Mesenchymal stem/stromal cells as a therapeutic intervention for COVID-19: a living systematic review and meta-analysis

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PREFACE

Aidan Kirkham was primarily reasponsible for all aspects of this thesis including conceptualization, methodology, validation, data-analysis, and writing. Aidan Kirkham designed the study and methodology in consultation with the primary supervisor, Dr. David Allan, who is an associate scientist at the Ottawa Hospital Research Institute and an Associate Professor, in the Departments of Medicine and Biochemistry, Microbiology & Immunology. Primary study screening and data extraction was performed by Aidan Kirkham. Madeline Monahan (medical student, Saba University) and Adrian Joseph Micheal Bailey (medical student, University of Ottawa) performed the duplicate study screening and data extraction. Data analaysis and risk of bias assesment was performed by Aidan Kirkham. All included manuscripts were written by Aidan Kirkham and reviewed by relevant co-authors and the primary supervisor (Dr. David Allan). Other manuscript co-authors include Dr. Dean Fergusson, who is a Senior Scientist at the Ottawa Hospital Research Institute and Full Professor in the Departments of Medicine, Surgery, and the School of Epidemiology and Public Health at the University of Ottawa, Dr. Manoj Lalu, who is an Associate Scientiest at the Ottawa Hospital Research Institute and Assistant Professor in the department of Anesthesiology and Pain Medicine at the University of Ottawa, and Risa Shorr, who is a Health Sciences Librarian at the Ottawa Hospital Research Institute. Funding acquisition was performed by Dr. David Allan. Research ethics board approval and consent to participate was not required for this research as it did not examine primary data from patients.

Abstract

Background: Since its emergence in December 2019, SARS-CoV-2, the coronavirus responsible for COVID-19, has spread across the globe, infected millions of people and caused several million deaths. One promising intervention to combat the ongoing COVID-19 pandemic is mesenchymal stem/stromal cells (MSCs). Many trials were registered at the onset of the pandemic to determine the safety and efficacy of MSCs in COVID-19 patients. However, currently published studies are underpowered to provide an estimate of safety and efficacy on their own. Thus, a living systematic review (SR) is needed to establish the benefits and drawbacks of MSCs for COVID-19 on a relevant timescale.

Methods: Systematic literature searches were conducted on Feb 3rd, 2021 and November 15th, 2021 to identify all English-language, full-text, clinical studies examining MSCs to treat COVID-19. (PROSPERO: CRD42021225431).

Findings/Conclusions: Our first search identified nine studies (4 controlled) examining the use of MSC derived products to treat COVID-19 patients. This first iteration of our SR revealed that MSCs were safe and reduced mortality in patients suffering from COVID-19. However, risk of bias (RoB) and poor adherence to ISCT cell product characterization guidelines limited the strength of our conclusions. In the second iteration of our living SR, we only included controlled studies to strengthen our conclusions. We identified eleven controlled studies (5 RCTs). MSCs continued to demonstrate safety and efficacy at reducing mortality at study endpoint (RR: 0.50 [0.34 to 0.75, 95% CI, p=0.0006, I^2=0%]). However, we continued to encounter barriers which prevented us from drawing more definitive conclusions. A master protocol appears necessary to facilitate the accelerated accumulation of high-quality evidence where standardized outcome reporting and consistent product characterization allow for a more definitive and timely estimate regarding the safety and efficacy of this cell-based therapy for COVID-19.

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1. INTRODUCTION & THESIS OUTLINE

Biology and Epidemiology of SARS-COV-2

SARS-CoV-2, the etiological agent responsible for coronavirus disease 2019 (COVID-19), is a positive sense single stranded RNA beta coronavirus that is thought to have originated in bats and spread to humans during a zoonotic spillover event[1]. The first cases of COVID-19 were reported in the city of Wuhan China in December 2019 when several patients presented to the hospital with pneumonia like symptoms of unknown origin[1]. Real-time polymerase chain reaction (RT-PCR) and next-generation sequencing of samples from the lower respiratory tract of these patients revealed the presence of a novel coronavirus, which was named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)[2]. Since its emergence, SARS-CoV-2 has spread across the world, infected millions of people, and caused several million deaths[3]. SARS-CoV-2 is closely related to Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV)[4], the coronavirus responsible for the SARS outbreak of 2003[5]. The genome of SARS-CoV-2 encodes 29 proteins in total (16 nonstructural, 4 structural and 9 accessory)[6]. One particularly important SARS-CoV-2 protein is the spike (S) protein[7]. The S protein is composed of two subunits, S1 and S2[8]. SARS-CoV-2 cellular entry involves binding of the receptor binding domain (RBD) on the S1 subunit of S to the angiotensin converting enzyme 2 (ACE2) receptor on the surface of target cells[9]. Upon binding of the RBD to ACE2, the S protien is primed through a secondary cleavage step in which S is cleaved at two different sites, S1/S2 and S2', by the transmembrane serine protease 2 (TMPRSS2) enzyme[9]. This cleavage step primes the S protein and allows SARS-CoV-2 to enter target cells through fusion of the viral and cellular membranes[9]. Although the S proteins of SARS-CoV-2 and SARS-CoV are similar, the RBD of the SARS-CoV-2 S protein has a much greater affinity for ACE2 receptor compared to the RBD of SARS-CoV[10].

Moreover, SARS-CoV can only enter cells which have both ACE2 and TMPRSS2 co-localized on their cell surface[11]. In contrast, SARS-CoV-2 possesses a novel furin protease enzyme cleavage site on its S protein[12]. The presence of this novel cleavage site means that furin can carry out S protein priming and cleavage, allowing SARS-CoV-2 to enter cells where ACE2 and TMPRSS2 are not co-localized[12]. Because ACE2 and TMPRSS2 enzymes only co-localize in the cells of the lungs, the complications of SARS-CoV are strictly pulmonary in nature[13]. However, ACE2 and furin are co-localized in cells in a wide range of tissues and organs throughout the body including the heart, liver, kidneys and intestines[14]. This wide tropism means that in addition to pulmonary complications, patients that become infected with SARS-CoV-2 can experience a wide range of extrapulmonary complications including myocarditis, acute kidney injury, liver dysfunction and diarrhea[15]. Moreover, the severity of symptoms in COVID-19 patients can vary greatly, with some patients presenting as asymptomatic or mildly symptomatic and other presenting with severe life-threatening symptoms[16]. Though the exact contributors to varying COVID-19 severity between patients is an area of ongoing research, patients who possess certain co-morbidities (diabetes, hypertension, obesity, etc.), are unvaccinated, are immunocompromised and/or are of advanced age generally have worse outcomes after contracting COVID-19 compared to the rest of the general population[17, 18].

Current Status of COVID-19 Therapeutics

During the initial stages of the COVID-19 pandemic, a variety of conventional therapies were repurposed to tackle COVID-19 including antiviral drugs (remdesivir, lopinavir–ritonavir, etc.), immunomodulatory drugs (dexamethasone, hydroxychloroquine, etc.), anti-cytokine drugs (tocilizumab, anakinra, etc.) and combination therapies[19]. However, many of these repurposed therapies have proved to be relatively ineffective, were only approved for use in certain patient

populations and occasionally resulted in adverse side effects[20]. This early repurposing stage of the COVID-19 pandemic was followed by the development of several SARS-COV-2 specific interventions including vaccines (AstraZeneca ChAdOx1-S, Pfizer BNT162b2), monoclonal antibodies (bamlanivimab, sotrovimab) and antibody cocktails (casirivimab and imdevimab, bamlanivimab & etesevimab)[21]. Although these SARS-CoV-2 specific therapeutics have dramatically lowered death rates and prevented countless patients from progressing to severe life threatening COVID-19, they are not universally effective across patients with different disease severity, patients of advanced age and/or patients who are immunocompromised[22,23]. Additionally, many of these therapeutics carry substantial costs and are not available in all parts of the world[22,23]. With regards to vaccines, although they have proven to be the most effective way to prevent COVID-19 related hospitalization and mortality[24,25], they are not equitably distributed or universally available across the globe and are susceptible to escape variants[26, 27]. With large portions of the population remaining unvaccinated in certain areas across the world and the rise of escape variants raising the possibility of living with COVID-19 for an extended period of time, new therapeutics are desperately needed to diversify therapeutic options and supplement ongoing vaccination efforts[28-30].

MSCs as a Therapeutic Intervention for COVID-19

One potentially useful intervention that was viewed with immense therapeutic promise at the onset of the COVID-19 pandemic is mesenchymal stromal cells (MSCs)[31,32]. MSCs are multipotent stem-like cells that can be isolated from a variety of adult and neonatal tissues, including bone marrow, adipose, umbilical cord and placenta[33,34]. The remarkable immunomodulatory and tissue reparative capabilities of these cells have been demonstrated in a number of preclinical and clinical studies of COVID-19 related diseases and conditions[35-36].

Additionally, MSCs are minimally immunogenic, allowing them to be administered to patients in an allogeneic manner[37]. Furthermore, the occurrence of adverse events associated with MSC therapy is extremely rare[38]. Moreover, MSCs may achieve their immunomodulatory and regenerative capabilities through the release of paracrine factors[39,40]. These paracrine factors may allow MSCs to achieve their therapeutic capabilities without cell engraftment and are mediated by many of the same important biomolecules (proteins, lipids, nucleic acids) as their parent cells[39,40]. Lastly, certain MSCs populations have been shown to lack ACE2 and TMPSS2, which may prevent them from being infected by SARS-CoV-2 when being used in a therapeutic context[41,42].

