-10) with the release of soluble factors like transforming growth factor-β1 (TGF-β1) prostaglandin E2 (PGE2), hepatocyte growth factor (HGF), indoleamine-pyrrole 2,3-dioxygenase (IDO), and nitric oxide (NO) [54]. These in turn decrease the proliferation of activated T-cells and NO on the other hand causes cell cycle arrest by repressing the phosphorylation of signal transducers and transcription of STAT-5 in T-cells. Further, IDO secretion by MSCs leads to apoptosis of activated T-cells and converts tryptophan into kynurenine causing suppression of proliferation of effector T-cells [55].

Regeneration and repair

The abnormal immune response in SARS-CoV-2 infection leads to acute and chronic lung injury often involving fibrosis either as a cause or consequence. MSCs promote repair through paracrine signaling inducing secretion of growth factors such as Hepatocyte Growth Factor (HGF), transforming growth factor beta 1 (TGF-β1), Vascular Endothelial Growth Factor (VEGF) etc. which are necessary for tissue repair. Through their regenerative and reparative potential, they may protect alveolar epithelial cells, bring about recovery of pulmonary microenvironment, improve lung dysfunction and pulmonary fibrosis.

Multiple pro-survival pathways converge and death-inducing pathways are attenuated on a cellular level to bring about improvements in the lungs. Inhibition of pulmonary fibrosis and reduced collagen deposition by these cells [56-58] may lead to decreased edema and opacity in chest X-rays. Further, MSCs trapped in pulmonary circulation undergo differentiation over the long term to yield a multitude of alveolar cell types that integrate into the pulmonary tissue and improve lung function [41, 59]; we may thus also expect improved tissue microarchitechture in the chest CT scans along with improved air exchange and lung function.

Antimicrobial properties

Along with immunomodulation and regeneration, MSCs also possess antimicrobial properties. Besides, these cells have shown to be ACE-2 negative and therefore cannot be infected by SARS-CoV-2 [60].

Angiogenesis

VEGF secreted by stem cells is a pro-angiogenic factor. Angiogenesis is critical for tissue regeneration [61]. In the lung, angiogenesis is crucial since the blood-air interface is the source of oxygenation and oxygen delivery to the body. Through secretion of VEGF, stem cells can help recovery from lung injury. Animal studies have shown that administration of VEGF improved aeration and prevented the development of respiratory distress syndrome and mortality in premature animals [62].

Rationale for Intravenous injection

Safety and efficacy have been satisfactorily demonstrated in the intravenous administration of these cells in the human body for all the conditions mentioned above at different doses, reviewed exhaustively by Can et al. [22]. Intravenous route of administration is consid-

ered to be an ideal approach given its broad biodistribution and easy access. It has been used as the route of cell delivery for a large number of preclinical and clinical studies. The first organ through which intravenously injected MSCs pass are the lungs [63]. Engraftment in the lungs is a very rapid event; cells can be detected already seconds or minutes after intravenous transplantation [64, 65]. Cell fate tracking studies in sheep [66, 67] and rats [68, 69] have shown the superiority of the intravenous route for administering these cells, with the cells primarily distributed in the lungs. Studies have shown that the detainment of MSCs in the lungs is due to the combination of mechanical and physiological conditions and may be due to the small capillary size, the large capillary network and the strong adhesion properties of MSC. Cultured MSCs are more than 20 μm in diameter, which does not allow them to pass through the lungs as they are larger than the width of the micro-capillaries of the lungs [70]. These cells thus may improve the pulmonary microenvironment and lung function by differentiating into different types of alveolar epithelial cells as well as immunomodulation, are safely tolerated, and efficacious in improving tissue microarchitecture. These data taken together rationalize the intravenous administration of umbilical cord and placenta mesenchymal stem cells for the treatment of COVID-19.

