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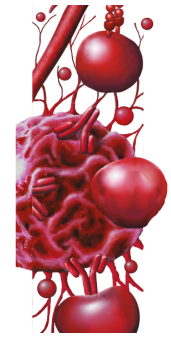

Mesenchymal stem cell treatment for hyperactive immune response in patients with COVID-19

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The human immune system protects the body against invasive organisms and kicks into a hyperactive mode in COVID-19 patients, particularly in those who are critically sick. Therapeutic regimens directed at the hyperactive immune system have been found to be effective in the treatment of patients with COVID-19. An evolving potential treatment option is therapy with mesenchymal stem cells (MSCs) due to their regenerative and reparative ability in epithelial cells. Clinical trials have reported the safe usage of MSC therapy. Systemic effects of MSC treatment have included a reduction in pro-inflammatory cytokines and a decrease in the levels of CRP, IL-6, and lactase dehydrogenase, which function as independent biomarkers for COVID-19 mortality and respiratory failure.

Plain language summary: Treatment of COVID-19 is becoming increasingly difficult because of new variants, such as Delta, and more recently Omicron. Each virus variant becomes smarter at being able to evade the body's immune system, vaccines and drug treatments. The biggest challenge in treating COVID-19 is when the body's immune system starts to become hyperactive. In such a scenario, the immune system releases the compounds that are supposed to be released in small doses all at once. Thus, overwhelming the body and causing many complications. One possible solution to this is the mesenchymal stem cell. Multiple clinical trials have shown that mesenchymal stem cells can heal all different cell types in the body and stop the hyperactive immune system.

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Keywords: biomarkers • clinical immunology • COVID-19 • endothelitis • mesenchymal stem cells

The onslaught of the coronavirus pandemic has precipitated a calamitous increase in the number of patients who need immediate and effective treatment for COVID-19 caused by the SARS-CoV-2. Despite medical advances in vaccines and antiviral therapy, treating COVID-19 patients remains a challenge. Treatment of COVID-19 patients is difficult because of the many point mutations that may arise when the virus jumps from person to person, resulting in 'variant[s] of concern' [1,2]. The most recent variant at the time of this writing, Omicron, was confirmed on 9 November 2021. Omicron is a global threat due to its ability to evade antibodies in vaccinated and unvaccinated individuals [3]. Although prophylactic measures such as wearing masks, social distancing and vaccines help prevent some transmission, it is not known whether they will continue to help with future variants. Furthermore, there has been unprecedented vaccine hesitancy, precipitated by political and religious reasons as well as a lack of awareness and the rapid spread of misinformation on social media supported by individuals, misinformed or misguided, pushing their own agendas. The incidence of Omicron and potential future variants highlight the need for therapies such as mesenchymal stem cells (MSCs) in treating patients infected with COVID-19.

COVID-19 presents with a wide range of disorders such as lung injuries, venous/arterial thromboses, neurological injuries, stroke, and much more [4]. Particularly susceptible to mortality are elderly individuals, who often present with acute respiratory distress syndrome (ARDS) and multiple organ failure [5]. The physiological process of SARS-CoV-2 infectivity is not yet clear, but there is a general understanding that the SARS-CoV-2, through angiotensin-

converting enzyme 2 (ACE2) receptors, uses serine protease *TMPRSS2* to infiltrate endothelial/epithelial cells [6]. Studies have shown a correlation between the severity of SARS-CoV-2, and the location and the density of ACE2 receptors expressed. For instance, the lungs and the heart both exhibit high levels of ACE2 receptors, causing COVID-19 patients to have cardiovascular complications in addition to pulmonary problems [4]. COVID-19 progression is divided into three phases: the viremia, acute, and recovery phases. As the infection progresses, T- and B-cell levels are reduced while levels of circulating pro-inflammatory cytokines are increased, culminating in phenomena led by the immune system called the hyperactive immune response, a hallmark sign among severe COVID-19 patients, that results in disseminated intravascular coagulation, causing multiorgan failure and death [5].

Hyperactive immune response is not a cytokine storm

The term 'cytokine storm' became popular once the media started reporting on the high levels of cytokines in the circulatory system of patients with COVID-19 due to the lack of terminology available to describe the hyperactive immune system response. The levels of pro-inflammatory cytokines are elevated in patients, especially those who are very sick, but not as extreme as those seen in patients with ARDS [7]. Because COVID-19-associated cytokine storm presents with lower cytokines compared with ARDS, a novel term that accurately describes this phenomenon is hyperactive immune response, and this is the term used in this article.

Mesenchymal stem cell therapy

One possible solution to counter hyperactive immune response is the use of MSCs due to their immunomodulatory properties, which allow them to detect changes, such as inflammation, in the environment [8,9] (Figure 1). In the presence of a hyperactive immune response, the endothelial layer releases tissue factors into the systemic circulation that trigger factors VII and X as well as vWFs of the coagulation cascade, leading to clot formation [10] (Figure 2). Any therapy directed at COVID-19 should treat the hyperactive immune response, either specifically or nonspecifically. An artificial intelligence study by Sahoo *et al.* analyzed genes released during a hyperactive immune response and identified the root cause of the response: airway epithelium [11]. The therapeutic targeting of the airway epithelium in a COVID-19 patient could potentially prevent the hyperactive immune response (Figure 3) [11].