Why Use of MSCs for Therapeutic Indications Remains a Challenge

As a biological therapeutic, MSCs possess many inherent challenges to making them a widely used and available therapeutic compared to other classes of drugs. These include issues such as obtaining cells from the tissues of healthy donors, verifying cellular identity, maintaining cell viability, complicated storage and transportation needs, standardization of active therapeutic components (proteins RNAs, miRNAs, etc), dosing challenges, unpredictable *in vivo* activity, substantial cost to manufacture and lack of on-site expertise with regards to production and use[43-45]. For instance, it has been demonstrated that MSCs originating from different tissue sources possess varied therapeutic efficacy across different diseases and conditions[46,47]. Moreover, MSCs exposed to certain chemicals, growth factors or environmental conditions may display altered efficacy compared to MSCs cultured under neutral conditions[48,49]. The observed differences in therapeutic efficacy resulting from these factors may be due to variations in cellular activity, viability, gene expression profile or contamination of the therapeutic product[50,51]. In an attempt to develop a standardized methodology to characterize MSCs and verify their cellular identity for

therapeutic use, a set of minimal criteria was put forth by the International Society of Cellular Therapy (ISCT) in 2006 position paper[52]. This position paper detailed three criteria that must be met in order for a population of cells to be considered MSCs[52]. Specifically, this paper states that cells must be adherent to plastic when grown in culture, express and lack expression of specific surface antigens (positive (≥95%) for CD105, CD73 and CD90; negative (≤2%) for CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR) and possess the capability to differentiate into osteoblasts, adipocytes, chondroblasts *in vitro*[52]. Moreover, this position paper was updated in 2019 to include a fourth criterion[53]. This fourth criterion requires that MSC cell viability be verified before employing cells in a therapeutic context[53]. Verification of MSC viability prior to therapeutic use is essential to ensure that expected therapeutic outcomes will be achieved through administration of an efficacious cell product[54]. Moreover, this updated ISCT criteria also detailed several recommendations to improve transparency in MSC research such as highlighting whether the cells being employed are mesenchymal stromal cells or mesenchymal stem cells and denoting the tissue origin of the MSCs being used[53].

Clinical Trials of MSCs for COVID-19

The rapid and unprecedented emergence of SARS-CoV-2 combined with the immense therapeutic promise of MSCs lead to the rapid registration of many clinical trials of MSCs for COVID-19 across several clinical trials databases. These trials were captured in a scoping review published by our group in July 2020[55]. This scoping review revealed 41 studies (27 controlled) examining the use of MSC derived products for the treatment of COVID-19[55]. Across all planned studies, 1129 patients were to receive MSCs, with 801 patients receiving MSCs in the controlled studies[55]. In terms of MSC product characteristics, cells were derived from a wide range of known MSC tissue sources, including bone marrow, adipose, umbilical cord and

placenta[55]. However, many of the registered trials failed to mention important aspects of MSC product manufacturing, including whether xenogeneic growth factors were used, how many times the MSCs were passaged and whether MSCs were characterized according to minimal ISCT criteria[55]. Additionally, many of the studies possessed significant risk of bias (RoB)[55]. Thus, due to the varying study methodology, small sample sizes, heterogeneity in product characterization and notable RoB, we concluded that none of the registered trials possessed sufficient statistical power on their own to provide an estimate of the safety and efficacy of MSCs as a therapeutic intervention for COVID-19. Therefore, we determined that the only way to ascertain the safety and efficacy of MSCs for COVID-19 on a timescale relevant to the ongoing pandemic was to perform a systematic review and meta-analyses.

Role of Systematic Reviews/Meta-analyses, and Why a Living Systematic Review is Needed

A search of any biomedical repository or database will reveal an innumerable quantity of systematic reviews and meta-analyses examining a wide range of thereputics for nearly every human disease and condition imaginable[56]. Systematic reviews and meta-analyses form the backbone of evidence-based medicine, providing a robust and unbiased source of evidence through which the reader can determine the safety and efficacy of a given intervention or technique for a disease or condition or interest[57]. Additionally, many systematic reviews contain detailed subgroup analysis, which can help the reader delineate specific subpopulations and indications for which the intervention or technique of interest may show differential effects within the same disease or condition[58,59]. Moreover, high-quality systematic reviews and meta-analyses provide risk of bias and study quality assessments for both the individual studies and the body of evidence as a whole, allowing the reader to decide whether the evidence presented warrants local or widespread changes in clinical practice or policy[60,61]. Altogether, high quality

systematic reviews serve as an invaluable resource to guide the decisions and practices of scientists, healthcare professionals and policymakers alike[62,63]. However, the onset of the COVID-19 pandemic has led to an unprecedented surge in publications as investigators across the globe have diverted significant time and resources towards discovery of repurposed and novel therapeutics to help combat the COVID-19 pandemic[64,65]. In light of this unprecedented publication rate, traditional systematic reviews and meta-analysis would become outdated quickly due to the infrequent rate at which they are typically updated[66]. One emerging way of conducting systematic reviews in a rapid manner is through use of the living systematic review framework[66]. A living systematic review is a type of systematic review that is updated frequently as new evidence becomes available[66]. This can involve updating the systematic review at regular prespecified intervals (3 months, 6 months, etc.) or through use of artificial intelligence to alert researchers when new relevant evidence becomes available[66]. In certain cases, researchers may even use artificial intelligence to automate the study screening, data extraction and data analysis steps to provide real-time updates[67, 68]. Despite the wide range of techniques that may be used to perform living systematic reviews, the recommendations for when a living systematic review should be conducted in place of a traditional systematic review are well defined[66]. A guideline paper published by Elliot *et al* in 2017 recommends that living systematic reviews should be performed in place of traditional systamtic reviews when the systematic review is of priority for decision-making, the certainty in the existing evidence is low and there is likely to be new research emerging at a rapid rate[66]. Due to the speed at which new evidence is emerging, low certainty of the existing evidence and the importance of timely decision making, the topic of MSCs as a therapeutic intervention for COVID-19 is well suited to the conduct of a living systematic review. Use of the living systematic review framework will provide researchers,

clinicians, and policymakers with the most up to date information surrounding the use, efficacy and safety of MSCs as a therapeutic intervention for COVID-19.

Goal, Hypothesis, and Aims

The goal of this research was to determine the strength of the evidence for the use of MSCs as a therapeutic intervention for patients with COVID-19. We hypothesized that repeated meta-analysis was needed to determine safety and efficacy due to the rapidly evolving evidence base. To address our goal, we developed a protocol for a living systematic review, identified all relevant studies, performed meta-analysis on data extracted from identified studies and developed a framework for rapid identification of high-quality evidence.

This thesis consists of three manuscripts, either published or accepted to peer-reviewed journals:

1. Mesenchymal stromal cells as a therapeutic intervention for COVID-19: a living systematic review and meta-analysis protocol (published in *Systematic Reviews*). **Kirkham AM**, Monaghan M, Bailey AJM, Shorr R, Lalu MM, Fergusson DA, Allan DS. Mesenchymal stromal cells as a therapeutic intervention for COVID-19: a living systematic review and meta-analysis protocol. *Syst Rev*. 2021 Sep 15;10(1):249. doi: 10.1186/s13643-021-01803-5. PMID: 34526123; PMCID: PMC8441251.

2. Mesenchymal stem/stromal cell-based therapies for COVID-19: First iteration of a living systematic review and meta-analysis (published in *Cytotherapy*). **Kirkham AM**, Monaghan M, Bailey AJM, Shorr R, Lalu MM, Fergusson DA, Allan DS. Mesenchymal stem/stromal cell-based therapies for COVID-19: First iteration of a living systematic review and meta-analysis:

MSCs and COVID-19. *Cytotherapy*. 2022 Jan 31:S1465-3249(22)00012-3. doi: 10.1016/j.jcyt.2021.12.001. Epub ahead of print. PMID: 35219584; PMCID: PMC8802614.

3. Updated living systematic review and meta-analysis of controlled trials of mesenchymal stromal cells to treat COVID-19: A framework for accelerated synthesis of trial evidence for rapid approval – FASTER Approval (accepted in *Stem Cells Translational Medicine*). **Kirkham AM**, Bailey AJM, Monaghan M, Shorr R, Lalu MM, Fergusson DA, Allan DS.

2. MESENCHYMAL STROMAL CELLS AS A THERAPEUTIC INTERVENTION FOR COVID-19: A LIVING SYSTEMATIC REVIEW AND META-ANALYSIS PROTOCOL

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Kirkham AM, Monaghan M, Bailey AJM, Shorr R, Lalu MM, Fergusson DA, Allan DS. Mesenchymal stromal cells as a therapeutic intervention for COVID-19: a living systematic review and meta-analysis protocol. *Syst Rev*. 2021 Sep 15;10(1):249. doi: 10.1186/s13643-021-01803-5. PMID: 34526123; PMCID: PMC8441251.