Clinical Evidence

The first study was conducted in China by Leng, et al. to assess if cell therapy using MSC could improve outcome of patients with COVID-19. 7 patients, including 1 critically ill, 4 severe and 2 non-severe cases, received a single dose of MSCs (1×10^6 cells/kg body weight) by IV infusion; 3 severe cases forming the control group received placebo. Significant improvement in pulmonary function was noted in all patients in the MSC treatment group within 2 days. There were no MSC infusion associated adverse events. Two non-severe and one severe patient were discharged within 10 days following recovery. As compared to the control group, pro-inflammatory TNF-α significantly decreased while the anti-inflammatory IL-10 levels increased in the MSC treatment group [37].

Another study including 31 patients with severe COVID-19 pneumonia demonstrated improved clinical outcomes following IV infusion of human umbilical cord-derived MSC (hUC-MSC) in a dose of 1×10^6 cells/kilogram of weight [71]. The SARS-CoV-2 PCR test results turned negative in a mean time of 10.7 (4.2) days. Clinical data and laboratory parameters showed that UC-MSC therapy improved oxygenation and attenuated the hyper-inflammatory state in these patients.

Multiple case reports showing benefit of hUC-MSCs in patients with Covid 19 have been published. No adverse events were reported. Patients showed improved symptoms alongwith improved inflammatory markers [72-74].

Sengupta et al. conducted a prospective nonrandomized open-label cohort study using exosomes derived from allogenic BMMSCs in 24 confirmed COVID-19 patients. 83% survival rate was observed with a recovery rate of 71% (17/24), stability in 13% (3/24) and mortality unrelated to treatment was 16% (4/24). Laboratory investigations showed significant reduction in absolute neutrophil count with alleviated levels of acute phase reactants, C-reactive protein, downregulating cytokine storm and restoring immunity [75].

Another cell-based therapy, derived from allogenic cardiospheres was assessed for its safety and effectiveness in 6 critically ill COVID-19 patients by Singh et al. All patients were on ventilatory support. All patients survived with 4 discharged and 1 on respiratory support compared to 18% mortality in control group. Results were well correlated with diminished levels of ferritin and absolute lymphocyte counts, suggesting the role of cell-based therapies in modifying the immune responses [76].

Clinical outcome of this study

Tenforde et al., in their study, reported that the median duration for symptom resolution of Covid 19 patients was 16 days [77], however, we noticed that all symptoms of our study group resolved within 10 days. Thus, use of UC-MSCs significantly decreased the time required for COVID-19 symptoms to resolve. (**Table 2**). Cough, sore throat, sputum, chest pain, loss of appetite, taste and smell, giddiness, resolved within 5 days. Generalized fatigue, shortness of breath and requirement for supplemental oxygen was resolved within

10 days. 9 out of 10 patients were discharged within 9 days of their admission. Better alleviation of symptoms could be attributed to the ability of UC-MSCs to improve lung damage via their differentiational and regenerative properties. Along with improved clinical symptoms, levels of inflammatory biomarkers such as C-reactive protein, interleukin 6, ferritin and D-dimer also improved in all patients after intervention. There was no deterioration observed in clinical and laboratory parameters. None of the patients progressed to severe stage of Covid. MSCs migrate to the site of inflammation and impart anti-inflammatory and immunomodulatory effects through cell interactions and paracrine mechanisms thereby, reducing the inflammation caused due to COVID-19 and inhibiting cytokine storm. This can be corroborated by the improved inflammatory markers recorded in all patients after MSC transplantation. Improved oxygenation was recorded in all the patients which was demonstrated by improvement in the SpO2/FiO2 ratio and PaO2/FiO2 ratio. Improvement in lung damage alongwith angiogenesis may result in overall improvement in hypoxemia in all the patients. None of the patients showed any major or minor adverse events immediately after intervention or on follow up after 6 months. One patient with history of diabetes mellitus expired due to cardiac arrest after 3.5 months post intervention which was unrelated to cell therapy. Cardiac manifestations have been reported in patients recovering after COVID-19 [78]. While patients with pre-existing cardiovascular disease and risk factors are more likely to experience cardiac sequelae, those with no cardiovascular history have also shown signs of cardiac complications because of COVID-19. Mechanisms responsible for cardiovascular sequelae in post COVID-19 may include direct viral invasion, downregulation of ACE2, inflammation and the immunologic response affecting the structural integrity of the myocardium, pericardium and conduction system [79].