MSCs influence the cellular physiology of other cell types via paracrine effects through secreted factors and extracellular vesicles [12]. MSCs stimulate both innate and adaptive immune systems affecting the physiology of dendritic cells, T cells, B cells, and macrophages [6]. In an interesting clinical observation, Leng *et al.* [13] showed that MSCs suppressed the accumulation of macrophages in the SARS-CoV-2 infected lung tissue and stimulated the proliferation of regulatory dendritic cells. MSCs also suppressed recruitment of neutrophils to the infected lung tissue and through secretion of IL-10, reduced secretion of pro-inflammatory cytokine TNF-α. Th17 cell lineage can act as the source of the hyperactive immune response and thus is an important target for MSC therapy [14]. MSCs secrete immunosuppressive factors such as TGF-ß1, enabling proliferation and transformation of Th17 cells to anti-inflammatory *FOXP3* Tregs [15]. MSCs stimulate the polarization of pro-inflammatory M2 to anti-inflammatory M1 macrophage phenotype and downregulate the expression of chemokines CXCL1/2, and pro-inflammatory cytokines such as IL-6 and TNF-α [16].

In addition, MSCs can exert their immunomodulatory effects independent of the hyperactive immune response. First, they prevent infiltration of the pro-inflammatory cytokines. Second, MSCs coupled with TSG-6 reduce pro-inflammatory macrophages and release protective molecules such as kynurenic acid, spermine, and lactate. Coupled with B and T cells, MSCs increase the production of *FOXP3* and IL-10, and further, MSCs regulate the immune response via extracellular vesicles and exosomes [17,18].

MSCs: a potential treatment option for hyperactive immune response

MSCs do not express or exhibit ACE2 receptors and have been deemed to be SARS-CoV-2 resistant [19]. Ellison-Hughes *et al.* provided several clinical cases as examples of the use of MSCs in the treatment of hyperactive immune response [17]. In the first MSC clinical study in China, administration of MSCs reduced COVID-19-related symptoms in 2–4 days with oxygen saturation stabilizing at 95% without the need for mechanical ventilation. In this study, severe and critical patients saw a notable switch in the presence of pro-inflammatory cells such as CXCR3+CD4+T cells to CD14+CD11C+CD11b regulatory dendritic cells [17,20].

A case study in China reported significant improvement in a 66-year-old patient who developed ARDS, pulmonary edema and septic shock from COVID-19. The study reported a significant increase in anti-inflammatory