2.1 Abstract

Background: Mesenchymal stromal cells (MSCs) have significant immunomodulatory and tissue repair capabilities, mediated partly by conditioned media or through secreted extracellular vesicles (MSC-EVs). Infection with SARS-CoV-2 can cause mild to life-threatening illness due to activated immune responses that may be dampened by MSCs or their secretome. Many clinical studies of MSCs have been launched since the beginning of the global pandemic, however, few have been completed and most lack power to assess efficacy. Repeated systematic searches and meta-analyses are needed to understand, in real time, the extent of potential benefit in different patient populations as the evidence emerges.

Methods: This living systematic review will be maintained to provide up-to-date information as the pandemic evolves. A systematic literature search of Embase, MEDLINE, and Cochrane Central Register of Controlled Trials databases will be performed. All clinical studies (e.g., randomized, pseudorandomized and non-randomized controlled trials, uncontrolled trials, and case series) employing MSCs or their secretome as a therapeutic intervention for COVID-19 will be included. Patients must have confirmed SARS-CoV-2 infection. Study screening and data extraction will be performed in duplicate. Information concerning interventions, patient populations, methods of MSC isolation and characterization, primary and secondary clinical and/or laboratory outcomes, and adverse events will be extracted. Key clinical outcomes will be pooled through random-effects meta-analysis to determine the efficacy of MSCs and their secreted products for COVID-19.

Discussion: Our systematic review and subsequent updates will inform the scientific, medical, and health policy communities as the pandemic evolves to guide decisions on the appropriate use of MSC-related products to treat COVID-19.

Systematic review registration: PROSPERO CRD 42021225431.

Keywords: ARDS; Acute respiratory distress syndrome; COVID-19; Coronavirus disease 2019; Exosomes; Extracellular vesicles; MSCs; Mesenchymal stem cells; Mesenchymal stromal cells; Microvesicles; Pneumonia; SARS-CoV-2; Sepsis; Severe acute respiratory syndrome coronavirus 2.

2.2 Introduction

Severe acute respiratory syndrome coronavirus 2 (SARSCoV-2), which is responsible for coronavirus disease 2019 (COVID-19), is a pathogenic beta coronavirus that has now spread to over 200 countries around the world and infected millions of people [1]. Cells and tissues infected with SARS-CoV-2 experience a rapid recruitment of pro-inflammatory immune cells to the affected tissue [2]. The ensuing surge of inflammatory cytokine production (termed "cytokine storm"), causes significant tissue damage through apoptosis and pyroptosis of cellular constituents [3, 4]. A large portion of patients who contract COVID-19 remain asymptomatic or experience mild symptoms [5]. However, many patients experience more severe symptoms [6]. These severe symptoms can manifest as serious respiratory complications such as acute lung injury [7], pneumonia [8], pulmonary fibrosis [9] and acute respiratory distress syndrome (ARDS) [10], or extrapulmonary complications including thrombosis [11], myocarditis [12], pericarditis [13], liver dysfunction [14], acute kidney injury [15], sepsis [16], and multiorgan failure [17]. Since its rapid emergence in December 2019, researchers from across the globe have been working to develop strategies to combat COVID-19. In an attempt to discover effective

COVID-19 therapeutics, several living systematic reviews and network meta-analyses have been performed on a variety of repurposed therapeutic agents with diverse mechanisms of action. Some agents including hydroxychloroquine, lopinavir–ritonavir, and convalescent plasma have demonstrated little to no benefit over standard of care in terms of improving important clinical outcomes such as all-cause mortality, duration of hospitalization, and risk of requiring mechanical ventilation [18, 19]. Other therapeutic agents including dexamethasone, glucocorticoids, remdesivir, and tocilizumab have demonstrated benefits over standard of care in terms of improving important clinical outcomes such as all-cause mortality, risk of requiring mechanical ventilation, and progression of clinical symptoms [18– 20]. However, the magnitude of these benefits is marginal at best, and the quality of most studies included in these meta-analyses was generally low, drastically reducing the certainty in these observed modest effects [18– 20]. Thus, a proven safe and effective therapeutic agent for combatting COVID-19 has yet to be discovered. One potentially useful therapy for COVID-19 is mesenchymal stromal cells (MSCs) [21–26]. MSCs are multipotent stem cells that can be isolated from a variety of adult and neonatal tissues [27]. Several preclinical and clinical studies have demonstrated that MSCs possess remarkable immunomodulatory properties and the capacity for tissue repair [28–35]. Additionally, MSCs are poorly immunogenic and elicit minimal immune response when administered to patients [36, 37], allowing for third party "off the shelf" use. Furthermore, the occurrence of serious adverse events associated with MSC therapy is rare [38–40]. MSCs may achieve their immunomodulatory and regenerative capabilities without cell engraftment through paracrine mechanisms [41], including MSC-derived extracellular vesicles (MSC-EVs) [42] and conditioned medium (MSC-CM) [43]. The rapid pace of the evolving COVID-19 pandemic combined with ongoing issues such as variants of concern and the potential for illness in

vaccinated individuals means that systematic reviews can become outdated quickly, underscoring the need for a living systematic review that can be updated regularly [44]. The use of the living systematic review framework will provide researchers, clinicians, and policymakers with the most up-to-date information surrounding the use, efficacy, and safety of MSCs and their secretome as therapeutic interventions for COVID-19 [44].

2.3 Research Objectives

This review will examine all clinical studies of MSCs or their secretome (MSC-EVs, MSC-CM) as a therapeutic intervention for COVID-19. Our primary objective is to assess the improvement of clinical symptoms and outcomes in patients with confirmed SARS-CoV-2 infection who have been administered MSCs or their secretome as a therapeutic intervention for COVID-19. Our secondary objective is to describe how study designs and aspects of MSC manufacturing and administration differ across the studies and the extent to which these differences impact patient outcomes (e.g., MSC tissue source, dosage, route of administration, patient characteristics, severity of COVID-19, co-morbidities), and to describe adverse events associated with MSC treatment. Given the expected heterogeneity of methods used (e.g., cell sources, isolation techniques) and variability in outcomes assessed across studies, a systematic review and meta-analysis is needed to provide context on the effectiveness of this therapy and how MSC treatment can be refined to optimize patient outcomes.

2.4 Methods and Design

Protocol

This systematic review protocol is reported in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analysis Protocol (PRIMSA-P) reporting guidelines [45]

(see Additional file 1). A summary of the protocol has been registered at the International Prospective Registry of Systematic Reviews (PROSPERO CRD 42021225431). The first systematic search of the literature is planned for February 3rd, 2021. An updated search will be performed 6 months after this initial search (August 3rd, 2021) to capture any new articles that have been published. A third and final update to the search will be performed on February 3rd, 2022 (6 months after the second search, 12 months after the first search).

Eligibility criteria

Population

Inclusion — Patients of any age (adults or pediatrics) with suspected and/or confirmed SARS-CoV-2 infection (by nucleic acid amplification testing, antibody assay, or antigen testing) and who are experiencing symptoms of coronavirus disease (COVID-19) or asymptomatic patients with confirmed SARS-CoV-2 infection. Exclusion — Studies of patients without confirmed SARS-CoV-2 infection will be excluded.

Intervention

Inclusion — We will include studies of MSCs or their secretome to treat COVID-19 in symptomatic or asymptomatic patients with confirmed SARS-CoV-2 infection. MSCs or their secretome can be derived from any tissue source (e.g., bone marrow, adipose, umbilical cord, dental pulp, placenta, etc.). Tissues from which MSC/secretome are obtained may be syngeneic, allogeneic, or xenogeneic. All routes of MSC/secretome administration will be considered (intravenous injection, aerosol inhalation, intramuscular injection, etc.). MSCs/secretome may be administered along with other therapeutic agents (antivirals, anti-cytokine drugs, immunomodulatory agents, etc.). Exclusion — Studies in which only non-MSC-based

therapeutics are administered to treat COVID-19, such as non-MSC cells (natural killer cells, T cells, artificial antigen-presenting cells), antivirals (remdesivir, sofosbuvir, ribavirin), immunomodulatory drugs (dexamethasone, sarilumab, baricitinib), combination therapies that do not include MSC-based treatment, and anti-cytokine drugs (anti-IL-6, anti-IL-1, anti-TNF-α, etc.). Studies that administered concomitant treatments along with MSC-based therapies will be included.

Comparators

Patients receiving conventional therapies for COVID-19 treatment (antivirals, immunomodulatory drugs, anticytokine drugs, combination therapies, etc.) or placebo will be included. Studies that do not include a comparator arm (i.e., Single-arm studies, observational studies) will also be included and may be analyzed or described separately from studies with comparator arms, as appropriate.

Outcomes

The primary outcomes for this study are overall mortality, the need for ICU admission, and the need for mechanical ventilation. These outcomes will be measured post-intervention (i.e., after administration of MSCs, MSC secretome, or comparator treatments). Dichotomous outcome measures will be expressed as risk ratios at the study endpoint (RR). The secondary outcomes for this study include COVID-19 severity according to the World Health Organization Ordinal Scale for Clinical Improvement (WHO-OSCI) [46], length of time in the intensive care unit (ICU), length of time on mechanical ventilation, length of time in hospital, presence and severity of clinical symptoms (fever, cough, shortness of breath, chest pain, etc.), presence and size of pulmonary lesions (CT scan) and change in oxygenation levels (e.g., PaO2/FiO2 ratio),

viral load, body temperature, organ failure assessment score (e.g., SOFA), circulating levels of immune cells (lymphocytes, neutrophils, macrophages, regulatory dendritic cells, NK cells), pro-inflammatory cytokines, (IL-6, TNF-α, IFN-γ, etc.), anti-inflammatory cytokines (IL-10, TGF-β, etc.) and inflammatory markers (C-reactive protein, Ferritin, D-dimer, etc.), and adverse events arising from MSC/secretome administration (tumorigenesis, thromboembolism, ectopic tissue formation, etc.). These outcomes will be measured pre-intervention and post-intervention (intervention = administration of MSCs or MSC secretome). Dichotomous, endpoints will be expressed as risk ratios (RR) and continuous endpoints will be expressed as mean difference (MD) or standardized mean difference (SMD).

