Review

Are mesenchymal stem cells able to manage cytokine storm in COVID-19 patients? A review of recent studies

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Abstract

The Covid-19 disease has recently become one of the biggest challenges globally, and there is still no specific medication. Findings showed the immune system in severe Covid-19 patients loses regulatory control of pro-inflammatory cytokines, especially IL-6 production, called the “Cytokine storm” process. This process can cause injury to vital organs, including lungs, kidneys, liver, and ultimately death if not inhibited. While many treatments have been proposed to reduce cytokine storm, but the safety and effectiveness of each of them are still in doubt. Mesenchymal stem cells (MSCs) are multipotent cells with self-renewal potential capable of suppressing overactive immune responses and leading to tissue restoration and repair. These immuno-modulatory properties of MSCs and their derivatives (like exosomes) can improve the condition of Covid-19 patients with serious infectious symptoms caused by adaptive immune system dysfunction. Many clinical trials have been conducted in this field using various MSCs around the world. Some of these have been published and summarized in the present article, while many have not yet been completed. Based on these available data, MSCs can reduce inflammatory cytokines, increase oxygen saturation, regenerate lung tissue and improve clinical symptoms in Covid-19 patients. The review article aims to collect available clinical data in more detail and investigate the role of MSCs in reducing cytokine storms as well as improving clinical parameters of Covid-19 patients for use in future clinical studies.

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1. Introduction

The Covid-19 is the biggest challenge in the medical world in the first half of the twenty-first century, and all scientists around the world are trying to find a way to tackle this problem. The prevalence of new coronavirus (SARS-CoV-2) causing lung infection (Covid-19) started in December 2019 in China [1], and the virus spread rapidly around the world. SARS-CoV-2 is an enveloped virus, having a positive-sense, single-stranded RNA, as a member of the B lineage of coronaviruses family. The most critical targets of the virus are epithelial cells, the airways, and alveoli [2]. This virus can access pulmonary alveolar cells through Angiotensin-Converting Enzyme 2 (ACE-2) receptors and enter the cell via endocytosis. Since the ACE-2 receptors exist on various cells, including epithelial lung cells and kidney, heart, and liver parenchymal cells, it can cause multi-organ failure in the final progressive stages of Covid-19 disease [3]. Covid-19 has a wide range of clinical symptoms, including cough, fever, lymphopenia, sore throat, fatigue, headache, and sense of taste or smells' loss [4]. One of the well-known facts about SARS-CoV-2 infection is its multi-organ downfall and Acute Respiratory Distress Syndrome (ARDS) in critical patients [5]. This infection is correlated with a destructive inflammatory response, including releasing many pro-inflammatory cytokines called “Cytokine storm.”. There is much evidence from the cytokine profile of Covid-19 patients, indicating that cytokine storm is directly linked to lung injury, multi-organ failure, and the disease’s severity [6–8].

So far, many treatment strategies for this disease have been proposed [9–11], including hydroxychloroquine and combinations [11–13], neutralizing antibodies [14], repurposing various antiviral treatments [9,15–17], and passive antibody transfer from convalescent patients’ sera [18,19]. There are still controversies about these treatments, and they have a complementary role; besides, they seem insufficient due to the rapid progression of the disease and the increase in the number of patients, and certain limitations of any treatment method. For example, using high doses of steroids is not only useful in treating Covid-19 patients but can also cause severe side effects like the increased risk of bacterial infections or diabetes [20–22]. On the other hand, considering differences in the severity of the disease and individual discrepancies in the incidence of immune responses to some existing treatments, the need to find new treatment strategies is felt. In addition to inhibiting virus replication, perhaps one of the strategies to improve critically Covid-19 patients with severe lung involvement is to prevent cytokine storms formation.