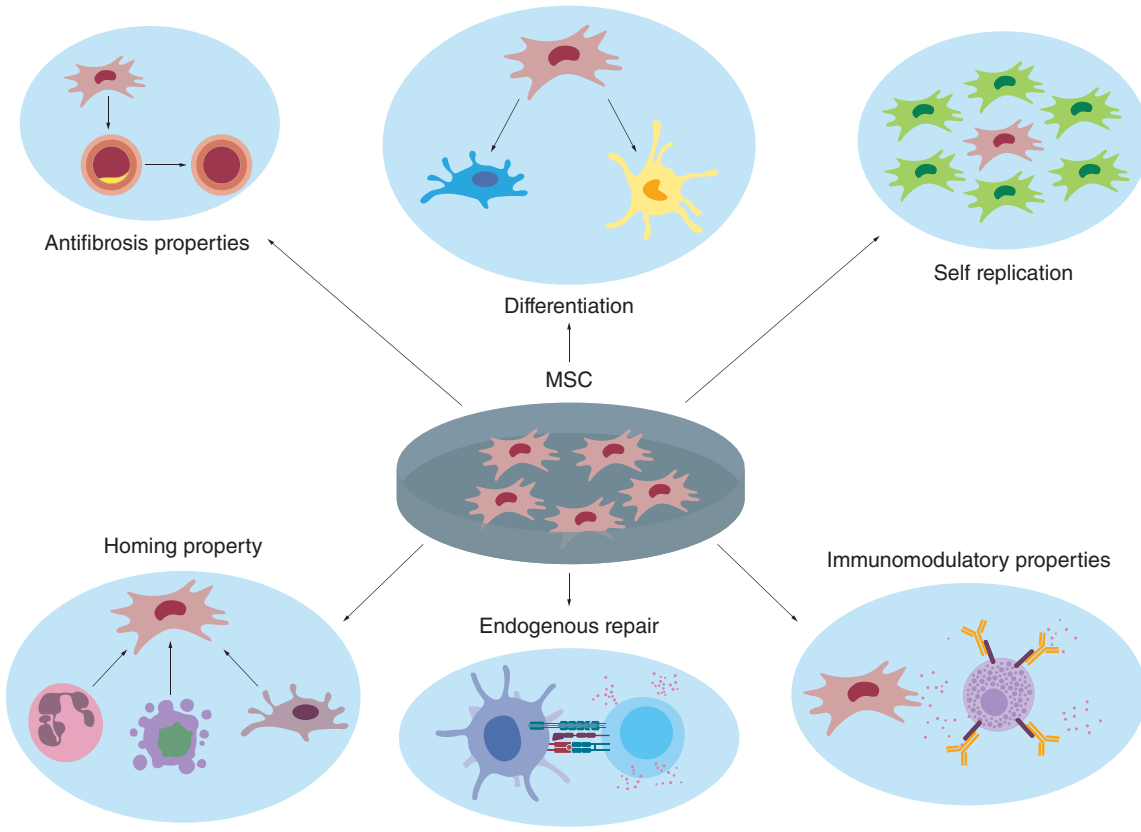

Figure 1. Abilities of mesenchymal stem cells. MSCs are stromal progenitor cells that can self-renew and differentiate into other native environment cells. Essentially, MSCs take the properties of one lineage and transfer them to another lineage. MSCs primarily act on T and B cells, macrophages, and dendritic cells, thus exerting their effects such as antifibrosis, endogenous repair, immunomodulation, and homing effects on almost all immune cells. Image created with BioRender.com.
MSC: Mesenchymal stem cell.

cytokine IL-10 and a decrease in pro-inflammatory cytokine IL-6, both involved in hyperactive immune response [12].

A case study from Brazil reported a patient who showed no response to O_2 supplementation and treatment with dexamethasone and enoxaparin. The patient was then administered two doses of tocilizumab, an IL-6 receptor antagonist, followed by intravenous infusion of MSCs. The patient showed improvement specifically after MSC infusion [21]. Another study from Brazil [22] reported the case of a 30-year-old man who presented with COVID-19; this patient was placed on mechanical ventilation before therapy with human umbilical cord MSC (hUC-MSC). There were no serious adverse events (AEs) with MSC therapy, and CT scan imaging showed decreased fibrosis. The lack of AEs is especially important due to the thromboinflammatory state COVID-19 patients present with. Three days after MSC infusion, a significant reduction in proinflammatory cytokines was observed [22].

Primorac *et al.* treated a patient with lung fibrosis, limited response to O_2 supplementation, and high D-dimer levels (>35.2 mg/l) with ImmnoART, a bone-marrow-derived MSCs, and after 22 days of hospitalization, leukocyte count and levels of D-dimer and CRP returned to normal. This case report serves to highlight the potential use of MSCs in critically ill COVID-19 patients [23]. Similarly, a 48-year-old patient in China was treated successfully with hUC-MSCs after presenting with lung fibrosis, high levels of neutrophils, CRP, ARDS and low blood oxygen levels [24].

Zengin *et al.* published a report of a COVID-19 patient who presented with late-stage COVID-19 and abnormally high IL-6 levels and was treated with MSCs. The treating physicians reported no AEs but stressed the importance of extrapolating data from clinical trials rather than case reports [25].

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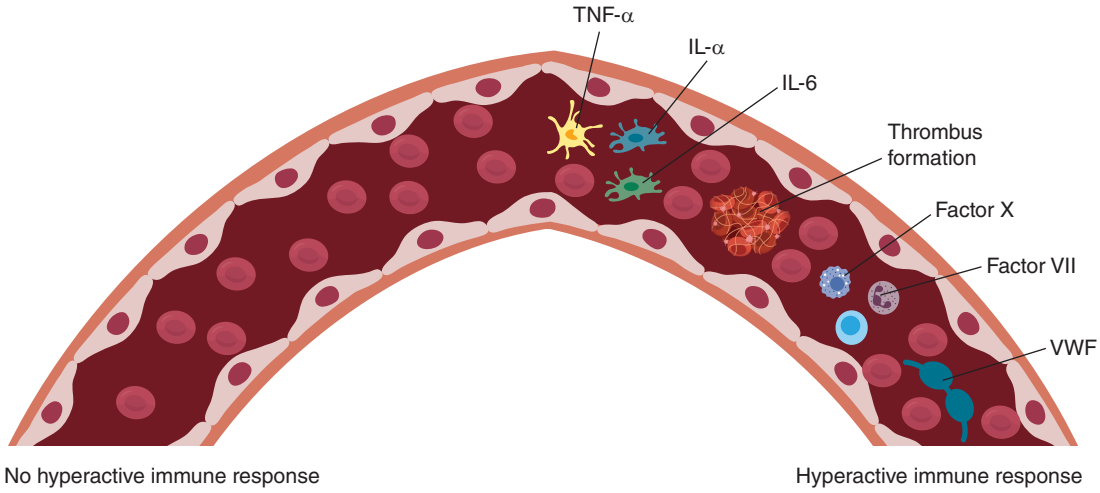