Consistent decline in disease severity within a short duration alongwith normalization of oxygen saturation can be attributed to the anti-inflammatory, immunomodulatory, angiogenic and anti-viral effects of MSCs. Administration of MSCs in moderate stage Covid patients resulted in prevention of the cytokine storm and halting the disease progression.

Radiological findings

Improvement in lung damage after MSC transplantation was demonstrated by radiological investigations like Chest X-ray and Chest CT scan. These scans showed no adverse effects of the MSCs transplantation on lung tissue. Additionally, no post-covid fibrosis was observed on Chest CT done 28 days after the treatment and Chest X ray done 6 months after the treatment.

On chest X-ray, 8 out of 10 patients showed significant bilateral lung infiltrates. 7 (87.5%) out of these 8 patients resolved completely in average 19 days. Improvement was noted in the radiographs at Day 7 in most patients. Warissara Kiththiworaphongkich, 2021 showed that the improvement was seen in X-rays post 13 days of the illness [80]. However, in this study improvement was seen post 7 days of treatment suggesting early resolution of lung pathology.

Temporal changes in the lung tissue involvement studied previously on a CT scan shows that CT scores peak during illness days 6-11 [81, 82]. In this study, 90% of the patients receiving MSCs transplantation showed reduction in the CT score at day 7 and the scores continued to reduce thereafter, suggesting better resolution of lung pathology post treatment.

One of the limitations of this study was that the sample size was small. Secondly, the study did not include a control group. Future research should include a larger sample size with a control group.

Conclusion

Stage 1 of this phase 1 clinical trial wherein 10 patients with moderate COVID-19 infection were administered two doses of MSCs (100 million cells each on Day 1 and Day 4) was found to be safe and effective. No adverse events were noted. Early resolution of symptoms, improved inflammatory markers, improved oxygenation and halting of disease progression observed in the study population after intervention, can be attributed to the ability of MSCs to immunomodulate, reduce inflammation, prevent cytokine storm, promote angiogenesis, improve oxidative stress and oxygenation in moderate COVID-19. The regenerative

and repair potential of these cells may also help in better post Covid recovery. The combination of MSCs derived from umbilical cord and placenta with standard treatment should be further explored as an effective therapeutic strategy for COVID-19.

Disclosure of conflict of interest

None.

Address correspondence to: Pooja Kulkarni, Department of Research & Development, NeuroGen Brain & Spine Institute, Plot 19, Sector 40, Nerul (W), Next Seawoods Grand Central Station. Navi Mumbai 400706. India. E-mail: publications@neurogen.in