Mesenchymal Stem Cells (MSCs) and their derivatives may be able to prevent or mitigate this cytokine storm through their Immunomodulatory capacity [23] and lack of expression of ACE2. Many clinical trials have been registered in line with MSCs therapy for Covid-19 patients (https://clinicaltrials.gov), many of which are still in the process, and only a few results have been published. Many review articles have also pointed to registered trials [24–27], but the article that summarizes the published results is not yet available. This review paper aims to investigate the role of MSCs in reducing and managing cytokine storms in Covid-19 patients based on summarizing the available results from recent studies that can provide useful information for clinicians and researchers in this field.

2. Cytokine Storm

Cytokine storm is an inflammatory phenomenon which is marked by the systemic release of large numbers of cytokines including: Interleukin-6 (IL-6), Interferon-gamma (IFNγ), Tumor Necrosis Factor-alpha (TNFα), Interferon-alpha (IFNα), Monocyte Chemotactic Protein-1 (MCP-1), Interleukin-1β (IL-1β), Interferon-Beta (IFNβ), and Interleukin-2 (IL-2). The release of a large number of free radicals from immune cells is induced by these cytokines, which are the primary cause of Acute Respiratory Distress Syndrome (ARDS) and multiple organ failure [28].

It has been proven that the internalization of the virus in tissue and immune cells leads to activation of the nuclear factor-kappa B (NF-kB) pathway and secretion of a myriad of inflammatory factors leading to Cytokine storm [29]. Indeed, a complex set of interactions between cytokines, cell types, and signaling pathways are involved in cytokine storm formation. The most important cells of the innate immune system involved in cytokine storms’ pathogenesis are neutrophils, macrophages, and Natural killer cells (NK). Also, some T-lymphocyte subsets are involved in the cytokine storm process among adaptive immune system cells, including T-helper 1 (TH1), Cytotoxic T Lymphocyte (CTL), and T-helper 17 (TH17). T regulatory cell (Treg) discharge leads to an intensification in the levels of IFN-γ, IL-17, and IL-6, and through the secretion of IL-8, decreases the neutrophils’ clearance [30]. Taking together all these observations is responsible for lung damage in Covid-19 and ARDS [31]. B cells are rarely contributed to the cytokine storm pathogenesis [32]. Since cytokine storm is the main reason for morbidity in patients infected with SARS-CoV and MERS-CoV, recognizing it is vital in diagnosing and treating the disease by physicians. The medicinal problems of patients involved with cytokine storm can progress rapidly and have consequences such as hypoxemia, hemorrhages, anemia, thrombocytopenia, dyspnea, hypotension, vasodilatory shock, and death [33]. Immunosuppression is crucial in the treatment of cytokine storm in Covid-19 patients, especially those with severe conditions. Blocking IL-6, IL-1 and TNFα may prevent or inhibit the disease [34].

As immune-suppressive, steroids were widely used to treat SARS to ameliorate the intensity of inflammatory injuries [35]. However, high doses of steroids were not sufficient for treating extreme pulmonary injury in SARS and Covid-19 patients [20,21]. Instead, they may lead to destructive side effects, including an increased risk of diabetes, osteonecrosis, severe bacterial infections, and cardiovascular disease [22], dramatically affecting the prognosis. So finding new and safe treatment ways to suppress the immune system can be very helpful for patients.
3. Important cytokines involved in cytokine storm