Figure 2. Role of hyperactive immune response in endothelial damage. Functioning endothelial cells regulate the integrity of the barrier, vasodilation, anticoagulation, anti-inflammatory, antioxidant and profibrinolytic properties. The combination of anticoagulant and antithrombotic properties of the endothelial layer is responsible for the endothelial layer's ability to prevent or destroy clot formation. However, when the immune system's actions become hyperactive, the cytokines (e.g., IL-1α, IL-1β, IL-6 and TNF-α) disrupt the balance between the endothelial layer's prothrombotic and antifibrinolytic properties. Such disruptions contribute to clot formation and disseminated intravascular coagulation (DIC) in young and older age COVID-19 patients [5].
Image created with BioRender.com.

Completed MSC clinical trials in COVID-19 patients

Data on clinical trials with MSCs were obtained from clinicaltrials.gov, chictr.org.cn, clinicaltrialsregister.eu and cochranelibrary.com. The following search terms were used: 'mesenchymal stem cells', 'MSC', 'cytokine storm', and 'cytokine release syndrome'. Upon compilation of clinical trials, 'completed' was chosen in the 'Status' box as a criterion. Once completed, clinical trials were chosen to be analyzed if 'coronavirus', 'COVID-19' and similar terminology were seen. As of this writing, there have been 28 successfully completed MSC clinical trials with 14 clinical trials having posted the results of their study (Table 1).

The primary outcomes measured in all the completed trials were the safety and efficacy of MSC treatment – that is, whether the patient presented with serious or nonserious AEs post-treatment. In addition to the primary outcome, the secondary outcomes measured were pro- and anti-inflammatory markers (Table 2). The majority of trials were in phase II, administering hUC-MSCs through intravenous infusion. (Figure 4)

Adas *et al.* [26] conducted a trial that examined alterations in hyperactive immune response with MSC administration. Thirty patients were divided into three groups. Group 1 was composed of moderately sick patients treated with standard therapy, group 2 was composed of critically sick patients treated with standard therapy, and group 3 was composed of critical patients treated with standard therapy with MSC therapy [26]. The standard therapy administered to the patients included antibiotics (piperacillin-tazobactam), antivirals (Favipiravir), dexamethasone, hydroxychloroquine, and enoxaparin. The MSCs were administered in three doses of 3 x 10^6 cell/kg via intravenous infusion every 3 days for 6 days. IL-6 and CRP levels were higher in groups 1 and 2. However, group 3 patients who received MSC therapy exhibited significantly lower levels of IL-6 and CRP [26]. The other cytokines measured- IFN-γ, IL-2, IL-12 , and IL-17A declined significantly after the third dose of MSCs. The anti-inflammatory cytokines such as IL-10, IL-13, and IL-1ra levels increased significantly in Group 3 patients. There were no AEs or complications during administration of MSCs or during the follow-up period. The investigators concluded that MSC therapy resulted in reduction in mortality and ICU admission time concurrent with reduction in pro-inflammatory cytokines and an increase in trophic factors that aid in endogenous repair of endothelial cells [26].

An alternative to MSCs is a recently approved adjuvant therapy, MSC extracellular vehicles termed exosomes. Clinically, exosomes provide several advantages such as intranasal or inhalation route of administration; elimination of the risk of uncontrolled variables such as proliferation, hemocompatibility issues, and risk of transmission; and