Study types

Inclusion — All clinical studies (controlled and uncontrolled) examining the use of MSCs or their secretome as a therapeutic intervention for COVID-19 will be included. Studies may be randomized, pseudo-randomized, or non-randomized. Studies may be single-armed, observational, or have a comparator or control.

Data sources

We will search Embase Classic+Embase, Ovid MEDLINE(R), Ovid EBM Reviews, and Cochrane Central Register of Controlled Trials from inception onwards (Additional file 2). Preprint servers (e.g., medRxiv) will not be searched for this review. Other sources that become available during future iterations of the search will be considered, including search platforms that are specific for COVID-19. Additionally, two reviewers (AMK, MM) will perform manual searches of the bibliographies of included articles and relevant reviews will be performed.

Search strategy

The search strategies (Additional file 2) used for this review will be generated by a health sciences librarian (RS) with experience designing systematic literature searches. A second information specialist with no association to the project will also review the strategy using Peer Review of Electronic Search Strategies (PRESS) before the final search procedure is executed [47, 48]. The search strategy will utilize calculated vocabulary and MeSH terms (e.g., mesenchymal stem cells, mesenchymal stromal cells, mesenchymal stromal cell extracellular vesicles, mesenchymal stromal cell conditioned medium, severe acute respiratory syndrome coronavirus 2, coronavirus disease 2019, acute respiratory distress syndrome) and acronyms (e.g., MSCs, MSC-EVs, MSC-CM, SARS-CoV-2, COVID-19, ARDS), with alterations as required per database. No language or publication date restrictions will be applied for the initial search. Keywords for the search will include mesenchymal stromal cells, mesenchymal stem cells, MSCs, mesenchymal stromal cell conditioned medium, MSC-CM, mesenchymal stromal cell extracellular vesicles, MSC-EVs, exosomes, microvesicles, coronavirus disease 2019, COVID-19, severe acute respiratory syndrome coronavirus 2, SARSCoV-2, acute respiratory distress syndrome, ARDS, sepsis, pneumonia. The search will be updated every 6 months to capture any new relevant citations for inclusion in future iterations of this living systematic review. Unpublished studies, abstracts, review articles, editorials, and letters will be excluded.

Study records

Data management

All citations obtained from the systematic search of the literature will be uploaded to Rayyan QCRI systematic review software (https://www.rayyan.ai/) [49].

Selection process

Two reviewers (AMK, MM) will screen the titles and abstracts in an independent and blinded fashion using the a priori inclusion and exclusion criteria outlined above. Reasons for study exclusion during this initial screening will not be recorded. Studies that appear to meet the inclusion criteria, or where there is any uncertainty, the full-text articles will be obtained. The same two reviewers (AMK, MM) will assess the eligibility of full-text articles in an independent and blinded fashion using the same a priori inclusion and exclusion criteria. Any disagreement between the two reviewers will be resolved through discussion with a third-party member (DSA). Reasons for study exclusion at this level will be recorded. Study screening for updates to this review will shortly after the search is completed. For our initial iteration of the review, all clinical studies (both controlled and uncontrolled), examining the use of MSCs or their secretome as a therapeutic intervention for COVID-19 will be included. As more studies become available, future iterations of the review will utilize only controlled studies, with the eventual goal being to use only randomized controlled trials (RCTs).

Data collection process

A standardized data extraction form will be designed in Microsoft Excel (Microsoft Corporation, Seattle, USA) by the primary review author (AMK). Relevant information from each study will be extracted from each eligible study by two reviewers (AMK, MM) in an independent and blinded fashion. After data extraction is complete, any disagreements will first be discussed between the two independent reviewers (AMK, MM). If a resolution cannot be reached, a third-party member will be consulted (DSA).

Data items

Broad categories of data items to be extracted include study characteristics, baseline patient characteristics (age and gender breakdown, severity of COVID-19, comorbidities, etc.), intervention details (MSC tissue source, dose, route of administration, timing of administration, etc.), methods of MSC/secretome isolation and characterization (ultracentrifugation, scanning electron microscopy, western blot, flow cytometry, etc.), whether MSCs met the ISCT criteria [50] (plastic adherence, trilineage differentiation potential, positive and negative surface markers), primary and secondary clinical outcomes, and adverse events arising from MSC therapy.

Risk of bias assessment

The Cochrane risk of bias tool (RoB) for randomized trials version (2.0) will be used to assess the risk of bias for all included randomized studies (RCTs) [51]. The ROBINS-I tool for non-randomized studies will be used to assess the risk of bias for all other interventional controlled studies [52]. The EBM tool will be used to assess study quality in observational or uncontrolled studies [53]. Risk of bias assessment will be assessed at the study level by two independent, blinded reviewers (AMK, AJMB). Any discrepancies will be resolved by a third reviewer (DSA).

Confidence in cumulative evidence

We will use the GRADE criteria [54] to rate the strength of the body of evidence with regards to the effect of MSCs and their secretome on our primary outcome (mortality). This will strengthen the quantitative and narrative conclusions of the review, which will allow scientists,

clinicians, and policymakers to make informed decisions regarding the design of future clinical studies, regulatory approval, and clinical translation of this novel cellular therapy.

Data analysis

Meta-analysis is planned should there be enough controlled studies reporting our primary outcomes. Data will be pooled from multiple studies if there are three or more studies that report the same outcome. Data synthesis and analysis will be performed using RevMan 5 (Version 5.4) Systematic Review Software (https:// training.cochrane.org/online-learning/core-softwarecochrane-reviews/revman/revman-5-download). All primary outcomes will be analyzed and plotted separately as forest plots. Outcomes that will be attempted to be synthesized will include mortality rate, number of patients requiring ICU admission, and number of patients requiring mechanical ventilation. Because all primary outcomes are dichotomous variables (mortality rate, number of patients requiring ICU admission, number of patients requiring mechanical ventilation), the results from each study will be pooled and described as risk ratios (RR) with 95% confidence intervals using the DerSimonian and Laird random-effects method [55]. Before a meta-analysis is executed, the number of included studies and the outcomes they report will be assessed in consultation with a senior team member (DSA, DAF). If a sufficient number of studies exist with homogeneity in terms of outcome reporting, a meta-analysis will be performed. Statistical heterogeneity of effect sizes will be assessed using the I2 statistic [56]. The thresholds for interpretation of are I2: 0–40% (low heterogeneity), 30– 60% (moderate heterogeneity), 50–90% (substantial heterogeneity), and 75–100% (considerable heterogeneity) [56]. Should considerable heterogeneity exist, sources of heterogeneity will be explored through subgroup analysis. Evidence tables will be used to provide an overall description of included studies with regards to characteristics such as study design, participants, and intervention/control

groups [57]. Furthermore, evidence tables will be used to provide an overview of the number of studies reporting our primary/secondary outcomes and the number of studies reporting the occurrence of adverse events [57]. New data from subsequent searches of the literature will be incorporated into the review upon publication. The review authors will manually generate figures and update the results section of the review accordingly.

Subgroup analyses

Subgroup analysis is planned should a sufficient number of studies exist. Planned product-related subgroup analyses include efficacy of MSCs isolated from different tissue sources (bone marrow, adipose, umbilical cord blood, dental pulp, etc.), MSCs compared to their secretome (MSC-EVs, MSC-CM), and MSCs that have been treated (chemical agents, hypoxia, etc.) and/or genetic modified (e.g., altered expression of specific RNA species) before administration or isolation of paracrine factors. Planned patient-related subgroup analyses will address the efficacy of MSCs or their secretome according to COVID-19 severity (moderate, severe, critical), presence of co-morbidities (no-comorbidities, one comorbidity, multiple co-morbidities), patient age (over 55 vs under 55), and patient sex (male vs female). In addition, we will plan to analyze subgroups based on geographic location (China, Europe, North America, other) and by type of funding support for the study (public funding via government or charity, private funding from industrial sponsor, other).

Meta-biases assessment

Evaluation for publication bias will be conducted using a funnel plot [58]. These funnel plots will be generated using RevMan 5 (Version 5.4) Systematic Review Software

(https://training.cochrane.org/online-learning/coresoftware-cochrane-reviews/revman/revman-5-download).

Knowledge dissemination

Our findings will be disseminated through a widely read, open access, peer-reviewed scientific journal (e.g., PLOS Medicine, JAMA). Furthermore, we will share our findings at scientific meetings (e.g., American Society for Transplantation and Cellular Therapy (ASTCT) meetings, Till & McCulloch meetings (Stem Cell Network)) and through the preparation of evidence briefs that will be shared with health policy groups including Health Canada and the World Health Organization.

