

Focused Review

Overview of Stem Cell Therapy for Acute Respiratory Distress Syndrome with Focus on COVID 19

Rachel J. Kaye, BA, MD (anticipated)

From: Medical University of South Carolina Medical School, Charleston, SC

Address Correspondence:
Rachel Kaye, BA
Medical University of South Carolina Medical School,
171 Ashley Ave
Charleston, SC 29425
E-mail:
rachelkaye17@hotmail.com

Disclaimer: There was no external funding in the preparation of this manuscript.

Conflict of interest: Each author certifies that he or she, or a member of his or her immediate family, has no commercial association (i.e., consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted manuscript.

Manuscript received: 06-16-20
Accepted for publication:
06-29-2020

Free full manuscript:
www.painphysicianjournal.com

Objective: There are as yet no effective strategies to treat the novel COVID-19 and to stem its symptoms, including ARDS. This review examines recent research studies in humans to determine whether mesenchymal stem cells (MSCs) may be used effectively and safely to target potentially deadly lung damage that may follow infection.

Methods: A literature search was conducted to find published manuscripts on the treatment of ARDS and COVID-19 symptoms, disease presentation, and available treatment regimens. Electronic data bases of scientific articles and records of printed documents of JAMA journals were searched to find research publications on MSC treatment of ARDS and COVID-19. Outcome variables included mortality over varying time periods, hospital days, days on ventilator, and biological factors.

Results: Two randomized double-blind clinical trials, 2 pilot studies, and 2 case reports described MSC use to treat ARDS with specific focus on COVID-19 and lung symptoms of cytokine storm. The MSCs were well-tolerated across studies. No significant differences in treatment outcome were found in randomized double-blind trials; however, results of 1 pilot study and 1 case report showed that MSCs led to lung symptom resolution and survival in severely ill treatment patients.

Conclusions: There is little published research on disease and survival outcomes among patients suffering severe lung disease associated with ARDS and COVID-19, and studies available are limited by lack of consistency in design and numerous flaws and limitations. Comparisons across studies are difficult. Nevertheless, it is documented that 8 ARDS patients with COVID-19 experienced symptom recovery and survival subsequent to MSC administration. MSCs are potentially life-saving treatment approaches for some patients who exhibit severe lung distress and have not responded to standard treatments. This is an obviously exciting research and treatment option for COVID-19 and other life-threatening diseases.

Key words: stem cells-ARDS-mesenchymal stem cells-stem cell therapy-COVID-19

Pain Physician 2020; 23:S421-S431

Severe acute respiratory syndrome coronavirus 2 infection (SARS-CoV-2), the cause of coronavirus disease 2019 (COVID-19), has led to a pandemic and public health emergency with an estimated 3 million cases and 1 million deaths worldwide as of May 1, 2020 (1). Among the most significant clinical manifestations are fever, dry cough, dyspnea, and atypical pneumonia, identified on CT radiologic exam with bilateral ground glass opacity of the lungs (2). Of patients diagnosed with COVID-19 pneumonia, approximately 15 to 30 percent go on to develop acute

respiratory distress syndrome (ARDS) (3). A study of 1,591 critically ill patients admitted to the ICU with COVID-19 in Lombardy, Italy, documented that the majority had ARDS, and mortality was 26% (4). In general, ARDS is a rapid onset of diffuse lung injury caused by varied insults (5) and affects about 3 million people worldwide each year (6). ARDS can be triggered by pneumonia; sepsis; aspiration; massive trauma; pancreatitis; pathogens such as Anthrax, Spanish flu virus, H5N1 avian influenza virus, and SARS-CoV (7), etiologies which damage the alveolar capillary barrier

of the lungs impairing gas exchange. ARDS is associated with significant morbidity and mortality (5).

The pathophysiology of ARDS can be explained as a result of an imbalance between proinflammatory and anti-inflammatory cytokines; oxidants and antioxidants; procoagulants and anticoagulants; neutrophil recruitment, activation, and clearance; and proteases and protease inhibitors (8). In coronavirus infections, the immune system can undergo systemic overreaction called a cytokine storm which is a release of cytokines TNF α , IL1 beta, IL2, IL6, IFN α , IFN beta, IFN gamma, and MCP1, leading to the release of free radicals, a major etiology of ARDS, and multiple organ failure (9). The first pathologic examination of the lungs of a patient who died of COVID-19 on hospital day 14 showed bilateral diffuse alveolar damage with hyaline membrane formation (consistent with ARDS), interstitial lymphocytic inflammation, and intra-alveolar multinucleated syncytial cells with atypical enlarged pneumocytes and viral cytopathic effect (10). There are no specific pharmacologic treatments for ARDS, and supportive care is the mainstay treatment approach, including protective mechanical ventilation, prone positioning ventilation, and fluid management (6).