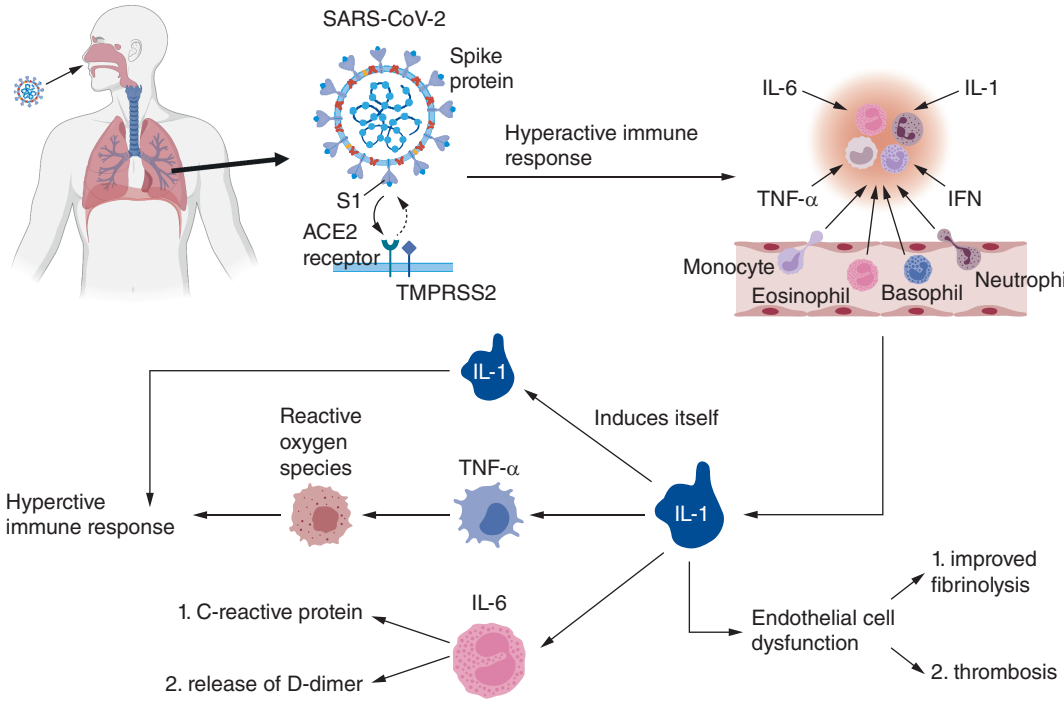

Figure 3. Positive feedback loop involved in the hyperactive immune response. Reactive oxygen species alert cells of oxidative stress and help in the upregulation of antioxidants. When a patient presents with a hyperactive immune response, the subsequent release of cytokines, such as TNF-α and IL-1, causes an increase in the levels of hydrogen peroxide and superoxide. IL-1 is produced by macrophages and Th17 cells differentiation, and its involvement in the TNF-α pathway allows it to include itself in the feedback loop. Due to the interaction between the cytokines and endothelial cells, NAD(P)H oxidized-derived reactive oxygen species accumulate causing more oxidative stress and are firmly entrenched into a positive feedback loop that activates excess pro-inflammatory cytokine production. The activation of neutrophils and macrophages by cytokines helps illustrate its role in the positive feedback loop. The advent of the feedback loop allows for widespread inflammation, which in turn leads to the loss of vascular integrity, onset of endotheliitis, infiltration of the virus into the systemic circulation, and ultimately respiratory failure. Image created with BioRender.com.

ease of storage because of extended shelf life [39–42]. The first clinical use of bone marrow MSC-derived exosomes was tested in an open-label study conducted using ExoFlo (a product derived from allogeneic bone marrow) [30]. Laboratory results showed significant reduction in neutrophil number and acute phase proteins such as CRP, ferritin and D-dimer without any AEs. The survival rate of the cohort was 83%, indicating that ExoFlo could be a potential therapeutic agent for COVID-19 treatment. Similarly, groups 1 and 2 in NCT04491240 were each given MSC exosomes twice daily for 10 days. Group 3 was given placebo twice daily. Group 1 had the lowest time to clinical recovery from COVID-19 infection detection, followed by the placebo group and then group 2. The first biomarker measured was CRP, which has been found to be an accurate, sensitive, and early predictor of COVID-19 severity in patients [43–45]. COVID-19 patients in both groups 1 and 2 saw significant decreases in CRP levels of 72.47 and 69.57 mg/l (vs. reduction of 53.21 mg/l in the placebo group). Another biomarker studied was lactate dehydrogenase (LDH), a nonspecific indicator of cell injury [45,46]. Like CRP, LDH levels decreased the most in groups 1 and 2: 332 and 367 Ul, vs. a reduction of 239 U/l in the placebo group.

Kouroupis *et al.* [27] completed a trial with 24 participants split into two groups. Group 1 was given hUC-MSC and heparin. Group 2 was given placebo along with supportive care. Trial participants were administered two doses of either hUC-MSC or placebo in 100 x 10^6 cells/infusion intravenously at 24 and 72 hours after study enrollment. The investigators noted significant differences in survival, serious AEs, and time to recovery compared with the control group. After 6 days, sTNFR2 levels were significantly higher, and TNF levels were significantly lower. These results are important because increased sTNFR2 levels have been shown to increase the Treg, Foxp3+ [27].

Dilogo *et al* [29] conducted a trial that examined changes in 40 critically ill COVID-19 patients with ARDS, PaO_2/FiO_2 levels below 300 mm Hg, shock, and/or multiorgan failure. Patients were randomly assigned to either

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Table 1. Completed mesenchymal stem cell clinical trials in COVID-19 patients.