Living systematic review approach

Living systematic reviews are defined by Cochrane as a method of updating systematic reviews frequently to incorporate new evidence regarding an intervention of interest as soon as it becomes available [59]. While living systematic reviews and non-living systematic reviews both adhere to standard rigorous systematic review methodology (e.g., screening, data extraction, and risk of bias assessment), living systematic reviews also include explicit, transparent, and pre-specified decisions on how frequently the literature will be searched for new evidence and when new updates of the review are expected to be published [59]. Several criteria should be met to ensure that the intervention under consideration is well suited for performing a living systematic review [59]. For a living systematic review to be performed, the review question should be a priority for decision-making, there should be considerable uncertainty surrounding the existing evidence, and there should be a substantial chance that emerging evidence will impact the conclusions of the review [59]. In the case of MSCs and their secretome as a therapeutic

intervention for COVID-19, these pre-specified criteria would be met, making this topic well suited for a living systematic review. There remains an urgent need for clarity regarding optimal management and many ongoing studies are actively recruiting and are anticipated to contribute to the evolving evidence base over the next 12 months. Additionally, the skill level and workload for the review team should be considered carefully before initiating the review to ensure that the appropriate personnel are available to support the ongoing maintenance of the living systematic review [59]. The bulk of project management will be undertaken by a graduate student (AMK) who is completing the review as the main component of their graduate studies. Our review team also has access to several medical students who will be available to perform tasks which must be done in duplicate (MM, AJMB) for the duration of the review. Furthermore, a medical information specialist (RS) is also available for the whole expected duration of the project. Lastly, our team has several experienced senior members (DSA, DAF, MML) who can assist in moving the review forward and offering advice should problems arise.

Updates

The literature search for this living review will be updated every 6 months (see Fig. 1). Article screening and data analysis will be performed immediately following updates to the literature search. The review itself will be updated every 6 months or as required depending on the amount of new evidence that emerges. New data will be incorporated into the review upon publication. The review authors will manually generate all figures/tables and update the results section of the review accordingly. We will not utilize any techniques to update this living systematic review in a rapid manner (e.g., machine learning) as soon as individual studies are published.

Amendments

If any amendments to this protocol are necessary, the date and specific changes to the protocol will be documented on PROSPERO (CRD42021225431) with rationales as to why the alterations were required.

2.5 Discussion

This systematic review will summarize all published clinical studies investigating the safety and efficacy of MSCs and their secretome as a therapeutic intervention for COVID-19. Due to their immunomodulatory and regenerative capabilities, MSCs and their secretome have the potential to dampen the inflammatory immune response initiated by SARS-CoV-2 infection and heal damaged tissues [60, 61]. MSC-EVs and MSC-CM represent a particularly promising approach to harness the therapeutic benefits of MSCs with lower manufacturing/storage costs and an improved safety profile [41–43]. Of particular significance, MSCs and their secretome have demonstrated promise for a number of COVID-19-related ailments including acute respiratory distress syndrome (ARDS) [62], acute lung injury [63], pneumonia [64], pulmonary fibrosis [65], acute kidney injury [66], myocarditis [67], and sepsis [68].

Despite extensive funding allocation and research, only two therapeutics are approved to date, for the treatment of COVID-19 in Canada [69], Bamlanivimab, a monoclonal antibody directed against the spike protein of SARSCoV-2 [70], and Remdesivir, a broad-spectrum adenosine analog [71]. Additionally, three antibody cocktails have been granted emergency use authorization in the USA: bamlanivimab and remdesivir, bamlanivimab combined with etesevimab, and REGEN-COV (casirivimab and imdevimab) [72]. Although remdesivir effectively reduces the recovery time of COVID-19 patients [73], it is only approved for use in

severe COVID-19 patients [71]. In contrast, although bamlanivimab and other antibody therapies prevent the worsening of COVID-19 symptoms and reduce hospitalizations [74], their use is currently restricted to patients with mild–moderate COVID-19 that are at high risk of progressing to severe COVID-19 [70]. Furthermore, both these therapies are not approved for use in pediatric populations [70, 71], are resource intensive [75, 76], and may be susceptible to escape mutants [77, 78].

Though living systematic reviews are important for disseminating critical information in a timely fashion, they require a significant commitment from the primary author and the rest of the review team to keep up to date with rapidly evolving bodies of literature [59]. This is particularly true for COVID-19 related reviews as the rate at which new studies are being published [79] may make it challenging to keep pace with the evolving knowledge base and provide readers with versions of the review which incorporate all relevant evidence at any given timepoint. Though our review team currently possesses the required resources to maintain a living systematic review of this nature, circumstances could change that affect our ability to update the review in the prespecified time intervals laid out in this protocol. Furthermore, with the uncertainty surrounding the timeline of the COVID-19 pandemic, it may be necessary to transition this review from a living systematic review to a standard systematic review should the rate at which studies in this area are being published fall dramatically. At this point, we will follow the guidelines recommended by Cochrane for transitioning out of a living systematic review for our final update of the review to inform readers that the review is no longer in living mode [59].

Our planned systematic review and meta-analysis is not without limitations. Firstly, informative practice changing systematic reviews and meta-analyses often incorporate many

high-quality studies with an overall patient population large enough to achieve the statistical power necessary to detect differences between control and experimental groups [80, 81]. Although a large number of trials examining the use of MSCs and/or their secretome as a therapeutic intervention for COVID-19 have been registered across clinical trial databases globally [82], most of the published literature to this point is in the form of case reports, case series, uncontrolled studies, or small early-phase controlled trials. With these low-quality trials appearing to make up the bulk of published studies at this point, it is possible that this review will not produce any meaningful conclusions, especially the first iteration. Secondly, the rapid onset of the pandemic has led to a rush by researchers to register, run and publish trials without following robust clinical trial methodology [83]. This lack of methodological rigor may increase the risk of bias of the individual studies included in this review, which will in turn decrease the certainty of the conclusions of the review as a whole [84]. Lastly, with the duration of the pandemic being unknown and vaccine rollout well underway in many countries, it is possible that all these registered trials may never enroll enough patients to reach completion. Thus, the possibility exists that there may never be enough high-quality controlled studies published to determine the true extent to which MSCs and their secretome are an effective therapeutic intervention for COVID-19.

To diversify the available therapeutic options and fill unmet therapeutic needs, there has been an enormous surge in the number of registered clinical trials examining COVID-19-related interventions. Among these registered trials, several studies examining the use of MSC based therapies for COVID-19 can be found. However, significant heterogeneity can be observed in terms of the study design, intervention characteristics, and outcome reporting across these trials. This systematic review will capture this heterogeneity and discern the most frequently employed

and effective approaches. A meta-analysis of the primary outcome will allow for a better interpretation of the true clinical efficacy of MSCs and their secretome as a therapeutic intervention for COVID-19 than could be achieved in any individual study alone. With the majority of studies not planning to enroll enough patients to reach adequate statistical power to determine efficacy, the findings presented in this review should be useful in guiding the design and implementation of more definitive clinical trials in the future or may possibly provide sufficient evidence to support approval of this class of cell-based therapy. Taken together, our planned systematic review and meta-analysis will help accelerate approval of this novel cellular therapy by relevant regulatory bodies and propel MSC-based therapy for COVID-19 towards use in the broader population should there be sufficient evidence demonstrating its safety and efficacy.

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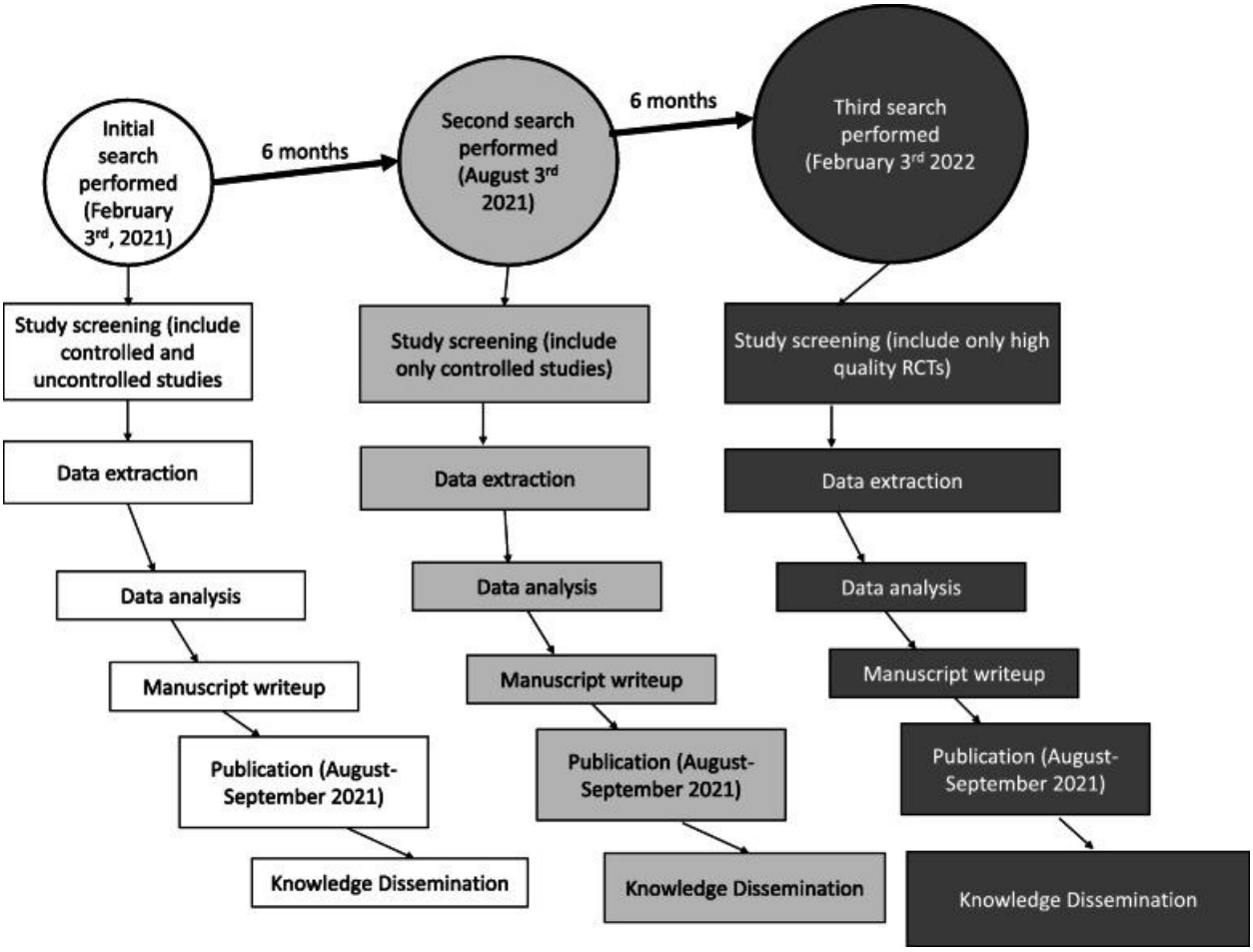
2.7 Figures and Tables