Mesenchymal Stem Cells (MSCs)

Mesenchymal stromal cells are fibroblast-like multipotent cells, most often used in cell therapy for immune mediated and inflammatory diseases and isolated from various sources, such as bone marrow, umbilical cord, and adipose tissues (11,12). The International Society for Cellular Therapy defines the criteria for a MSC as a plastic adherent cell; expressing cell surface markers of CD105, CD73, and CD90 and absence of CD45, CD34, CD14, CD19, CD124, and HLA-DR; and, having the capacity to differentiate into osteoblasts, adipocytes, and chondroblasts in vitro conditions (13). In addition to their potential for differentiation, MSCs have immunomodulatory capabilities (14). Preclinical studies in animal models show that MSCs promote tissue recovery primarily by paracrine mechanisms, initiating anti-inflammatory cytokines, immunomodulation, angiogenesis, antimicrobial peptide secretion and extracellular vesicle release (15-17). MSCs mediate such paracrine factors as keratinocyte growth factor (KGF), prostaglandin E2 (PGE2), angiopoietin-1 (Ang-1), interleukin-10 (IL-10), hepatocyte growth factor (HGF), and other trophic cytokines (18). MSCs secrete soluble factors that have immunomodulatory properties on the innate and adaptive immune systems (19). Proin-

flammatory cytokines, interferon gamma and tumor necrosis factor α prime MSCs and through cell-to-cell contact or through production of soluble factors such as TGF beta 1, HGF, PGE2, IDO, NO, and others mediate immune response (11). These soluble factors produced by the MSCs in turn inhibit maturation of dendritic cells and suppress T lymphocyte, B lymphocyte and NK cell function/activation (19, 20).

MSCs in Treating ARDS and Other Illnesses.

MSCs are immuno-privileged, expressing low levels of HLA antigens on their cell membranes, which initially allows escape from destruction and use for allogenic transplantation in acute disease (21). A benefit to treating lung injury, about 30 minutes after intravenous administration, is that most MSCs accumulate in the pulmonary vascular bed (22). Intravenously administered MSCs mainly act on injured host cells within the microenvironment of repair by cell-to-cell contact and paracrine secretion of soluble mediators and transfer of mitochondria containing vesicles (21). In the lung, MSCs release antimicrobial peptides, anti-inflammatory cytokines, angiogenic growth factors, and extracellular vesicles (15-17). Further, the protective effect of MSCs has been demonstrated by the direct cell transfer of mitochondria from MSCs to respiratory epithelial cells restoring alveolar bioenergy (23). Angiopoietin-1 and keratinocyte growth factor secreted by MSCs have been shown to help repair alveolar-capillary walls in ARDS induced by bacterial infection (24,25).

Questions remain as to MSCs protective benefits in viral-induced ARDS infections (26). In preclinical studies of viral induced lung injury, activated MSCs have been shown to release anti-inflammatory mediators and suppress T cell function and lymphocyte proliferation which could compromise the antiviral response leading to a prolonged infection (26). On the other hand, in vitro studies on models of Cytomegalovirus and Epstein Barr virus infection show that MSCs allow virus specific T cells to proliferate and produce interferon gamma to destroy virus infected cells (27). In general, MSCs can either promote or suppress inflammation based on the inflammatory environment to which they are exposed (11). Researchers have observed that MSCs are resistant to viral infection and do not rely on interferon (INF) signaling for antiviral protection, unlike differentiated cells, keeping stem cells safe and multiplying for the organism's lifespan (28). Interferon stimulated genes (ISGs) are one of nature's most sophisticated antiviral defense systems (29). ISGs in stem cells are intrinsically

expressed and code for antiviral proteins that prevent the virus from entering the cell (28). However, some studies show that MSCs permit viral infection through viral receptors on MSCs cell surface, and MSCs may become infected (30-32).

REVIEW AIMS

Despite these possible concerns, mesenchymal stem cells, with their immunomodulatory functions, have been shown to be safe and effective in clinical trials of immune mediated inflammatory diseases such as perianal Crohn's disease (33), graft-versus-host disease (34), and systemic lupus erythematosus (35). Numerous animal model preclinical studies of mesenchymal stem cells (MSCs) for acute lung injury have demonstrated improvements in lung injury of infectious and non-infectious etiologies (24,36-38). Preclinical data support MSCs as a potential therapy for ARDS, and clinical trials phase I/II have begun to assess safety of human MSC infusions in patients with ARDS with no adverse effects identified. However, there are very few preclinical studies investigating MSCs in viral respiratory infections, and these have shown conflicting results (26). In addition,

none of these preclinical studies involved coronavirus. With the current widespread outbreak of the novel coronavirus, its severe ARDS inducing pneumonia, and no vaccine yet available, effective and targeted treatments are needed to prevent lung injury and promote repair. The aim of this review is to examine studies of the clinical efficacy and safety of mesenchymal stem cell therapy in ARDS resulting from COVID-19 infection. Studies were reviewed in human patients to determine whether MSCs may be applied effectively and safety to aid in the treatment of COVID-19.