3.1. IL-6

Interleukin-6 (IL-6), among all cytokines, is more suspected to be involved in the cytokine storm induced by a coronavirus. IL-6 is tightly linked to mediate various immunomodulatory and inflammatory pathways [36]. It is produced by approximately all stromal cells and competent immune cells, including macrophages, mast cells, T lymphocytes, dendritic cells (DC), monocytes, B lymphocytes, as well as some other non-lymphocytic cells, such as glomerular mesangial cells, endothelial cells, tumor cells, keratinocytes, and fibroblasts [37]. IL-6 performs as an alarming intermediary for the announcement of the occurrence of some urgent occasion. IL-6 is produced in infectious damage and transmits an alerting sign to the entire body [38]. IL-6 is responsible for activating TH17 cells in the interaction between T cell-dendritic cell [39]. In Covid-19 patients, an increased percentage of activated TH17 cells may be due to increased production of IL-6 in response to the virus’s entry into the immune system [40]. It is recognized that IL-6 and D-Dimer levels dramatically increase in severe Covid-19 patients, and so they can be good diagnostic predictors for finding the severity of the disease [41]. It has been proven that the viral nucleocapsid SARS-CoV N protein is responsible for the elevated IL-6 levels and lung injury in the severe stage of disease progression in SARS patients [42]. Besides, laboratory tests have indicated that IL-6 boosts macrophage activation syndrome (MAS), triggering mass manufacture of pro-inflammatory cytokines and inducing fibroblasts and neutrophils migration into the alveolar epithelium. This results in an enlarged deposition of fibrin and collagen, leading to harm to underlining lung tissue [43,44].

3.2. TNFαs

As a dominant pro-inflammatory cytokine, Tumor Necrosis Factor-α (TNF-α) is applied pleiotropic impacts on distinct cell types and critically is involved in viral diseases associated with the pathogenesis of chronic inflammatory [45]. It is known to be released by activated monocytes and is cytotoxic to tumor cells [46]. It has been proven that TNF-α triggers macrophage-induced angiogenesis, which is a virtual event throughout inflammation, tumor growth, and wound repair [47].

As another mechanism, it is accepted that TNF and other cytokines in the TNF–TNF receptor superfamily are potent inducers of NF-κB, leading to multiple expression pro-inflammatory genes [32]. Since TNFαs is one of the most critical hyaline-production inducer cytokines in alveolar lung cells and fibroblasts, its high uncontrolled rate in Covid-19 patients can cause serious lung damage [48]. Also obtained data from several studies show that anti-TNF therapy reduces morbidity and mortality in Covid-19 patients [49,50].

3.3. Interferons (IFNs) family

The interferons (IFNs) are a set of cytokines secreted by cells over a specific situation like exposure to viruses, polypeptides, or double-stranded RNA and have crucial immunomodulatory, antiviral, antitumor, and anti-proliferative properties [51]. In mammals, this system is the first protective barrier against viral infection. This system’s design is such that even at the cost of increasing the death rate of virus-infected cells, prevents the spread of the virus in the body [52]. Type 1 IFNs (IFN-α/β), as an essential part of the intrinsic antiviral innate immune system, are the fastest and most effective antiviral response as soon as the virus enters the body. Viral infections, including SARS-CoV-2, can suppress the production of IFN types 1 and 3 but induce the production of IL-6 and TNF. IFN type 1 might remarkably reduce viral replication [53]. Current studies show that IFN disorder is an important marker to discover Covid-19 pathogenesis. Efficient IFN stimulation or prophylactic direction of IFNs at the early stage before severe Covid-19 may bring about an autonomous antiviral state, limit the virus infection, and inhibit Covid-19 progression [54].

3.4. IL-1 family

The interleukin-1 (IL-1) cytokine family is consists of 11 members. Some more important of them are: IL-1α, IL-1β, IL-1 receptor antagonist (IL-1Ra), IL-18, and IL-33 [55]. During the cytokine storm, IL-1β, IL-18, and IL-33 are the three most essential cytokines from this family [56]. IL-1 family members boost the activity of the innate immune system cells. They also have crucial roles in provoking and amplifying the performance of polarized T cells. Regardless of some exceptions, as a general rule, IL-18 mainly affects T helper 1 (TH1) cells, IL-33 mainly affects TH2 cells, and IL-1 has a crucial role in TH17 cell differentiation and maintenance [55].

The benefits or disadvantages of IL-1 depends on the dosage. Although low doses may be protective, when it is generated in high doses in infectious diseases, it can be destructive; therefore, blocking it is necessary. When activating by SARS-CoV-2, IL-1 provokes the release of IL-6 and TNF-α, pro-inflammatory network that can cause cytokine storms and be detrimental both in the lung and systemically [57].