Fig. 1 Diagram of anticipated updates of the living systematic review. Updates will increase the quantity and quality of evidence to refine conclusions



2.8 Appendix

Additional file 1. PRISMA-P 2015 Checklist.

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review	☒	☐	Page 1, line 2
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	☐	☒	NA
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	☒	☐	Page 5, line 8
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	☒	☐	Page 1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	☒	☐	Page 15, line 10
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	☒	☐	Page 13, line 12
Support					
Sources	5a	Indicate sources of financial or other support for the review	☒	☐	Page 15, line 19
Sponsor	5b	Provide name for the review funder and/or sponsor	☐	☒	NA
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	☐	☒	NA
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	☒	☐	Pages 3-5
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	☒	☐	Page 4, line 13
METHODS					
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report	☒	☐	Page 5, line 9

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
		characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review			
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	☒	☐	Page 7, line 19
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	☒	☐	Page 8, line 3; Additional file 2
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	☒	☐	Page 9, line 2
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	☒	☐	Page 9, line 7
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	☒	☐	Page 10, line 3
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	☒	☐	Page 10, line 10
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	☒	☐	Page 6, line 16
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	☒	☐	Page 11, line 1
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	☒	☐	Page 11, line 8
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2, Kendall's tau)	☒	☐	Page 11, line 8
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	☒	☐	Page 12, line 9

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	☐	☒	NA
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	☒	☐	Page 12, line 16
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	☒	☐	Page 9, line 20

Additional file 2. Search Strategy.

Database: Embase Classic+Embase <1947 to 2021 February 03>, Ovid MEDLINE(R) ALL <1946 to February 03, 2021>, EBM Reviews - Cochrane Central Register of Controlled Trials <December 2020>
Search Strategy:
---
1 COVID-19/ (17886)
2 (exp coronavirus/ or coronavirus*.mp. or corona virus*.mp.) and (wuhan or beijing or shanghai).mp. (9261)
3 ((coronavirus or corona virus) adj3 "2019").tw. (41532)
4 (covid or covid2019).tw,kf. (175983)
5 covid19.tw,kw. or covid 19.kf. (42778)
6 sars cov 2.tw,kw. (62711)
7 (ncov or n cov).tw,kw. (3072)
8 novel coronavirus.tw,kw. (14551)
9 sars cov2.tw,kw. (3114)
10 Coronavirus Infections/ and Pandemics/ (39146)
11 (ncov19 or ncov-19 or 2019-novel CoV).tw,kf. (119)
12 or/1-11 (198342)
13 Mesenchymal Stromal Cells/ (49672)
14 Mesenchymal Stem Cell Transplantation/ (24831)
15 Multipotent Stem Cells/ (8889)
16 (MSC or MSCs or ADMSC or ADMSCs or BM-MSC or BM-MSCs or BMD-MSC or BMD-MSCs or BMDMSC or BMDMSCs).tw,kw. (88902)
17 (mesenchymal adj5 (cell* or stem or stromal or progenitor* or multipotent or bone marrow or adipose or placenta*)).tw. (199459)
18 (mesenchymal and (cell* or stem or stromal or progenitor or multipotent or bone marrow or adipose or placenta*)).kw. (33543)
19 ((multipotent or multi-potent) adj (stroma* cell* or stem cell*)).tw,kf. (4692)
20 (msc ev or msc cm).tw,kf. (1071)
21 (colony-forming adj2 fibroblast*).tw. (1675)
22 marrow stroma* cell*.tw. (17531)
23 Mesoderm/cy (5853)
24 Exosomes/ or Extracellular Vesicles/ (42505)
25 exosom*.tw,kw. (41425)
26 Cell-Derived Microparticles/ (8876)
27 (microvesicle* or micro vesicle*).tw,kw. (11432)
28 ((cell or cells or extracellular) adj2 vesicle*).tw. (29239)

29 microparticle*.tw,kw. (42112)
30 (extracellular and vesicle*).kf. (5278)
31 ((cell or cells) and vesicle*).kf. (2195)
32 or/13-31 (354252)
33 12 and 32 (743)
34 33 use medall (306) Medline

35 (Coronavirinae/ or coronavirus*.mp. or corona virus*.mp.) and (wuhan or beijing or shanghai or hubei).mp. (9894)
36 ((coronavirus* or corona virus* or coronavirus* or coronaviridae or coronaviridae or betacoronavirus*) adj3 ("19" or "2019")).tw. (52354)
37 (covid or covid19).tw. (173537)
38 sars cov 2.tw. (53939)
39 (ncov or n cov).tw. (2696)
40 (novel coronavirus* or novel corona virus*).tw. (14310)
41 (CoV 2 or CoV2 or sarscov2 or 2019nCoV or novel CoV or wuhan virus).tw. (56040)
42 (coronavirus infection/ or severe acute respiratory syndrome/) and (pandemic/ or pandemic*.tw.) (51355)
43 limit 42 to yr="2019 -Current" (50254)
44 35 or 36 or 37 or 38 or 39 or 40 or 41 or 43 (197531)
45 exp mesenchymal stem cell/ (102855)
46 exp mesenchymal stem cell transplantation/ (24831)
47 exp mesenchymal stroma cell/ (14237)
48 (MSC or MSCs or ADMSC or ADMSCs or BM-MSC or BM-MSCs or BMD-MSC or BMD-MSCs or BMDMSC or BMDMSCs or BMSC or BMSCs or HBMSC or HBMSCs or ABMSC or ABMSCs or MAPC or MAPCs).tw. (106309)
49 (((multipotent or multi-potent) adj3 (stem or stroma$1 or progenitor*)) and (cell$1 or cell-induced)).tw. (16261)
50 multipotent stem cell/ (9199)
51 (mesenchymal adj5 (cell* or stem or stroma* or progenitor* or multipotent or bone marrow or adipose or placenta*)).tw. (199944)
52 (colony-forming adj2 fibroblast*).tw. (1675)
53 (marrow stroma* adj2 cell*).tw. (18363)
54 exosome/ (31880)
55 exosom*.tw. (37895)
56 (microvesicle* or micro vesicle*).tw. (10182)
57 microparticle*.tw. (40494)
58 ((cell or cells or extracellular) adj2 vesicle*).tw. (29239)
59 exp membrane microparticle/ (8167)
60 (msc ev or msc cm).tw. (1070)
61 or/45-60 (359808)
62 44 and 61 (735)
63 62 use emczd (370) Embase
64 COVID-19/ (17886)
65 (exp coronavirus/ or coronavirus*.mp. or corona virus*.mp.) and (wuhan or beijing or shanghai).mp. (9261)
66 ((coronavirus or corona virus) adj3 "2019").tw. (41532)
67 (covid or covid2019).tw,kw. (175722)
68 covid19.tw,kw. or covid 19.kw. (75538)
69 sars cov 2.tw,kw. (62711)
70 (ncov or n cov).tw,kw. (3072)
71 novel coronavirus.tw,kw. (14551)
72 sars cov2.tw,kw. (3114)
73 Coronavirus Infections/ and Pandemics/ (39146)
74 (ncov19 or ncov-19 or 2019-novel CoV).tw,kw. (125)
75 or/64-74 (199082)