METHODS

Electronic databases including PubMed and the Cochrane Library were searched to find relevant articles as well as records from printed documents of the JAMA on stem cell therapy for ARDS and COVID-19 (Fig. 1). Six original articles on MSCs therapy of ARDS and COVID-19 pneumonia were identified (Table 1). An overview of these recent studies is provided to describe investigative work and to allow assessment of the efficacy of the clinical applications on quantifiable factors and patient outcomes.

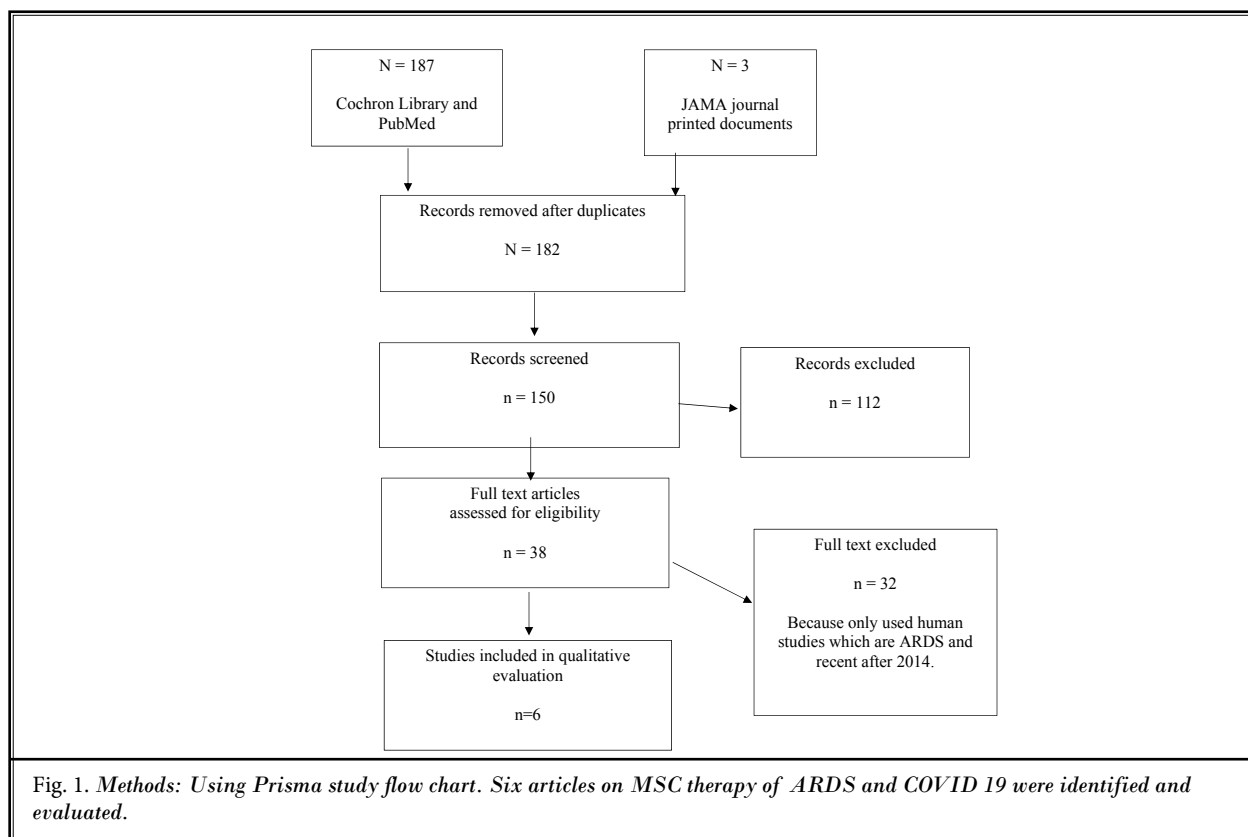


Table 1. Summary of recent MSC double-blind clinical trials, pilot studies, and case-reports of ARDS treatment.