3.5. Mesenchymal stem cell (MSC) therapy

Mesenchymal stem cells (MSCs) have been used in laboratory and clinical cell therapies for many years. MSCs are adult multipotent cells with self-renewal potential and multi-lineage differentiation ability into specialized cells [58]. The long-term culture proficiency, easy accessibility, and control of how such cells specialize in forming the body’s different tissues offer significant healthcare advances [59]. MSCs are accessible using simple methods and have been isolated from multiple tissues such as bone marrow [60], adipose tissues [61], umbilical cord [62], the dental pulp [63], menstrual-blood [64], Wharton’s Jelly [65] and periodontal ligament [66]. MSCs can reach the required volume for clinical trials at a suitable time [57].

The safety and efficacy of MSCs have been recorded in several clinical trials, especially in immune-mediated inflammatory diseases, like graft-versus-host disease (GVHD) and systemic lupus erythematosus (SLE) [68,69]. Mainly MSCs exert their beneficial therapeutic effects in two ways, including immunomodulatory and differentiation ability. MSCs can release various cytokines by paracrine method or make direct interactions with immune cells, including natural killer cells (NK), T cells, B cells, Dendritic cells, and macrophages leading to immune system regulation. MSC therapy can also theoretically suppress the over-activated immune systems and upgrade endogenous restore by improving the microenvironment [70]. Strong anti-inflammatory properties of MSCs are the main reasons for improving the health of Covid-19 patients after MSCs injection. Besides, as another beneficial mechanism, direct cell–cell transmission of mitochondria [71] from MSCs to respiratory epithelial and immune cells has also been described [72].

4. Effects of MSCs on ARDS and immune system of Covid-19 patients

As mentioned, in Covid-19 patients, in a positive feedback of the immune system, it produces many inflammatory factors that lead
to cytokine storms, and again the resulting cytokine storm causes overproduction of inflammatory cytokines and immune cells [73].

Acute Respiratory Distress Syndrome (ARDS) is the main typical intricacy of Covid-19 disease-causing by various events, including the renin-angiotensin system dysregulation, cytokine storm, over-activation of neutrophils, and elevated coagulation [74]. The ARDS usually involves a general lung injury that is associated with characteristics such as pulmonary edema and damage to the endothelium of the lungs [75]. Unfortunately, the mortality rate caused by the 2019 coronavirus, leading to the induction of this severe lung damage (ARDS), is relatively high [76]. For example, in Huang et al. report, among critically covid-19 patients, 67–85% of them had ARDS, which is one of the leading causes of high mortality rates (61.5%) [4]. Many studies showed that MSCs are also useful in the treatment of ARDS [77,78].

MSCs therapy can theoretically suppress the overactive immune system, and by improving the microenvironment, assist endogenous restoration. After intravenously reaching the body, some of the MSCs accumulate in the lung and potentially prevent pulmonary fibrosis, protect lung pneumocytes, improve pulmonary microenvironment and enhance lung function [70,76]. MSCs with raising autophagy through the phosphoinositol 3-kinase/protein B pathway may also defend alveolar cells [79]. Furthermore, it has been reported that MSCs can secrete some soluble bioactive factors and interact with damaged tissue leading to an increase in the population of T-regulatory cells and inhibit of Th1 and Th17 proliferation.

Besides, because of ATP levels reduction in injured alveolar epithelial cells, as another mechanism, MSCs by transporting mitochondria to these locations and refilling depleted ATPs, improve their functional status [72].