ID Number	Intervention	n	Results	Ref.
NCT04713878	MSCs	21	Not Posted	
NCT04573270	PrimePro (MSCs) and placebo	40	Not Posted	
NCT04898088	MSC transplantation	30	Not Posted	
NCT04349631	Hb-adMSCs	56	Not posted	
NCT04392778	MSC treatment and saline control	30	Published	[26]
NCT04355728	hUC-MSCs + heparin with supportive care and placebo (heparin with supportive care)	24	Published	[27]
NCT04492501	Convalescent plasma, MSC therapy, tocilizumab and remdesivir	600	Not posted	
NCT04535856	Allogeneic MSCs and placebo	9	Not posted	
NCT04491240	EXO 1 inhalation, EXO 2 inhalation and placebo inhalation	30	Posted on ClinicalTrials.gov	
NCT04288102	UC-MSCs and saline containing 1% human serum albumin	100	Published	[28]
NCT04276987	MSC-derived exosome	24	Results submitted	
NCT04457609	Oseltamivir, azithromycin and UC-MSCs	40	Published	[29]
NCT05122234	Injection of MSC secretome and placebo	40	Not posted	
NCT04382547	Allogenic pooled olfactory mucosa-derived MSCs and standard treatment	32	Not posted	
NCT04348435	Hb-adMSCs and placebo	55	Not posted	
NCT04625738	*Ex vivo* expanded Wharton's jelly MSCs and placebo	30	Not posted	
NCT04493242	DB-001 and saline	120	Not posted	
NCT05019287	Allogeneic human menstrual blood stem cells secretome	29	Not posted	
NCT04657458	Bone marrow MSCs derived extracellular vesicle	24	Published	[30]
NCT04416139	Intravenous hUC-MSCs	10	Published	[31]
ChiCTR2000029606	Human menstrual blood derived stem cells, artificial liver therapy, conventional therapy, artificial liver therapy + human menstrual blood-derived stem cells + conventional treatment	63	Published	[32]
ChiCTR2000031494	hUC-MSCs and standard therapy	28	Published	[33,34]
ChiCTR2000029990	Clinical grade MSCs	10	Published	[13]
DFG Projekt # 374031971	MSCs	13	Published	[35]
doi: 10.1007/s12015-021-10214-x†	UC-MSCs	210	Published	[36]
doi: 10.6061/clinics/2021/e2604†	hUC-MSCs	25	Published	[37]
IRCT20200217046526N2	hUC-MSCs	11	Published	[38]
2020-002772–12	Wharton's jelly MSCs	30	Not posted	

†Trial ID was not found in clinicaltrial.gov, chictr.org.cn, clinicaltrialsregister.eu or Cochrane database.
EXO: Exosomes; Hb-adMSC: Adipose-derived mesenchymal stem cell; hUC-MSC/UC-MSC: Human umbilical cord mesenchymal stem cell; MSC: Mesenchymal stem cell.

the MSC group or the control group with standard treatment. Of the 40 patients, 26 succumbed to COVID-19, and 10 from the MSC group and four from the control recovered. The length of stay for patients in the MSC group was higher compared with the control group. IL-6 levels in the MSC groups declined significantly in recovered patients. Analysis of CRP, D-dimers, ferritin, VEGF, IL-10, and CXCR3 in the two groups showed no significant difference [29].

In an open-label trial, Xu *et al.* [32] administered menstrual-blood-derived MSCs in a nonrandomized fashion to 26 patients compared with the hUC-MSCs administered in other trials. The use of menstrual-blood-derived MSCs circumvents the ethical issues that arise with other types of MSCs [32]. The survival rate in the experimental group was 91.31% compared with 66.67% in the control group. There were no significant differences in CRP and IL-6 levels before and after infusion of menstrual blood-derived MSCs [32].

Shu *et al.* [34] split 28 patients into a control group (standard treatment) and standard treatment plus HUC-MSCs (2×10^6 cells/kg for 1 hr). The time to recovery for the MSC group was shorter than the control group. Researchers found that CRP and IL-6 levels were significantly lower in the MSC group 7 days after infusion. Additionally, the MSC group saw lymphocyte count return to normal faster and chest CT scans showed reduced inflamed lobes.

Table 2. Major COVID-19 markers measured in completed clinical trials with results.

ID	CRP	IL-6	IL-10	LDH	Ferritin	D-Dimer	Ref.
NCT04392778	Decreased†	Decreased†	Increased†	NM	NM	Increased in control	[26]
NCT04491240	Decreased†	NM	NM	Decreased†	NM	NM	
NCT04288102	Increased	Increased‡	NM	Increased	NM	NM	[28]
NCT04457609	Measured	Decreased†	Increased	NM	Decreased	Measured	[29]
NCT04657458	Decreased†	NM	NM	NM	Decreased†	Decreased†	[30]
NCT04355728	Decreased	NM	NM	NM	Not measured	Increased	[27]
ChiCTR2000029606	Decreased	Increased	NM	NM	NM	NM	[32]
ChiCTR2000031494	Decreased†	Decreased†	NM	NM	NM	NM	[33,34]
ChiCTR2000029990	Increased	NM	Increased†	NM	NM	NM	[13]
DFG Projekt # 374031971	Decreased	Increased	NM	NM	Increased†	No change	[35]
doi: 10.1007/s12015-021-10214-x†	NM	NM	NM	NM	NM	NM	[36]
doi: 10.6061/clinics/2021/e2604†	Decreased	Decreased	No change	NM	NM	NM	[37]
IRCT20200217046526N2	Decreased†	Decreased†	Increased	NM	NM	NM	[38]

†Statistically significant difference between the MSC and control groups.
‡Increased overall interleukin levels but did not mention IL-6 specifically.
LDH: Lactate dehydrogenase; MSC: Mesenchymal stem cell; NM: Not measured.