Research study	Country	MSC source	Study design	Age/Gender	Number	Inclusion/Exclusion criteria	Control Group	Follow up period	Outcome/Safety	MOA	Quantifiable factors	Results
Zheng et al. 2014 (39)	China	Single donor allogenic adipose tissue derived 1×10^6 cells/kg IV	Single center double-blind placebo controlled randomized pilot study	>18 years 11 males, 1 female	12 (6 MSCs 6 Placebo)	ARDS by Berlin criteria Excluded 13 due to pre-existing conditions or no informed consent	Yes	28 days	No adverse events during infusion → Safe	Not sufficient to support that MSC alleviates lung inflammation	IL-8, IL-6, SPD, Oxygen ratio, number of hospital days, number of ventilator free days, number of ICU free days	-Significant short term improvement in oxygenation index from baseline in MSC vs Placebo at day 5 and 7 -No significant difference in length of hospital stay, ventilator free days, and ICU free days -SPD was reduced at day 5 after MSC
Matthay et al. 2019 (40)	USA	Single dose IV bone marrow derived from 3 donors 1×10^6 cells/kg IV	Multicenter Prospective double-blind randomized trial Phase 2a	>18 years	60 (40 MSC 20 Placebo)	ARDS excluded trauma, liver disease, cancer, pregnancy, breast feeding, innate, ARDS >96 hrs and DNRs	Yes	28 days	No hemodynamic or respiratory events during infusion or 6 hrs after → Safe	Measured biomarkers suggest endothelial injury is significantly reduced by MSC release of anti-inflammatory mediators	IL-8, IL-6, RAGE, protein C, angiotensin 2	-28 day mortality no difference between groups (30% MSC, 15% Placebo) -No statistical difference between MSC and Placebo for mortality at 28 days, ventilator free days, or ICU free days. -angiotensin 2 plasma levels had a significantly greater decrease in MSC group vs. placebo
Chen et al. 2019 (41)	China	Menstrual blood derived from 1 healthy donor 1×10^6 cells/kg X 3 IV	Single center pilot study	62.8 +/- 14.4 years for MSC 61.8 +/- 11.8 years for control	61 (17 in MSC 44 in control)	ARDS with H7N9 also on anti-viral oseltamivir or peramivir and standard therapy	Yes	1 year (all) 5 years for 4 patients	No adverse events → Safe, short and long term	MSCs reduce secretion of inflammatory factors which mediate effects of the cytokine storm	CRP, PCT	3 died in MSC group, 24 died in control -survival rate was significantly higher in MSC group (82.4% vs. 45.5% in control p=0.006 -1 year after MSC transplant all patients showed improvement on chest CT scan
Leng et al. 2020 (42)	China	Clinical grade MSC single dose 1×10^6 cells/kg IV	Pilot study	45-75 years old 4 male, 6 female	10 (7 MSC and 3 Placebo)	RT-PCR assay positive for SARS-CoV-2 pneumonia and Respiratory symptoms, fever Exclusion: cancer, critically severe or participating in another study	Yes	14 days	No infusion, allergy related, or delayed hypersensitivity reactions, no secondary infections → Safe	Immunomodulatory function of MSCs	Serum cytokine and chemokine measurements: IL-10, TNF- α , CRP, VEGF, IP-10	-2-4 days after MSC treatment symptoms disappeared in all 7 patients -CRP decreased, oxygen sat rose, pneumonia on CT scan largely went away, in the severe patients anti-inflammatory IL-10 ration increased significantly while TNF- α pro-inflammatory ratio decreased significantly compared to control
Simonson et al. 2015 (45)	Sweden	Bone marrow from 1 donor 2×10^6 cells/kg IV	Case Report	-58 year old man liver and kidney failure -40 year old man AML	2	ARDS by Berlin criteria Severe Refractory ARDS compassionate basis 1 also on ECMO	No	69 days for patient 1 and 28 days for patient 2	No adverse events during MSC infusion → Safe	Both patients' levels of surfactant protein B increased in BAL fluid during the 2 days after MSC infusion suggesting recovery of alveolar epithelium	Surfactant protein B, Proinflammatory microRNAs	-Both patients recovered from MOS failure with resolution of respiratory and hemodynamic failure -Both had decrease in pulmonary and systemic inflammatory markers in plasma and BAL fluid -Both had improved lung function 3 days after infusion
Liang et al. 2020 (47)	China	Allogenic hUCMSCs 5×10^6 cells IV X 3	Case Report	-65 year old female with HTN and Diabetes Type 2	1	COVID-19 ARDS On ventilator in ICU with multi-organ system failure for compassionate care -Also received lopinavir/ritonavir, IFN α , antibiotics, and glucocorticoids	No	13 days	No obvious side effects → Safe	Speculated MSCs home to repair injured tissues and reduce inflammatory cytokines	CRP	-Pneumonia resolved -discharged from ICU 8 days after 1 st dose of MSC -twice negative for COVID-19 on throat swab 10 days after 1 st dose of MSC

RESULTS

Recent Clinical Studies of MSCs for ARDS Including COVID-19 Pneumonia

Two randomized, double-blind, clinical trials, 2 pilot studies, and 2 case reports regarding the use of MSCs to treat ARDS were evaluated.