In the same direction, many clinical trials in the world were performed on the Covid-19 patients which stem cells were used to treat patients. Most recently, China, USA, Jordan, Iran (In this context, our research team is also conducting a cell therapy project for Covid-19 patients, IRC2016080902975N1: 30/05/2020), and many other countries have started cell-based therapy clinical projects, and some reports have been published [70,76,80]. For example, on 2/5/2020, Beijing 302 Hospital register the first trial in this field. This project is accomplished to examine the safety and efficacy of Umbilical Cord-Mesenchymal Stem Cells (UC-MSCs) therapy for pneumonia in covid-19 patients [70]. The results of this project were auspicious, and no specific side effects were reported. MSCs can modulate the immune system by various mechanisms, which the most important ones are shown as follows and briefly in Fig. 1. The immunomodulatory effects of MSCs are triggered by the activation of TLR (Toll Like Receptor) in MSCs, stimulated by pathogen-associated molecules such as LPS or double-stranded RNA from the virus [81,82] like the HCoV-19. In response to the virus entering the body, mesenchymal stem cells secrete several soluble factors, including Nitric oxide (NO), Indoleamine2,3-dioxygenase (IDO), prostaglandin E2 (PGE2), and Transforming growth factor $\beta$ (TGF-$\beta$) [83,84], which affect the immune system in two ways. On the other hand, these soluble factors inhibit activation and proliferation of Th1 and Th17 cells, which subsequently leads to a decrease in inflammatory cytokines IFN$\gamma$ and interleukin-17 [85]. Besides, with the help of these soluble factors MSCs exert immunosuppressive effects on DCs (the central antigen-presenting cells) by inhibiting DC activation, decreasing IL-12 production, decreasing endocytosis, and inhibiting dendritic formation cells from monocytes, and cell maturation arrest [84,85]. This results in an increase in interleukin-10 and a decrease in TNF-$\alpha$ levels. Also, MSCs can suppress NK cells in two ways: direct contact with them and indirect interaction by releasing mentioned soluble factors [83,86,87]. It has also been proven that during the progression of COVID-19 disease, CD8+ T cells become overactive and attack lung parenchymal to remove the virus. Mesenchymal stem cells prevent serious lung damage by inhibiting these activated lymphocytes. Moreover, in COVID-19 patients, MSCs prevent pulmonary fibrosis by decreasing the pro-fibrotic factors’ levels and improving the lung microenvironment [88]. Also, in another mechanism, MSCs cause re-modeling and shift the T-cell subsets to regulatory cells and subsequently, they increase the level of anti-inflammatory cytokine IL-10, which plays a crucial role in regulating immune responses.

To make these mechanisms of action more comfortable to understand, we have summarized the results from available studies in Fig. 1.

5. Effects of MSCs on clinical parameters of Covid-19 patients: results of recent clinical trials

So far, more than 90 clinical trials in the field of stem cell therapy have been registered for Covid-19 patients (https://clinicaltrials.gov). Most of them are in the process of being completed, and only a few have reached an end and published their results. The results are promising, and no serious side effects have been reported during the patients’ treatment period. For example, Liang B et al. found that injection of 3 doses (50 × 10^6) of Umbilical cord-derived MSC (UMSC) in a 65-year-old woman significantly improved the clinical symptoms of Covid-19 disease. Also, no side effects were observed from the mentioned intervention [89]. Another clinical experiment involving seven Covid-19 pneumonia patients revealed that injections of a single dose of mesenchymal stem cells (1 × 10^6 Cell/Kg BW) led to the normalization of oxygen saturation (PaO2) and the number of inflammatory markers and lung tissue repair, as chest CT imaging showed improvement mainly on the 9th day after MSC transplantation. Also, as we know, with the help of ACE2 receptors widely distributed on human cells, including alveolar and capillary endothelium, hCoV-19 can access the cells.