References

[1] Zhu H, Wei L and Niu P. The novel coronavirus outbreak in Wuhan, China. Glob Health Res Policy 2020; 5: 6.
[2] García LF. Immune response, inflammation, and the clinical spectrum of COVID-19. Front Immunol 2020; 11: 1441.
[3] Wu C, Chen X, Cai Y, Zhou X, Xu S, Huang H, Zhang L, Zhou X, Du C, Zhang Y and Song J. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med 2020; 180: 934-43.
[4] Fajgenbaum DC and June CH. Cytokine storm. N Engl J Med 2020; 383: 2255-73.
[5] Sanders JM, Monogue ML, Jodlowski TZ and Cutrell JB. Pharmacologic treatments for coronavirus disease 2019 (COVID-19): a review. JAMA 2020; 323: 1824-36.
[6] Mahalmani VM, Mahendru D, Semwal A, Kaur S, Kaur H and Sarma P. COVID-19 pandemic: a review based on current evidence. Indian J Pharmacol 2020; 1: 117. 52.
[7] Gupta RK, Peppa D, Hill AL, Gálvez C, Salgado M, Pace M, McCoy LE, Griffith SA, Thornhill J, Alrubayyi A and Huyveneers LE. Evidence for HIV-1 cure after CCR5Δ32/Δ32 allogeneic haemopoietic stem-cell transplantation 30 months post analytical treatment interruption: a case report. Lancet HIV 2020; 7: e340-7.
[8] Peng L, Xie DY, Lin BL, Liu J, Zhu HP, Xie C, Zheng YB and Gao ZL. Autologous bone marrow mesenchymal stem cell transplantation in liver failure patients caused by hepatitis B: short-term and long-term outcomes. Hepatology 2011; 54: 820-8.
[9] Xiao K, Hou F, Huang X, Li B, Qian ZR and Xie L. Mesenchymal stem cells: current clinical progress in ARDS and COVID-19. Stem Cell Res Ther 2020; 22: 305.
[10] Du J, Li H, Lian J, Zhu X, Qiao L and Lin J. Stem cell therapy: a potential approach for treatment of influenza virus and coronavirus-induced acute lung injury. Stem Cell Res Ther 2020; 24: 192.
[11] Gates B. Responding to COVID-19-a once-in-a-century pandemic? N Engl J Med 2020; 382: 1677-9.
[12] Wang Y, Wang Y, Chen Y and Qin Q. Unique epidemiological and clinical features of the emerging 2019 novel coronavirus pneumonia (COVID-19) implicate special control measures. J Med Virol 2020; 92: 568-76.
[13] Tay MZ, Poh CM, Rénia L, MacAry PA and Ng LF. The trinity of COVID-19: immunity, inflammation and intervention. Nat Rev Immunol 2020; 20: 363-74.
[14] Walls AC, Park JY, Tortorici AM, Wall A, McGuire TA and Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 2020; 181: 281-92, e6.
[15] Shang J, Ye G, Shi K, Wan Y, Luo C, Aihara H, Geng Q and Auerbach A, Li F. Structural basis of receptor recognition by SARS-CoV-2. Nature 2020; 581: 221-224
[16] Ghoda A and Ghoda M. Liver injury in COVID-19 infection: a systematic review. Cureus 2020; 12: e9487.
[17] Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR and Katze MG. Into the eye of the cytokine storm. Microbiol Mol Biol Rev 2012; 76: 16-32.
[18] Melo AK, Milby KM, Caparroz AL, Pinto AC, Santos RR, Rocha AP, Ferreira GA, Souza VA, Valadares LD, Vieira RM and Pileggi GS. Biomarkers of cytokine storm as red flags for severe and fatal COVID-19 cases: a living systematic review and meta-analysis. PLoS One 2021; 16: e0253894.
[19] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X and Cheng Z. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020; 395: 497-506.
[20] Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y and Yu T. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020; 395: 507-13.
[21] Matthay MA, Zemans RL, Zimmerman GA, Arabi YM, Beitler JR, Mercat A, Herridge M, Randolph AG and Calfee CS. Acute respiratory distress syndrome. Nat Rev Dis Primers 2019; 5: 18.
[22] Can A, Celikkan FT and Cinar O. Umbilical cord mesenchymal stromal cell transplantations: a