Randomized, Double-Blind, Clinical Trials

Zheng et al (39) conducted a randomized, double-blind, placebo-controlled pilot study of 12 patients diagnosed with ARDS by Berlin criteria. Six patients received 100 mL normal saline, and 6 patients received 1×10^6 cells/kg single donor adipose tissue derived MSCs in 100 mL normal saline. In total, 25 ARDS patients were assessed for study eligibility, and 13 were excluded for preexisting severe organ disease or for no informed consent. The final 12 patients were well-balanced for baseline criteria. Aspects of patient therapeutic management and of the control group were not specified. There were clearly defined secondary efficacy endpoints. The researchers found no significant differences in oxygenation ratios, hospital stay days, ventilator free days, ICU free days, or serum biomarkers (IL-8, IL-6, surfactant protein D). The small sample size and follow up of only 28 days limited study conclusions.

A prospective, double-blind, randomized phase 2a clinical trial was conducted by Matthay et al (40), and 60 patients were randomly assigned to conditions in a ratio of 2:1. Forty patients received MSC infusion, and 20 received placebo infusion to assess treatment of a single dose of 1×10^7 cells/kg of bone marrow derived MSCs for moderate to severe ARDS (within 7 days of diagnosis). The MSCs were well-tolerated. There were no statistical differences between MSC and placebo groups on 28-day mortality (30% for MSC vs 15% for placebo, odds ratio 2.4, 95% CI 0.5-15.1), mortality at 60 days (38% for MSC vs 25% for placebo, odds ratio 1.8, 95% CI 0.5-7.6), ICU free days to day 28, or number of ventilator free days to day 28. At baseline the MSC group showed higher (worse) mean scores on Acute Physiology and Chronic Health Evaluation III (a severity of illness score), but groups were well-balanced for age, sex, and etiology of ARDS. Biomarkers IL-6, IL-8, RAGE, and protein C were unchanged from baseline at 6 and 24 hours after infusion of MSCs. One biomarker, angiotensin 2, a mediator of lung and vascular injury, had significantly lower plasma concentration at 6 hours post MSC infusion compared to the placebo group. An important post-hoc clinical trial result was a large

variation in viability of MSCs at the time of intravenous administration (36%-85%). They found washing during preparation of MSCs was associated with reduced viability compared to simple thawing. There was no significant difference in the viability of MSCs among the 3 bone marrow donors at collection, but viability was higher at 1 medical center compared to the other 4 medical centers at administration (40). The variability in viability of MSCs warrants further study. The small sample size of this clinical trial limits conclusions about the efficacy of MSCs.

Pilot Studies

Chen et al (41) published a pilot study investigating the effect of allogenic menstrual blood derived MSCs from a healthy donor and administered to patients with H7N9 confirmed influenza respiratory infection with ARDS. This single center clinical study was conducted during the 2013-2014 outbreak of H7N9 in Hangzhou, China and enrolled 61 patients. Forty-four patients received conventional treatment while 17 received conventional treatment plus 1×10^6 cells/kg in 100 mL Plasmalyte in 3 separate infusions. Conventional treatment included all patients receiving oral antiviral medications, oseltamivir or peramivir. In contrast to the other studies, MSC infusions were administered at the acute and late stage of ARDS. No specifics were provided on time period between infusions in each patient. The study did not appear to be double-blind, and no information on whether the control group received a placebo was provided. The investigators did not find infusion-related toxicities or serious adverse events in any of the patients. Longer than the other studies cited, the patient follow-up period was 1 year for all patients and 5 years for 4 patients compared to a month or less cited in the other work. Study groups were well matched for baseline characteristics, including age, comorbidities, standard treatment regimens, multiorgan failure, and baseline routine laboratory values. The only complication, shock, was significantly more frequent in the experimental group ($P = 0.030$) than in the control group.

Results of the Chen et al (41) work revealed that 24 patients died in the control group (82.4%), and three died in the MSC group (45%), a significant difference in survival. At hospital discharge, several blood indices differed significantly between the groups. The procalcitonin, creatinine, creatine kinase, prothrombin time, and D-dimer were all significantly higher in the control group versus the experimental group, suggesting a

higher number of critically ill patients in the control group. Information omitted from the study was the timing of the MSC infusions as related to the clinical, laboratory, and radiologic findings, and cause of death. Specific biomarkers of inflammatory outcomes such as IL-6, IL-8, and surfactant protein D were not included. There was no statistically significant difference in the C reactive protein between the MSC and placebo group. At 24 weeks and 1 year, all patients showed improvement of lungs on chest CT scan. One to 5 year follow-up in 4 patients with H7N9- induced ARDS revealed no significant differences in lung function tests (measured at 8-12 weeks; 24 weeks; year 1; year 2; year 5) within the experimental group, suggesting that MSC transplantation did not exert harmful effects on the lung in long term follow-up. Chen et al (41) postulated that since SARS-CoV-2 ARDS has very similar pathological features as H7N9 induced ARDS, in addition to similar symptoms, some COVID-19 patients with severe ARDS may benefit from MSC therapy.