Interestingly, using an RNA seq survey to identify 12,500 transplanted MSC in this study, the results showed that mesenchymal stem cells were ACE2 negative during treatment [76]. Results reported in a different study by Lanzoni et al. on 24 Covid – 19 patients with ARDS symptoms revealed that intravenous injection of 2 doses (100 × 10^6 Cells) of UMSC with an interval of 3 days dramatically improved clinical and immunological symptoms of patients [90]. Similarly, the results of MSCs therapy in 60 patients with moderate to severe ARDS, using a single dose of 1 × 10^7 Cell/kg of bone marrow-derived MSCs revealed that the MSCs were well-tolerated, and a significant reduction in C-Reactive Protein (CRP), IL-6, and IL-8 levels was observed [77]. These successful outcomes in treating lung damage in these patients can be a hope for the treatment of pulmonary injuries in Covid-19 patients. The summary of the published results of recent trials on covid-19 patients given in Table 1. Generally, in the conducted studies, the dose of injectable cell varies from 1 × 10^6 (Cell/Kg BW) [91] to 4 × 10^7 (Cell/Kg BW) [92] depending on the treatment protocol and there are still controversies in this regard. However, more research and a larger patient population with high precautions are needed to validate MSC therapeutic interventions further. Also, as mentioned in Table 1, there are many laboratory abnormalities such as elevated CRP, liver enzymes (Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Bilirubin, serum ferritin, D-dimer, and lower leukocyte and lymphocytes count, as well as some other parameters like Albumin and Urea, in Covid-19 patients, which are useful in the diagnosis of the onset and progression of the disease [102].
In the same direction, it should be briefly explained, C-Reactive Protein (CRP) is classified as one of the classical acute-phase proteins by its biological properties. It is synthesized and secreted to the blood by the liver after initiating signals from the body, for example, infection, trauma, or tissue damage, mediated by inflammatory cytokines [103].

CRP levels are directly related to inflammation levels, and situations like sex, age, and physical conditions cannot affect its concentration [104]. Comparing the serum profile of Covid-19 patients with different disease severity levels suggested that CRP level is a good predictor of disease severity. In the early stage of Covid-19 disease progression, CRP levels are positively associated with the diameter of lung lesions and severe presentation [105]. Findings of many studies revealed that treatment with MSC decreased CRP levels and pro-inflammatory cytokines and chemokines, like IL-6 and TNF-α [76, 95, 101].

It has also been found that SARS-CoV-2 can affect liver cells by causing elevated levels of aminotransferase enzymes and liver impairment [106]. Indeed, like observation in coronavirus SARS, liver dysfunction is a common complication in Covid-19 patients. Based on prior results, up to 60% of patients had a liver dysfunction, with liver biopsy specimens suggesting viral nucleic acid and damage [107]. In Covid-19 patients, liver enzyme abnormalities have been reported to range from 20% to 50%, but there is still controversy about the correlation of these findings and the disease’s prognosis or progression [108, 109]. Two hypotheses have been proposed in different studies in liver dysfunction and infection with Coronaviridae family viruses: systemic inflammation and direct liver attack by the virus [110, 111]. For example, in a retrospective cohort study conducted by Cheuk-Fung et al., reporting data from 1040 Covid-19 patients showed that 22.5% of patients had elevated liver enzyme levels [112]. Several studies have demonstrated that MSCs can differentiate in vitro along the hepatogenic lineage, and so they can be useful in improving liver problems and hepatic tissue regeneration with different mechanisms. For example, in a study using bone marrow-derived MSCs, the results showed that MSCs transplantation significantly inhibited Reactive Oxygen Species (ROS) and improve liver injury [113]. The results in the above studies [91, 94, 98] also showed a decrease in the number of liver enzymes after MSCs injection, resulting from the anti-inflammatory and immunomodulatory properties of these cells. Besides, several studies have described renal involvement mainly due to Acute Kidney Injury (AKI), affecting up to 70% of Covid-19 patients [114, 115]. The kidney is the second most affected organ in this disease, behind the lung and followed by the heart and the liver [116]. In the Diao et al. study, SARS-CoV-2 nucleocapsid protein was observed in the kidney’s tubular structures of 6 patients involved in the study. Also, Cheng et al. have shown that
among 710 hospitalized Covid-19 patients, elevated serum creatinine and urea (BUN) were 15.5% and 14.1%, respectively [117]. In this context, several studies revealed that administering in vitro expanded MSCs defends against acute renal injury and enhances renal restoration. In acute and chronic kidney injury models, intravenously injected MSCs have moved to tubulin, glomeruli, renal interstitium, and peritubular capillaries [118,119]. The results obtained from several clinical trials show that MSCs have useful effects on cytokine storm and disorders involved in Covid-19 disease [98]. In response to these questions, we carefully summarized available clinical data from recent studies in this review article.