systemic analysis of clinical trials. Cytotherapy 2017; 19: 1351-82.
[23] Sharma A, Gokulchandran N, Sane H, Nagrajan A, Paranjape A, Kulkarni P, Shetty A, Mishra P, Kali M, Biju H and Badhe P. Autologous bone marrow mononuclear cell therapy for autism: an open label proof of concept study. Stem Cells Int 2013; 2013: 623875.
[24] Tsilimigras DI, Oikonomou EK, Moris D, Schizas D, Economopoulos KP and Mylonas KS. Stem cell therapy for congenital heart disease: a systematic review. Circulation 2017; 136: 2373-2385.
[25] Sharma A, Sane H, Gokulchandran N, Kulkarni P, Gandhi S, Sundaram J, Paranjape A, Shetty A, Bhagawanani K, Biju H and Badhe P. A clinical study of autologous bone marrow mononuclear cells for cerebral palsy patients: a new frontier. Stem Cells Int 2015; 2015: 905874.
[26] Sharma A, Sane H, Gokulchandran N, Gandhi S, Bhovad P, Khopkar D, Paranjape A, Bhagwanani K and Badhe P. The role of cell therapy in modifying the course of limb girdle muscular dystrophy- a longitudinal 5-year study. Degener Neurol Neuromuscul Dis 2015; 5: 93-102.
[27] Zhang J, Lv S, Liu X, Song B and Shi L. Umbilical cord mesenchymal stem cell treatment for crohn's disease: a randomized controlled clinical trial. Gut Liver 2018; 12: 73-78.
[28] Sharma A, Sane H, Paranjape A, Varghese R, Nair V, Biju H, Sawant D, Gokulchandran N and Badhe P. Improved survival in amyotrophic lateral sclerosis patients following autologous bone marrow mononuclear cell therapy: a long term 10-year retrospective study. J Neurorestoratology 2021; 9: 117-136.
[29] de la Torre P, Pérez-Lorenzo MJ and Flores AI. Human placenta-derived mesenchymal stromal cells: a review from basic research to clinical applications. In: Valarmathi MT, editor. stromal cells - structure, function, and therapeutic implications [Internet]. London: IntechOpen; 2018.
[30] Huang L, Zhang C, Gu J, Wu W, Shen Z, Zhou X and Lu H. A randomized, placebo-controlled trial of human umbilical cord blood mesenchymal stem cell infusion for children with cerebral palsy. Cell Transplant 2018; 27: 325-34.
[31] Wang J, Zeng XX, Cai W, Han ZB, Zhu LY, Liu JY and Xu JX. Safety and efficacy of placenta-derived mesenchymal stem cell treatment for diabetic patients with critical limb ischemia: A pilot study. Exp Clin Endocrinol Diabetes 2021; 129: 542-8.
[32] Arutyunyan I, Elchaninov A, Makarov A and Fatkhudinov T. Umbilical cord as prospective source for mesenchymal stem cell-based therapy. Stem Cells Int 2016; 2016: 6901286.
[33] Taghizadeh RR, Cetrulo KJ and Cetrulo CL. Wharton's Jelly stem cells: future clinical applications. Placenta 2011; 32 Suppl 4: S311-315.
[34] Matikainen T and Laine J. Placenta--an alternative source of stem cells. Toxicol Appl Pharmacol 2005; 207 Suppl 2: 544-9.
[35] Zhu H, Xiong Y, Xia Y, Zhang R, Tian D, Wang T, Dai J, Wang L, Yao H, Jiang H and Yang K. Therapeutic effects of human umbilical cord-derived mesenchymal stem cells in acute lung injury mice. Sci Rep 2017; 7: 39889.
[36] Wu M, Zhang R, Zou Q, Chen Y, Zhou M, Li X, Ran R and Chen Q. Comparison of the biological characteristics of mesenchymal stem cells derived from the human placenta and umbilical cord. Sci Rep 2018; 8: 5014.
[37] Leng Z, Zhu R, Hou W, Feng Y, Yang Y, Han Q, Shan G, Meng F, Du D, Wang S and Fan J. Transplantation of ACE2 -mesenchymal stem cells improves the outcome of patients with COVID-19 pneumonia. Aging Dis 2020; 11: 216-28.
[38] Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A and Müller MA. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020; 181: 271-280, e8.
[39] Zhao Y, Zhao Z, Wang Y, Zhou Y, Ma Y and Zuo W. Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan 2019-nCov. Am J Respir Crit Care Med 2020; 202: 756-759.
[40] Shulla A, Heald-Sargent T, Subramanya G, Zhao J, Perlman S and Gallagher T. A Transmembrane serine protease is linked to the severe acute respiratory syndrome coronavirus receptor and activates virus entry. J Virol 2011; 85: 873-82.
[41] Berger MJ, Adams SD, Tigges BM, Sprague SL, Wang XJ, Collins DP and McKenna DH. Differentiation of umbilical cord blood-derived multilineage progenitor cells into respiratory epithelial cells. Cytotherapy 2006; 8: 480-7.
[42] Wang Y and Zhao S. Vascular Biology of the Placenta. San Rafael (CA): Morgan & Claypool Life Sciences; 2010. Chapter 10, Placental Tissue and Cord Blood Stem Cells.
[43] Gupta N, Su X, Popov B, Lee JW, Serikov V and Matthay MA. Intrapulmonary delivery of bone marrow-derived mesenchymal stem cells improves survival and attenuates endotoxin-induced acute lung injury in mice. J Immunol 2007; 179: 1855-63.
[44] Lee JW, Fang X, Gupta N, Serikov V and Matthay MA. Allogeneic human mesenchymal stem cells for treatment of E. coli endotoxin-induced acute lung injury in the ex vivo perfused human lung. Proc Natl Acad Sci U S A 2009; 106: 16357-62.