A newly published pilot study by Leng et al (42) conducted single dose clinical grade MSC transplantation of 1×10^6 cells/kg in 100 mL of normal saline in 7 patients with common (2 patients), severe (4 patients) and critically severe (one patient) SARS-CoV-2 ARDS. MSCs were administered when patient signs and symptoms were worsening and as standard treatment was conducted. Three severe SARS-CoV-2 ARDS patients were enrolled as placebo controls. The follow-up period was 14 days for primary safety data and primary and secondary efficacy outcomes. No acute infusion reactions, delayed hypersensitivity, or secondary infections were observed after MSC treatment. In all 7 patients, 2 to 4 days after MSC transplantation, all symptoms reportedly disappeared (fever, shortness of breath, low oxygen saturation (rose to greater or equal to 95% at rest, with or without oxygen uptake (5 liters per minute). In the severe ARDS patient, the ratio of before and after MSC treatment serum anti-inflammatory cytokine IL-10 was significantly increased ($P = 0.0282$), while the proinflammatory chemokine TNF α significantly decreased ($P = 0.0269$) compared to the control group. The sample size was small, and the study lacked specific information for evaluation of secondary efficacy on 9 out of the 10 patients overall, such as clinical course, comorbidities, and inflammatory cytokine plasma levels. Detailed information is provided in the only 1 critically severe patient in the experimental group.

Nevertheless, results showed that all 7 experimental patients were free of symptoms at 2-4 days after MSC

treatment, impacting the cytokine storm. An interesting aspect of the study revealed captured MSCs were rarely positive for ACE2 and TMPRSS2 on 10 x RNA-seq survey, suggesting these MSCs were free of SARS-CoV-2 infection. The reasoning behind the MSCs being free of viral infection is based on the model for SARS coronavirus entry into the cell. The transmembrane spike (S) protein on the virus binds to the host cell receptor angiotensin-converting enzyme 2 (ACE2) using the serine protease TMPRSS2 for host cell entry (43). On the other hand, MSCs genetically enhanced with ACE2, in preclinical studies in the mouse ARDS model, have been shown to improve lung histopathology, alleviate LPS-induced lung inflammation, and improve pulmonary endothelial functions (44). Leng et al (42) also performed a 10 x RNA-seq survey which demonstrated anti-inflammatory and growth factors such as TGF beta, HGF, LIF, GAL, NOA1, FGF, VEGF, EGF, BDNF, and NGF were expressed in MSCs reflecting immunomodulatory functions, which they postulated could prevent the cytokine storm.

Case Reports

Simonson et al (45) published a case report studying the in vivo effects of MSCs in 2 adult men with severe refractory ARDS diagnosed by Berlin criteria who were on mechanical ventilation and extracorporeal membrane oxygenation (ECMO) support, with multi-organ failure. Both compassionately received a total of 2×10^6 cells/kg of bone marrow derived MSCs from one donor administered in the same manner. Patient 1 had Influenza AH1N1 related lung injury, and patient 2 suffered Acute Myelogenous Leukemia (AML) with probable transfusion related lung injury. No adverse events were seen in the patients during MSC infusion. Both patients recovered with improved lung function. Patient 1 was discharged from the hospital and returned to work. Patient 2 returned to the hospital general ward off ventilator and ECMO and then received chemotherapy for AML. The researchers analyzed in detail the immunomodulatory features and proteins expressed by the MSCs which were infused into the 2 patients and correlated them with in vivo inflammatory actions. They found a decrease in pulmonary and systemic inflammatory markers, including epithelial apoptosis, alveolar capillary fluid leakage, proinflammatory cytokines, microRNAs, and chemokines in bronchioalveolar lavage fluid and plasma. In both patients, surfactant protein B levels increased in BAL fluid during the 4 days after MSCs were administered, a sign of alveolar epithelial recovery (45).

MicroRNA (miRNA) profiling of blood extracellular vesicles (EVs) *in vivo* was performed in both patients. Once thought to be pieces of debris, EVs are small circular structures surrounded by a phospholipid bilayer released by nearly all cells containing a variety of substances including inhibitory miRNAs which play a role in vital cell to cell communication (46). Simonson and colleagues found that in both patients levels of pro-inflammatory miRNAs in circulating EVs (miR-409-3P, 886-5P, 324-3P, 222, 125A-5P, 339-3P, 155) that were elevated before MSC administration, declined significantly within 24 hours of infusion of MSCs. The proteome characterization of the donor MSCs and their secreted EVs revealed an overlap of 754 proteins. Several proteins were only detected in EVs, supporting previous reports that EVs result from selective packaging into vesicles (45). Further, a GO enrichment analysis of the identified EV proteins revealed a significant enrichment of metabolic processes such as the tricarboxylic acid cycle, the glycolysis pathway, and adhesion and integrin signaling, which the authors believe could aid the energy deficits in ARDS lung injury. Even though this study included only 2 patients, incorporation of *in vitro* mechanisms of action in parallel with clinical study of MSCs brought out significant quantifiable biomarkers.