76 Mesenchymal Stromal Cells/ (49672)
77 Mesenchymal Stem Cell Transplantation/ (24831)
78 Multipotent Stem Cells/ (8889)
79 (MSC or MSCs or ADMSC or ADMSCs or BM-MSC or BM-MSCs or BMD-MSC or BMD-MSCs or BMDMSC or BMDMSCs).tw,kw. (88902)
80 (mesenchymal adj5 (cell* or stem or stromal or progenitor* or multipotent or bone marrow or adipose or placenta*)).tw. (199459)
81 (mesenchymal and (cell* or stem or stromal or progenitor or multipotent or bone marrow or adipose or placenta*)).kw. (33543)
82 ((multipotent or multi-potent) adj (stroma* cell* or stem cell*)).tw,kw. (4749)
83 (msc ev or msc cm).tw,kw. (1070)
84 (colony-forming adj2 fibroblast*).tw. (1675)
85 marrow stroma* cell*.tw. (17531)
86 Mesoderm/cy (5853)
87 Exosomes/ or Extracellular Vesicles/ (42505)
88 exosom*.tw,kw. (41425)
89 Cell-Derived Microparticles/ (8876)
90 (microvesicle* or micro vesicle*).tw,kw. (11432)
91 ((cell or cells or extracellular) adj2 vesicle*).tw. (29239)
92 microparticle*.tw,kw. (42112)
93 (extracellular and vesicle*).kw. (5106)
94 ((cell or cells) and vesicle*).kw. (2780)
95 or/76-94 (354775)
96 75 and 95 (739)
97 96 use cctr (79) Cochrane
98 34 or 63 or 97 (755)
99 remove duplicates from 98 (487)
100 99 use medall (295)
101 99 use emczd (115)
102 99 use cctr (77)

3. MESENCHYMAL STEM/STROMAL CELL-BASED THERAPIES FOR COVID-19: FIRST ITERATION OF A LIVING SYSTEMATIC REVIEW AND META-ANALYSIS

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3.1 Abstract:

Background: Mesenchymal stem/stromal cells (MSCs) and their secreted products are a promising therapy for COVID-19 given their immunomodulatory and tissue repair capabilities. Many small studies were launched at the onset of the pandemic and repeated meta-analysis is critical to obtain timely and sufficient statistical power to determine efficacy.

Methods and Findings: All English language published studies identified in our systematic search (up to February 3, 2021) examining the use of MSC-derived products to treat patients with COVID-19 were identified. Risk of bias (RoB) was assessed for all studies. Nine studies were identified (189 patients) and four were controlled (93 patients). Three of the controlled studies reported on mortality (primary analysis) and were pooled through random effects meta-analysis. MSCs decreased the risk of death at study endpoint compared to controls (RR: 0.18, 0.04-0.74; p=0.02; I^2=0%) although follow-up differed. Among secondary outcomes, IL-6 levels were most commonly reported and were decreased compared to controls (SMD: -0.69, -1.15 to -0.22, p=0.004; I^2=0%) (n=3 studies). Other outcomes were not reported consistently and pooled estimates of effect were not performed. Substantial heterogeneity was observed between studies in terms of study design. Adherence to published ISCT criteria for MSC characterization was low (2 of 9 studies RoB analysis revealed a low to moderate risk of bias in controlled studies and uncontrolled case series were of good (3 studies) or fair (2 studies) quality.

Conclusion: Use of MSCs to treat COVID-19 appears promising, however, few studies were identified and potential risk of bias was detected in all studies. More controlled studies that report uniform clinical outcomes and use MSC products that meet standard ISCT criteria should

be performed. Future iterations of our systematic search should refine estimates of efficacy and clarify potential adverse effects.

Keywords: mesenchymal stromal cells, mesenchymal stem cells, extracellular vesicles, exosomes, microvesicles, coronavirus disease 2019, COVID-19, severe acute respiratory syndrome coronavirus 2, SARS-CoV-2, acute respiratory distress syndrome, sepsis, pneumonia

3.2 Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the pathogenic beta-coronavirus that causes coronavirus disease 2019 (COVID-19), has spread rapidly around the world, creating an urgent need for effective therapies that can prevent excessive mortality (1). SARS-CoV-2 infects cells via attachment of its spike (S) protein to the angiotensin converting enzyme 2 (ACE2) receptor on the surface of target cells (2). The subsequent endocytosis of the ACE2 complex leads to increased free serum Angiotensin II (Ang II) which can induce profound inflammatory responses through binding with angiotensin receptor type 1 and activation of nuclear factor kappa-light chain enhancer of activated B cells (NF-kB) pathway (3) and the conversion of membrane bound IL-6Rα to soluble IL-6 (sIL-6), activating signal transducer and activator of transcription 3 (STAT3) (3). The synergistic activation of NF-kB and STAT3 creates a positive feedback loop that further augments the production of pro-inflammatory cytokines and chemokines (3), attracting pro-inflammatory immune cells to infected tissues (4) and resulting in a dramatic augmentation in pro-inflammatory cytokine production termed the “cytokine storm" (5). This cytokine storm causes considerable tissue damage through apoptosis, necroptosis (6) and pyroptosis (7). Pro-inflammatory responses and the cytokine storm induced by SARS-CoV-2 infection can lead to pulmonary complications including acute lung injury, pulmonary edema

and acute respiratory distress syndrome (ARDS) (8,9) which may require intubation and ventilator support in intensive care units.

Since SARS-CoV-2 first emerged in December 2019, few approved therapies have emerged to supplement the ongoing COVID-19 vaccination efforts that have been launched (10). With many people remaining vulnerable due to slower uptake of vaccinations in some areas, combined with the emergence of increasing variants of concern, the need for effective therapy remains a pressing issue.

Mesenchymal stem/stromal cells (MSCs) were quickly viewed with significant promise to treat COVID-19 (11) and many studies were launched rapidly with several now completed and reported. MSCs are multipotent stem like cells which can be isolated from a number of adult and neonatal tissues including bone marrow, adipose, umbilical cord and placenta among the others (12). MSCs have demonstrated immunomodulatory, antimicrobial and tissue regenerative capabilities across a wide variety of diseases in both preclinical and clinical studies ([13], [14], [15], [16], [17]) (see Box 1 for a summary of mechanisms of immune modulation by MSCs). Following migration to sites of infection or injury, MSCs can reduce neutrophil infiltration (18), suppress CD8+T cell proliferation, polarize pro-inflammatory M1 macrophages and Th1 CD4+ T cells to anti-inflammatory M2 macrophages and Th2 CD4+T cells, promote regulatory T cell (T regs) and B cell (B regs) function, suppress dendritic cell maturation and function, and modulate natural killer cell activity (19). The immunomodulatory actions of MSCs on these immune cells are mediated through the secretion of many soluble factors (18,20), through direct cell-cell contact and MSCs may target the IL-6 amplifier protein directly by attenuating the hyperactivation of NF-kB and STAT3 (21,22). The vast majority of preclinical and clinical studies examining the use of MSC-based therapeutics have found that MSCs have minimal

immunogenicity when administered to patients (23,24) and do not lead to further inflammation or worsening of the cytokine storm. Moreover, the occurrence of adverse events resulting from MSC therapy is rare (25,26).

Living systematic reviews are an emerging method of conducting systematic reviews which involves frequent updating to incorporate new evidence as soon as it becomes available, providing clinicians, scientists and policymakers with the most up to date high quality information surrounding specific topics (27). Living systematic reviews have been recently conducted to provide estimates regarding the safety and efficacy for many re-purposed therapeutics in the context of COVID-19 ([28], [29], [30]) and seems most appropriate for the analysis of MSCs in COVID-19 due to the expectation that many studies were launched early in the pandemic and will be published over the next 12 to 18 months (31).

Pooled estimates regarding the use of MSCs to treat patients with COVID-19 are needed as nearly all studies in this area are small and lack sufficient statistical power to determine efficacy on their own. Meta-analysis may be limited, however, by heterogeneity in aspects of study design, product characterization, outcome measures and differences in participant populations enrolled between studies. Timely regulatory approval and clinical translation will likely require meta-analysis of similar high quality well-designed studies identified through a systematic search of the literature to determine whether MSC-based therapeutics are safe and effective for the treatment of COVID-19. A living systematic review and meta-analysis is needed to keep pace with the rapid evolution of new information related to the pandemic and to provide insight from a combined sample size that will have sufficient power for determining efficacy.

3.3 Methods

This systematic review is reported in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) guidelines (32) (Table S4.). The study protocol has been published (33) and is registered at the International Prospective Registry of Systematic Reviews (PROSPERO; CRD42021225431).

Literature Search Strategy

A systematic search of all clinical studies (controlled and uncontrolled) examining the use of MSCs and/or their secretome (which includes conditioned media (MSC-CM), or extracellular vesicles (MSC-EVs) derived from MSCs) as a therapeutic intervention was conducted from 1947 to February 3, 2021 in Embase Classic+Embase, Ovid MEDLINE(R), Ovid EBM Reviews and the Cochrane Central Register of Controlled Trials. The search strategy was developed in collaboration with a health sciences librarian (RS) specializing in systematic review searches and was peer reviewed by a second librarian according to the Peer Review of Electronic Search Strategies (PRESS) framework (34). The reference lists of included studies and relevant reviews captured by the search were also examined by two independent reviewers (AMK, MM) to ensure that all relevant articles were captured. The full search strategy is outlined in Figure S1.