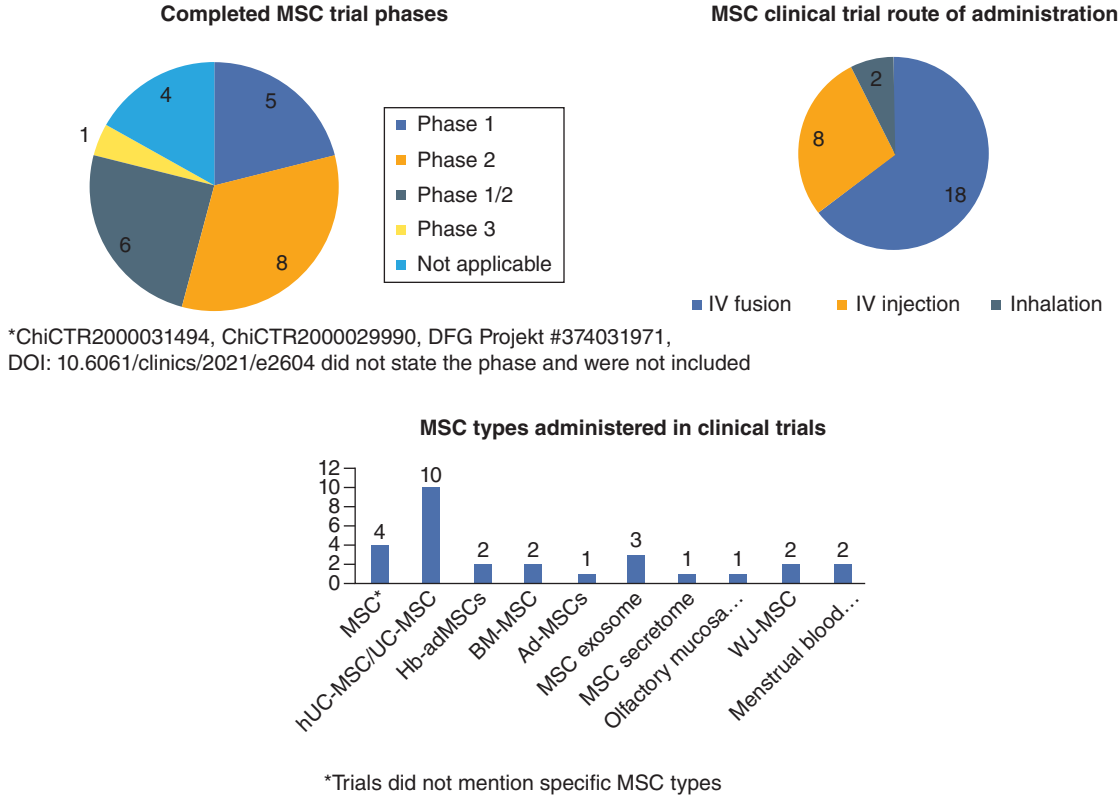

Figure 4. Mesenchymal stem cell types, clinical trial phases, and route of administration. Eight MSC clinical trials were conducted in phase II, six were conducted in phase I/II, five were in phase I, four were not US FDA-defined phases, and one trial was in phase III. Eighteen trials used intravenous infusion as the route of administration, eight trials used intravenous injection, and two trials used inhalation. The most common MSC type used in the clinical trials was human umbilical cord MSC.
MSC: Mesenchymal stem cell.

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Interestingly, Shu *et al.* also found patients with diabetes used less exogenous insulin after hUC-MSC infusion [34]. A 3-month follow-up found no significant difference in CRP and liver, kidney , and pulmonary functions [33].

Conclusion

Cytokine storm is a phenomenon that occurs in various diseases but the term's use in describing critical COVID-19 patients is being called into question. Hyperactive immune response is probably a better term. It is, however, difficult to identify the risk for COVID-19 complications based just on clinical findings, thus necessitating the use of biomarkers [47]. Melo *et al.* have noted several biomarkers such as albumin, neutrophils, alanine aminotransferase, ferritin, LDH , and many more that could impact patient outcomes [45]. Many of these biomarkers are also part of the hyperactive immune response [46].