Liang et al (47) presented a case report of a 65-year-old woman with COVID-19 pneumonia, who despite treatment with antiviral therapy, IFN α inhaler, intravenous antibiotics, methylprednisolone, immunoglobulin, and non-invasive mechanical ventilation, became critically ill with acute respiratory failure and acute diarrhea. She was intubated and transferred to ICU, and her condition deteriorated. She exhibited acute gastrointestinal bleed, anemia, and liver injury. Glucocorticoids and antiviral medications were discontinued. With family consent, the medical team attempted to treat her with allogenic human umbilical cord mesenchymal stem cells (hUCMSCs). Three intravenous doses each of 5×10^7 cells were given three days apart. No adverse effects were observed on infusions of hUCMSCs. Antibiotics and thymosin α 1 were also given. Two days after the second dose of MSCs, the patient was extubated, off the ventilator, and ambulating. After the second administration of MSCs the bilirubin, CRP, ALT and AST, WBC count, neutrophil and lymphocyte count returned to normal. The CT scan of her lungs improved 6 days after the last MSC infusion. Liang and colleagues (47) speculated that the mechanism by which the patient recovered was hUCMSCs homed to repair injured tissues and neutralized the proinflammatory cytokines

expressed by MSCs. The effects of coinciding treatment of the patient with thymosin α 1, an enhancer of the immune system, and hUCMSCs may limit speculation on the treatment efficacy.

DISCUSSION

Severe cases of COVID-19 may quickly result in ARDS, sepsis, and multiorgan failure, including kidney and cardiac injury (48). Although there are no specific treatments for ARDS or ARDS from severe COVID-19, treatment involves supportive care. A variety of therapies have been proposed, and there are protocols for compassionate use among the severely ill. One of these interventions is stem cell-based therapy. Since MSCs are known to home to injured tissues and then secrete soluble factors modulating the immune system (19), critically ill COVID-19 patients could benefit from this MSC tissue specific homing to injured lungs, kidneys, and heart. Increasing information is being collected to describe the damage to the heart and lungs.

One of the first autopsy series of the injuries to lungs and heart in patients with severe COVID-19 by Fox et al (49) showed diffuse alveolar damage characterized by hyaline membrane formation, predominantly interstitial lymphocytic infiltrate of CD4+ and CD8+ lymphocytes, and desquamated pneumocytes with viral cytopathic effect (cytomegaly, enlarged nuclei with eosinophilic nucleoli) in alveoli. Alveolar capillaries contained fibrin thrombi. CD61+ pulmonary megakaryocytes were present in alveolar capillaries associated with platelets, fibrin, and neutrophils (49). CD4+ lymphocytes were aggregated around thrombosed small blood vessels and suggested thrombotic microangiopathy in the lungs (49). Sections of the myocardium revealed atypical individual cell myocyte necrosis with rare lymphocytes surrounding degenerated myocytes (49). Laboratory findings of these COVID-19 patients within 24 hours of death included increased neutrophil count, relative lymphopenia, elevated aspartate aminotransferase, glucose, creatinine, D-dimer, fibrinogen, ferritin, and PT (49).

Taken together, data from autopsy and laboratory studies point to overactive cytokine pathway and platelet response among COVID-19 patients (49). Also, COVID-19 patients admitted to the ICU showed elevated plasma levels of cytokines and chemokines, IL2, IL7, IL10, GCSF, IP10, MCP1, MIP1A, and TNF α , compared to non-ICU patients, suggesting severe disease resulting from the cytokine storm (50). Thus, it is thought that when MSCs transplanted into an inflammatory

environment are exposed to tissue with high levels of pro-inflammatory cytokines they may adopt immunosuppressive features, modifying dendritic cell maturation and suppressing T lymphocytes and natural killer cells (51), which may in turn reduce the effects of the cytokine storm.

Results of the 6 studies reviewed show there is relative safety in administering MSCs in the short term, and no adverse events or toxicities were recorded across studies. Yet one long-term safety issue to be considered in MSC therapy is the potential of MSCs to promote tumorigenesis or metastasis in a tumor bearing host, as MSCs suppress anti-tumor immune response (IL-10 and TGF beta) and generate new blood vessels (VEGF and HGF) (11,19). Another long term MSC transplantation consideration is risk of MSCs suppression on the immune system in immunocompromised individuals (19). Follow-up studies over months and even years are needed to address these issues. Also concerns regarding MSC therapy include high cost, lack of standardization of isolation procedures, donor heterogeneity, ex vivo storage and expansion, variable dosing amount and intervals, and route of delivery (21,52). Such complexities are important but beyond the scope of this review.