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6. Discussion

Since the Covid-19 pandemic continues and the number of patients is increasing worldwide, understanding the mechanisms involved in the onset and treatment of the disease is one of the essential medical needs today. Many treatments have been suggested so far for COVID-19, which, while effective in some of them, are still inadequate to the increasing trend of disease. Many studies have emphasized that loss of immune system control (both innate and adaptive) and over-production of inflammatory cytokines called Cytokine storm respond to the virus’s arrival in critical Covid-19 patients are the leading causes of vital tissue damage and subsequently death [43,120]. Therefore, finding and using new therapeutic strategies based on immune system regulatory properties can effectively improve patients’ clinical status. In this context, Mesenchymal Stem Cells (MSCs) have been approved due to their safety and efficacy and the immune system’s regulatory properties in many diseases; for the Covid-19 patients, treatment has also been considered by many researchers and clinicians for the Covid-19 patients, treatment has also been considered by many researchers and clinicians. MSCs are accessible from various tissues and possess immunomodulatory functions as well as multipotency and self-renewal properties.

The question that arises here is: do mesenchymal stem cells have useful effects on cytokine storm and disorders involved in Covid-19 disease? What clinical evidence is available in this regard? In response to these questions, we carefully summarized available clinical data from recent studies in this review article.

As mentioned in Table 1, in many studies [93,94], the amount of serum creatinine, Blood Urea Nitrogen (BUN), and renal parameters after injection of MSCs returned to normal, which indicates the beneficial therapeutic effects of these cells in the regeneration of kidney tissue and reduce inflammation in it.

### Table 1

A summary of published results in the field of stem cell therapy for COVID-19 patients.

| Source of MSCs       | Autologous/     | Times of injection | Population size | Follow-up period (day) | Main therapeutic effects                                                                 | Ref |
|----------------------|------------------|--------------------|-----------------|------------------------|------------------------------------------------------------------------------------------|-----|
| UCB-MSCs             | Autologous       | Five times         | N = 1 (case report) | 10 days                | 1: Static pulmonary compliance †, lymphocyte number †                                     | [93]|
| UMSCs                | Autologous       | Once               | N = 1 (case report) | 19 days                | 1: The percentage and counts of CD3+ T cell, CD4+ T cell, and CD8+ T cell †             | [91]|
| UMSCs                | Autologous       | Three times        | N = 1 (case report) | 30 days                | 1: Inflammatory factors including CRP, TNF-α, and IL-6 †                              | [89]|
| UMSCs                | Autologous       | Once               | N = 1 (case report) | 30 days                | 1: CRP, ALT, AST, Bilirubin and D-dimer †                                                 | [90]|
| MB-MSCs              | Autologous       | Three times        | N = 2             | 24 days                | 1: Improvement in (PaO2/FiO2) ratio & CT images                                          | [95]|
| NM                   | Autologous       | Once               | N = 7             | 14 days                | 1: Oxygen saturation †, lung tissue repair †, peripheral lymphocytes † and IL-10†       | [76]|
| UMSCs (5 casees)     | Autologous       | Three times        | N = 11 (Case Series) | 19 days                | 1: Oxygen saturation †, Blood Urea Nitrogen ratio and CT images improvement †          | [96]|
| UMSCs                | Autologous       | Three times        | N = 2             | 14 days                | 1: CRP and TNF-α levels †                                                              | [96]|
| Exosomes-derived     | Autologous       | Once               | N = 18            | 23 days                | 1: CRP, IL-8, TNF-α, IFN-γ, and IL-6 levels †                                           | [98]|
| NM                   | Autologous       | Case series, different for cases (one, two, or three times) | N = 25            | 3 days                 | 1: Inflammatory factors (IL-6, procalcitonin and CRP) and IgG and IgM were not significantly changed. | [100]|
| UMSCs                | Autologous       | Once               | N = 41            | 28 days                | 1: Dendritic cell population, IL-10 levels, and oxygen saturation †                    | [90]|
| UMSCs                | Autologous       | Three times        | N = 101           | 28 days                | 1: CRP and IL-6 †                                                                      | [101]|