Both CRP and LDH are predictors of COVID-19 mortality and respiratory failure. Initial analysis of the NCT04491240 study indicated an association between the recovery of COVID-19 patients and decrease in CRP and LDH levels. It is interesting that there were no cases of bronchospasms, seizures, rashes or other serious AEs during inhalation. This study differed from those of Adas *et al.* [26], Dilogo *et al.* [29] and Shi *et al.* [28] primarily in that the route of admission was inhalation. Despite the differences in route of admission, Adas *et al.* [26] found statistically significant reduction in levels of pro-inflammatory cytokines IL-6, CRP, IFN-g, IL-2, IL-12 IL-17a and an increase in the levels of anti-inflammatory cytokines IL-10, IL-13, and IL-1ra, as well as significant increases in endothelial growth factors, which have been shown to directly increase the repair efficiency of airway epithelial cells [26]. Dilogo *et al.* [29] Shu *et al.* [34] and Hashieman *et al.* [38] reported safe administration of MSC treatment and significant reduction in IL-6 levels. Shi *et al.* [28] also reported safe use of MSC treatment. On the other hand, both Xu *et al.* [32] and Häberle *et al.* [35] found increased levels of IL-6 and decreased CRP levels. To understand how hUC-MSCs treat hyperactive immune response, Kouroupis *et al.* [27] measured soluble TNFR2 and TNF-α, and TNF-β. Kouroupis *et al.* [27] also found increased soluble TNFR2 levels indicating binding of soluble TNFR2 to TNF, leading to inhibition of TNF cytotoxicity and inflammation.

Of the 14 completed MSC trials, five reported AEs associated with MSC therapy. Grade 1 or 2 AEs are mild to moderate adverse events requiring minimal medical intervention, such as noninvasive interventions or transfusions [48]. Grade 3 AEs are medically severe but not immediately life-threatening and require hospitalization [48]. Grade 4 AEs are life-threatening complications that require immediate care, and grade 5 AEs are the cause of death [48]. Shi *et al.* [28] stated the most common grade 1/2 AEs in the MSC group was an increase in LDH, followed by an increase in alanine aminotransferase and hypokalemia. One grade 3 AE, pneumothorax, was also reported. Iglesias *et al.* [31] reported grade 1/2 AEs of muscle contractions in the extremities, decreased partial pressure of oxygen, and hypotension. Xu *et al.* [32] found 40 grade 1 AEs, six grade 2 AEs, three grade 3 AEs, four grade 4 AEs, and three grade 5 AEs. Leukopenia, abnormal liver function, increased cholesterol, anemia, and shock occurred most often [32]. In the trial conducted by Wei *et al.* [37], one patient died due to respiratory failure, secondary infection, and circulatory failure [37]. The death was not related to MSC treatment [37]. Finally, Hashemian *et al.* [38] reported two grade 1 AEs of shivering after initial MSC therapy.

The use of MSCs for COVID-19 treatment is still in its infancy, and the results of ongoing clinical trials or necessary to draw further conclusions.

Future perspective

Over the past decade, interest in MSCs has skyrocketed, as is evident by the number of MSC clinical trials being conducted. Coupled with the urgent need for COVID-19 treatment, the potential for MSCs-based therapies is high. The next step for MSC therapy is the completion of clinical trials with a large cohort of patients. However, before such large-scale trials can be approved, the establishment of a standardized framework addressing ethical concerns, the phase of COVID-19 to begin treatment (viremia phase, acute phase and recovery phase), route of administration, reproducibility, and standardized sources of MSCs is needed [49,50].

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants, patents received or pending, or royalties.

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Executive summary

- The coronavirus pandemic has led to an increase in COVID-19 patients and the need for effective treatment.
  - Treatment is difficult due to 'variants of concern,' such as Delta and Omicron;
  - Omicron was confirmed on 9 November 2021 and displays the ability evade the immune system of vaccinated and unvaccinated individuals;
  - SARS-CoV-2 infects through angiotensin-converting enzyme 2 receptors, which are present in both lungs and the heart, leading to pulmonary and cardiovascular complications.
- Hyperactive immune response should not be mistaken for cytokine storm.
  - Levels of pro-inflammatory cytokines are elevated in COVID-19 patients but not near the levels of those with acute-respiratory distress syndrome.
- Mesenchymal stem cells (MSCs) can immunomodulate the hyperactive immune response. MSCs act through secretomes and exosomes via paracrine effects. They secrete TGF, promoting the production of Tregs. MSCs prevent infiltrations of the pro-inflammatory cytokines TNF-α and IL-6. MSCs do not exhibit angiotensin-converting enzyme 2 receptors, making them SARS-CoV-2 resistant.
- Several case reports have shown safe and successful treatment of COVID-19 with MSC therapy.
  - A case study in China reported significant decrease in IL-6, a pro-inflammatory cytokine, and an increase in IL-10, an anti-inflammatory cytokine;
  - Two case studies from Brazil reported significant reduction in lung fibrosis upon MSC administration;
  - All case studies reported no serious adverse events after MSC therapy.
- Currently, there are 28 completed MSC clinical trials, with 14 clinical trials having posted the results.
  - The primary goal for the trials was to assess the safety and efficacy of MSC treatment.
    - Of the 14 MSC trials, five reported adverse events after MSC therapy;
    - Adverse events included increase in lactate dehydrogenase, leukopenia, abnormal liver function, anemia, shock and increased cholesterol.
  - Secondary goal measured pro- and anti-inflammatory markers.
- The next step for MSC therapy is the establishment of a standardized framework to conduct larger scale MSC trials.

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