[45] Lee JW, Gupta N, Serikov V and Matthay MA. Potential application of mesenchymal stem cells in acute lung injury. Expert Opin Biol Ther 2009; 9: 1259-70.
[46] Matthay MA. Therapeutic potential of mesenchymal stromal cells for acute respiratory distress syndrome. Ann Am Thorac Soc 2015; 12 Suppl 1: S54-7.
[47] ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L and Slutsky AS. Acute respiratory distress syndrome: the Berlin Definition. JAMA 2012; 307: 2526-33.
[48] Yang K, Wang J, Wu M, Li M, Wang Y and Huang X. Mesenchymal stem cells detect and defend against gammaherpesvirus infection via the cGAS-STING pathway. Sci Rep 2015; 5: 1-9.
[49] Bian P, Ye C, Zheng X, Yang J, Ye W, Wang Y, Zhou Y, Ma H, Han P, Zhang H and Zhang Y. Mesenchymal stem cells alleviate Japanese encephalitis virus-induced neuroinflammation and mortality. Stem Cell Res Ther 2017; 8: 38.
[50] Chandra PK, Gerlach SL, Wu C, Khurana N, Swientoniewski LT, Abdel-Mageed AB, Li J, Braun SE and Mondal D. Mesenchymal stem cells are attracted to latent HIV-1-infected cells and enable virus reactivation via a non-canonical PI3K-NFκB signaling pathway. Sci Rep 2018; 8: 1-17.
[51] Chen J, Hu C, Chen L, Tang L, Zhu Y, Xu X, Chen L, Gao H, Lu X, Yu L and Dai X. Clinical study of mesenchymal stem cell treatment for acute respiratory distress syndrome induced by epidemic influenza A (H7N9) infection: a hint for COVID-19 treatment. Engineering 2020; 6: 1153-61.
[52] Thanunchai M, Hongeng S and Thitithanyanont A. Mesenchymal stromal cells and viral infection. Stem Cells Int 2015; 2015: 860950.
[53] Perlman S and Dandekar AA. Immunopathogenesis of coronavirus infections: implications for SARS. Nat Rev Immunol 2005; 5: 917-927.
[54] Costela-Ruiz VJ, Illescas-Montes R, Puerta-Puerta JM, Ruiz C and Melguizo-Rodríguez L. SARS-CoV-2 infection: the role of cytokines in COVID-19 disease. Cytokine Growth Factor Rev 2020; 54: 62-75.
[55] Zhao W, Pi H, Feng W, Li X, Wang J, Liu Y, Jiang Y, Ma J, Xia Y, Wang C and Shao L. Contact or noncontact cocultures of articular chondrocytes with bone marrow mesenchymal stem cells: cell proliferation and differentiation. Chin J Tissue Eng Res 2019; 23: 24.
[56] Li Y, Li H, Cao Y, Wu F, Ma W, Wang Y and Sun S. Placenta-derived mesenchymal stem cells improve airway hyperresponsiveness and inflammation in asthmatic rats by modulating the Th17/Treg balance. Mol Med Rep 2017; 16: 8137-45.