**Abbreviations** (UMSCs: Umbilical cord-derived Mesenchymal Stem Cells/NM: Not Mentioned/CRP: C-Reactive Protein/ALT: Alanine aminotransferase/AST: Aspartate aminotransferase/MB-MSCs: Menstrual blood-derived MSCs/UCB-MSCs: Umbilical Cord Blood-derived Mesenchymal Stem Cells/AT-MSCs: Adipose Tissue derived mesenchymal Stem Cells/BM-MSCs: Bone Marrow-derived Mesenchymal Stem Cells/PL-MSCs: Placental derived mesenchymal stromal cells/IV: Intravenous/Alb: Albumin/BUN: Blood Urea Nitrogen).
Moreover, it has been reported that exosomes, which are derivatives of MSCs, also have beneficial therapeutic effects in the treatment of Covid-19 patients and reduction of cytokine storm. Exosomes are extra-cellular nanoparticles released by all cells and act through paracrine pathways. Exosomes have been proven to be mediators of immune regulation function of MSCs. MSC-Exos could shift macrophages from the M1 to the M2 phenotype, further suppressing pro-inflame-matory states [121]. Using a single dose of 15 ml ExoFlo from Bone Marrow-derived Mesenchymal Stem Cells (BM-MSCs) in a study performing by Sengupta et al. revealed that exosomes can significantly increase the number of lymphocytes and decrease inflammatory markers, as well as the number of neutrophils in Covid-19 patients [98]. The effective role of MSCs in cytokine storm reduction in Covid-19 patients has also been proven in several other clinical trials [96–98].

Briefly and as reflected in this article, MSCs, due to their immunomodulatory, anti-inflammatory, and regenerative properties, can control immune dysfunction and inflammation in Covid-19 patients. Interestingly, MSCs are not affected by the Covid-19 infection for not expressing the ACE2 receptor [76]. Besides, MSCs can help in remodeling the CD4 and CD8 T Cells depleting function in Covid-19 and thus improve pulmonary function. Most importantly, MSCs can decrease cytokine storms by reducing the proliferation of immune cells as well as regulating the balance of pro-inflammatory and anti-inflammatory cytokines.

7. Summary

The pandemic of Covid-19 infection caused by the highly pathogenic virus SARS-CoV-2 has prompted an urgent need for novel therapies. It is proved that SARS-CoV-2 in some patients leads to induction of excessive and prolonged inflammatory responses leading to hyper-inflammation called Cytokine storm. In Covid-19 patients, especially those with severe status, cytokine storm inhibition by immune-suppressives is urgent. Pre-clinical and preliminary clinical data suggest that Mesenchymal Stem Cells (MSCs), through their anti-inflammatory and immunomodulatory actions, can significantly inhibit this process, heal tissues, thereby enhancing recovery. Many clinical trials have been conducted in this field have reported significant results, and no specific side effects have also been reported in any of them. Of course, further clinical investigations with a larger sample size are still needed in this area.

Declaration of competing interest

All authors declare that they have no competing interests.

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