In the 2 most powerful clinical studies of MSC therapy for ARDS by Zheng et al (39) and Matthay et al (40), which were randomized, double-blind, placebo-controlled, there were no significant group differences on the quantifiable variables measured, such as biomarkers, mortality, ventilator free days, and ICU free days. Nevertheless, these nonsignificant findings could have been flawed by study limitations. Small numbers of patients in groups, group differences in clinical characteristics, and short follow-up times are some of these problems and underscores the need for larger, more carefully designed, controlled trials to determine both efficacy and safety of MSC therapy.

In the 2 pilot studies by Leng et al (42) and Chen et al (41) with control groups involving ARDS caused by viral infection several quantifiable factors were assessed. In Leng's study (42), serum cytokines, cytokine secreting immune cells, and CRP were measured. CRP decreased, and CXCR3+CD4+ T cells, CXCR3+CD8+ T cells, and CXCR3+ NK cells disappeared in 3-6 days after MSC treatment in one critically severe patient. IL10 increased and TNF α decreased, both significantly in the MSC severe COVID-19 patient group of 3, compared to controls. In Chen's study (41) the only measured biomarker, CRP, showed no difference between the 2

groups. However, mortality was significantly lower in the MSC group, yet other recognized endpoints were lacking.

The 2 case reports described suggest possible benefits and no harm to patient outcomes. They add quantifiable factors to the body of evidence for immunomodulatory mechanism of action of MSCs. In 2 adult male patients with severe illness, Simonson et al (45) quantified levels of pulmonary (BAL fluid) and systemic (serum) inflammatory markers; surfactant protein B, albumin, IL-6, IL-8, IFN gamma, lung epithelial recovery markers; K18, cck18, and microRNA profiling of extracellular vesicles in the blood. Findings suggested decrease in proinflammatory markers and recovery of alveolar epithelium in conjunction with improved lung function three days after infusion of MSCs. Both patients survived the episode of refractory ARDS. These results contrast with those of Zheng et al (39) who found insufficient evidence that MSCs alleviate lung inflammation.

Finally, Liang et al (47) presented one of the first study cases using hUCMSCs to treat a critically ill patient with COVID-19 and multiorgan failure. The patient also was treated with thymosin α 1, an immune booster, which of course, confounds the interpretation of results. Yet, the patient was discharged from ICU 8 days after the first dose of MSCs with CRP and most other clinical laboratory values having returned to normal. In this favorable outcome case report of only 1 patient, there can be no knowledge as to the possible mechanism of action of the MSCs. However, the Liang et al (47) case report evidence suggests an encouraging outcome that cannot be ignored in the search for better therapeutics for COVID-19. Unfortunately, as described in the Liang et al (47) case report, though the authors conclude that hUCMSCs assisted to repair injured tissue and attenuated proinflammatory cytokines, the precise mechanisms involved in the improvement of the patient cannot be certain. Clearly, as COVID-19 continues to spread throughout the world with critical and sometimes rapid development of ARDS and no specific cure, clinical trials are urgently needed to evaluate MSCs immune modulation and promotion of repair. Thus, studies of treatments for COVID-19 pneumonia have been expedited worldwide, especially for compassionate use reasons (53). Currently, there are 24 clinical trials evaluating stem cells for treatment of SARS-Cov-2 infected patients registered on ClinicalTrials.gov with researchers conducting studies around the world.

LIMITATIONS

Limitations of this review include the small number of case reports that make up the majority of information to date on stem cells and patients suffering from COVID-19. In all cases, these patients had other therapeutics making exact dose and therapeutic response ambiguous to some degree.

CONCLUSION

Limited numbers of patients have been treated with MSCs for ARDS, and even fewer have been offered MSCs for severe COVID-19 induced ARDS. The clinical studies discussed were designed to evaluate safety and potential efficacy of MSC therapy for ARDS, and some cases specifically for ARDS secondary to COVID-19 in adult patients. Probably of greatest interest are two recent reports. Leng et al (42) published a single dose clinical trial of MSC in 7 ARDS secondary to COVID-19 cases and 3 controls; and a case study by Liang et al (47) who described resolution of all COVID-19 symptoms in

a severely ill woman on ventilator who was administered three intravenous MSC doses. In these 2 reports, as many as 8 ARDS patients with COVID-19 showed symptom resolution subsequent to administration of MSCs, although the follow-up time periods varied, and there was no consistency in measurement of biological variables. None of the critically ill patients given MSCs perished from the ARDS induced pneumonia, however all patients had received other therapeutics making exact conclusions difficult. Moreover, MSC transplantation for ARDS and specifically COVID-19 induced acute respiratory distress syndrome appears safe. As time passes, there will be more studies addressing the specific symptoms and pathology associated with COVID-19 respiratory illness, including possibly therapeutic drugs and drug regimens; however, along with these investigations, it is clear that one path to recovery may involve better organized and rigorous use of MSCs driven by adequate validity and replicability of scientifically controlled studies.

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