[57] Li T, Xia M, Gao Y, Chen Y and Xu Y. Human umbilical cord mesenchymal stem cells: an overview of their potential in cell-based therapy. Expert Opin Biol Ther 2015; 15: 1293-306.
[58] Li X, Yue S and Luo Z. Mesenchymal stem cells in idiopathic pulmonary fibrosis. Oncotarget 2017; 8: 102600-16.
[59] Sueblinvong V, Loi R, Eisenhauer PL, Bernstein IM, Suratt BT, Spees JL and Weiss DJ. Derivation of lung epithelium from human cord blood-derived mesenchymal stem cells. Am J Respir Crit Care Med 2008; 177: 701-11.
[60] Cao Y, Wu H, Zhai W, Wang Y, Li M, Li M, Yang L, Tian Y, Song Y, Li J and Wang YA. Safety consideration of mesenchymal stem cell therapy on COVID-19. Stem Cell Res 2020; 49: 102066.
[61] Lee JH, Parthiban P, Jin GZ, Knowles JC and Kim HW. Materials roles for promoting angiogenesis in tissue regeneration. Progress in Materials Science 2021; 117: 100732.
[62] Matute-Bello G, Frevert CW and Martin TR. Animal models of acute lung injury. Am J Physiol Lung Cell Mol Physiol 2008; 295: L379-99.
[63] Wagner B and Henschler R. Fate of intravenously injected mesenchymal stem cells and significance for clinical application. Adv Biochem Eng Biotechnol 2013; 130: 19-37.
[64] Tögel F, Yang Y, Zhang P, Hu Z and Westenfelder C. Bioluminescence imaging to monitor the in vivo distribution of administered mesenchymal stem cells in acute kidney injury. Am J Physiol Renal Physiol 2008; 295: F315-21.
[65] Schrepfer S, Deuse T, Reichenspurner H, Fischbein MP, Robbins RC and Pelletier MP. Stem cell transplantation: the lung barrier. Transplant Proc 2007; 39: 573-6.
[66] Cardenes N, Aranda-Valderrama P, Carney JP, Sellares Torres J, Alvarez D, Kocydirim E, Wolfram Smith JA, Ting AE, Lagazzi L, Yu Z and Mason S. Cell therapy for ARDS: efficacy of endobronchial versus intravenous administration and biodistribution of MAPCs in a large animal model. BMJ Open Respir Res 2019; 12; 6: e000308.
[67] Asmussen S, Ito H, Traber DL, Lee JW, Cox RA, Hawkins HK, McAuley DF, McKenna DH, Traber LD, Zhuo H and Wilson J. Human mesenchymal stem cells reduce the severity of acute lung injury in a sheep model of bacterial pneumonia. Thorax 2014; 69: 819-25.
[68] McAuley DF, Curley GF, Hamid UI, Laffey JG, Abbott J, McKenna DH, Matthay MA and Lee JW. Translational research in acute lung injury and pulmonary fibrosis: clinical grade allogeneic human mesenchymal stem cells restore alveolar fluid clearance in human lungs rejected for transplantation. Am J Physiol Lung Cell Mol Physiol 2014; 306: L809.

[69] Zickri MB, Fadl SGA and Metwally HG. Comparative study between intravenous and intraperitoneal stem cell therapy in amiodarone induced lung injury in rat. Int J Stem Cells 2014; 7: 1-11.
[70] Crop MJ, Korevaar SS, de Kuiper R, IJzermans JNM, van Besouw NM, Baan CC, Weimar W and Hoogduijn MJ. Human mesenchymal stem cells are susceptible to lysis by CD8(+) T cells and NK cells. Cell Transplant 2011; 20: 1547-59.
[71] Guo Z, Chen Y, Luo X, He X, Zhang Y and Wang J. Administration of umbilical cord mesenchymal stem cells in patients with severe COVID-19 pneumonia. Critical Care 2020; 24: 420.
[72] Chen H, Zhang L, He Z, Wang D, Liu L, Zhang W, Chen T, Dai Z, Han Z, Chen M and Chen M. Systemic administration of human umbilical cord-derived mesenchymal stem cells effectively ameliorates the outcomes of a critically ill elderly patient with COVID-19 with multiple comorbidities: a case report. World Acad Sci J 2020; 2.
[73] Zhang Y, Ding J, Ren S, Wang W, Yang Y and Li S. Intravenous infusion of human umbilical cord Wharton's jelly-derived mesenchymal stem cells as a potential treatment for patients with COVID-19 pneumonia. Stem Cell Res Ther 2020; 11: 207.
[74] Peng H, Gong T, Huang X, Sun X, Luo H and Wang W. A synergistic role of convalescent plasma and mesenchymal stem cells in the treatment of severely ill COVID-19 patients: a clinical case report. Stem Cell Res Ther 2020; 11: 291.
[75] Sengupta V, Sengupta S, Lazo A, Woods P, Nolan A and Bremer N. Exosomes derived from bone marrow mesenchymal stem cells as treatment for severe COVID-19. Stem Cells Dev 2020; 29: 747-754.
[76] Singh S, Chakravarty T, Chen P, Akhmerov A, Falk J and Friedman O. Allogeneic cardiosphere-derived cells (CAP-1002) in critically ill COVID-19 patients: compassionate-use case series. Basic Res Cardiol 2020; 115: 36.
[77] Tenforde MW, Kim SS, Lindsell CJ, Billig Rose E, Shapiro NI, Files DC, Gibbs KW, Erickson HL, Steingrub JS, Smithline HA and Gong MN. Symptom duration and risk factors for delayed return to usual health among outpatients with COVID-19 in a multistate health care systems network - United States, March-June 2020. MMWR Morb Mortal Wkly Rep 2020; 69: 993-998.
[78] Carfì A, Bernabei R and Landi F. Gemelli against COVID-19 post-acute care study group. Persistent symptoms in patients after acute COVID-19. JAMA 2020; 324: 603-5.
[79] Nalbandian A, Sehgal K, Gupta A, Madhavan MV, McGroder C, Stevens JS, Cook JR, Nordvig AS, Shalev D, Sehrawat TS and Ahluwalia N. Post-acute COVID-19 syndrome. Nat Med 2021; 27: 601-615.
[80] Kiththiworaphongkich W. Disease course and temporal changes of chest X-ray findings in COVID-19 positive patients at Phayao Hospital. J Nakornping Hosp 2021; 12: 149-167.
[81] Wang Y, Dong C, Hu Y, Li C, Ren Q, Zhang X, Shi H and Zhou M. Temporal changes of CT findings in 90 patients with COVID-19 pneumonia: a longitudinal study. Radiology 2020; 296: E55-64.
[82] Wang C, Huang P, Wang L, Shen Z, Lin B, Wang Q, Zhao T, Zheng H, Ji W, Gao Y and Xia J. Temporal changes of COVID-19 pneumonia by mass evaluation using CT: a retrospective multi-center study. Ann Transl Med 2020; 8: 935.