Eligibility Criteria

All English-language, full-text, clinical studies examining the use of MSCs or their secretome (MSC-EVs, MSC-CM) as a therapeutic intervention for COVID-19 were included. Studies could be single armed (uncontrolled) or have a comparator or control groups (controlled). For the controlled studies, all randomization methods were considered acceptable

(randomized, pseudo-randomized, and non-randomized). Studies published in languages other than English, review articles, commentaries, editorials, letters, case reports, conference abstracts, unpublished gray literature and other study types (in vitro studies, preclinical animal studies, etc.) were excluded. All symptomatic or asymptomatic patients with confirmed SARS-CoV-2 infection (qRT-PCR, antibody assay, etc) were included. MSCs derived from any known applicable tissue source (bone marrow, adipose, umbilical cord, dental pulp, placenta etc.) were acceptable. MSCs could be obtained from syngeneic, allogeneic or xenogeneic tissues. All routes of MSC/secretome administration were acceptable (intravenous injection, aerosol inhalation, intramuscular injection, etc). MSC-based products could also be administered along with other therapeutic agents (antivirals, anti-cytokine drugs, immunomodulatory agents, etc.). Studies exclusively investigating other non-MSC based therapeutics were excluded.

Outcomes

The primary analysis of this study was mortality rate at study endpoint. Secondary analyses included number of patients requiring intensive care unit (ICU) admission, number of patients requiring mechanical ventilation, length of time in hospital, length of time in ICU, length of time on mechanical ventilation, presence and severity of clinical symptoms (fever, cough, shortness of breath, chest pain, etc), presence and size of pulmonary lesions on radiographic imaging (ie. CT scan) and change in oxygenation levels (e.g. PaO2/FiO2 ratio), viral load, body temperature, organ failure assessment score (e.g. SOFA), circulating levels of immune cells (lymphocytes, neutrophils, macrophages, regulatory dendritic cells, NK cells), pro-inflammatory cytokines, (IL-6, TNF-α, IFN-γ, etc.), anti-inflammatory cytokines (IL-10, TGF-β, etc.) and inflammatory markers (C-reactive protein, Ferritin, D-dimer, etc.), and adverse events arising from MSC-based product administration (tumorigenesis, thromboembolism, etc.)

Study selection

All citations identified in the search were imported into Rayyan, (https:// rayyan.qcri.org/) for management of search records. After duplicates were removed, the study titles and abstracts were screened in duplicate by two independent reviewers (AMK, MM). After all potentially relevant titles and abstracts were identified, the full texts of all potentially relevant studies were reviewed in duplicate to determine final eligibility. In cases of disagreement between the two reviewers, consensus was achieved through discussion with a third senior team member (DSA).

Data Extraction

All relevant data was extracted in duplicate by two independent reviewers (AK, MM) from the included studies using a standardized data extraction template in Microsoft Excel (Microsoft, Seattle, USA). In cases of disagreement between the two reviewers, the differences were resolved through consultation with a senior team member (DSA). Specific data extracted from studies included study characteristics (e.g. authors, publication year, country of study), study design (characteristics of control group, sample size, length of observation period, planned preconditioning or alterations to MSCs or secreted factors before therapeutic use, MSC/secretome isolation and characterization methods, etc.), patient characteristics (e.g. age, sex, co-morbidities, COVID-19 severity, symptoms upon hospital admission, etc), intervention characteristics (MSC tissue source, MSC/secretome dose, route of administration, number of doses, whether MSC-based products met all of the minimal criteria established in guidelines by the International Society for Cellular Therapy (ISCT) (35) and/or Minimal Information for Studies of Extracellular Vesicles (MISEV) (36) criteria), all data pertaining to primary and secondary outcomes, and details concerning risk of bias (RoB) determination were extracted.

RoB assessment was conducted using the Risk-of-Bias Tool for Randomized Trials (ROB 2) tool (37) for randomized controlled trials, the Risk of Bias In Non-randomized Studies of Interventions (ROBINS I tool) (38) for non-randomized controlled studies, and the Evidence Based Medicine (EBM) tool (39) for case series. Image J software was used to extract data in graphical format (https://imagej.nih.gov/ij/download.html).

Data Analysis

The results from individual studies were pooled for meta-analysis using Review Manager (Version 5.4) Systematic Review Software (https://training.cochrane.org/online-learning/core-software-cochrane-reviews/revman/revman-5-download). For dichotomous outcomes, risk ratios (RRs) were calculated to determine the risk of death between the control and experimental groups at study endpoint. For continuous outcomes, the standardized mean difference (SMD) between control and experimental groups was calculated using random effects meta-analyses. Significance in pooled analysis was performed using the DerSimonian and Laird random effects model. All data is presented with 95% confidence intervals (CIs). Meta-analysis was only performed when three or more controlled studies reported on the same outcome. Outcomes that were reported in less than three controlled studies or where adequate data for inclusion in meta-analysis was not provided were analyzed in a descriptive manner. Statistical heterogeneity was assessed using the I2 statistic. Potential subgroup analyses were determined a priori in our study protocol with the goal of determining if the effect of MSCs as a therapeutic intervention for COVID-19 was significantly different for studies that used MSCs from specific tissue sources, MSCs vs secreted factors (MSC-EVs, MSC-CM), or in patients with varying COVID-19 severity. Due to the small number of studies included in each of our quantitative analyses, we did

not perform a planned analysis for publication bias. Finally, $p<0.05$ was considered significant for all analyses.

3.4 Results

Literature search

A total of 459 unique records were identified in our systematic search of the literature after duplicates were removed. Nine articles met the criteria for inclusion in our analysis ([40], [41], [42], [43], [44], [45], [46], [47], [48]). Reasons for study exclusion were trial protocol only (n=57), reviews, editorials or commentaries (n=9), non-MSC cells (n=8), and uncontrolled case series in languages other than English (n=4; Spanish (n=1), Chinese (n=1), Persian (n=1), and Russian (n=1)). (Figure 3).

Study characteristics

The characteristics of the 9 included studies are summarized in Table 1. Four of the studies were controlled ([40], [41], [42], [43]) and five of the studies were uncontrolled ([44], [45], [46], [47], [48]). Two of the controlled studies were RCTs (41,43) and two were non-randomized controlled trials (40,42). All five of the uncontrolled studies were case series ([44], [45], [46], [47], [48]). Study publication date ranged from March 9th, 2020, to January 29th, 2021. Five of the studies were conducted in China, ([40], [41], [42],45,46), two in the USA (43,44), one in Iran (48) and one in Spain (47).

Patient characteristics

In total, there were 189 patients (mean age 58.3 ± 6.3; 124 male) enrolled across all study groups and 136 patients (mean age 58.5 ± 6.7; 96 male) were administered MSC-based therapy

as a therapeutic intervention for COVID-19. In the controlled studies, 40 patients (55.5 ± 7.1 years of age; 24 male) were treated with MSCs and 53 patients (57.9 ± 6.4 years of age; 28 male) served as controls. The distribution of patients with mild, moderate, severe, and critical COVID-19 at the time of treatment with MSC-based treatment was somewhat similar for patients in the intervention groups and controls, however, there were more patients with mild COVID-19 and fewer with severe disease in the intervention group compared to the control group (Table 1).

In terms of patient co-morbidities, there were more obese patients in the intervention group compared to controls (27.5% vs 9.4%). However, all other co-morbidities including hypertension, diabetes, chronic obstructive pulmonary disease (COPD), coronary artery disease and hyperlipidemia appeared well balanced between control and intervention groups. (Table 1).

Intervention characteristics

Intervention characteristics are summarized in Table 2. Eight studies used MSCs ([40], [41], [42], [43],[45], [46], [47], [48]) and one study used MSC-EVs (exosomes) (44). All MSCs were derived from allogeneic human tissues, including umbilical cord (n=5) ([41], [42], [43],45,46), bone marrow (n=1) (44) and adipose tissue (n=1) (47). One study used MSCs derived from both umbilical cord and placental tissue (48). One study did not report the tissue source from which its MSCs were obtained (40). The passage number of the MSCs varied widely between studies (see Table 2), with four studies not reporting how many passages were performed before harvesting MSCs from ex vivo culture. With regards to the extent that studies reported on specific ISCT criteria (35) for MSC characterization, only two of the nine studies addressed all three minimal criteria established by the ISCT. Specific details regarding the number of studies meeting each of the three individual ISCT criteria can be found in Table 2.

The study that used MSC-EVs (termed “exosomes” in the study) did not report sufficient details to allow classification of the EVs within the MISEV (36) criteria for characterization.

MSC doses varied and the format of reporting dose differed between studies, including cells/kg of body weight (n=4; 1 - 2 × 10^6 - cells/kg), total cells/injection (n=4; 0.3 - 2.0 × 10^8 cells), and mL of ExoFlo™ (n=1; 15 mL) in the case of MSC-EVs. All nine studies administered their product intravenously. Most patients (41.9%) received one infusion of MSCs, although other studies reported administering up to four MSC infusions (see Table 2). The reported time from COVID-19 diagnosis to MSC administration (median of 6.5 days across studies (n=8), range 1-15 days) was similar between control groups (4.0, range 1 – 14) and intervention groups (5.9, range 1 – 11.5) in the controlled studies.

Patients were administered other therapeutic agents in addition to MSCs or MSC-EVs in eight of the nine studies (88%). The specific therapeutic agents administered varied considerably between studies and are summarized in Table 2. Two of the studies stated that they used medications in addition to MSCs but did not specify which medications were used. The median period of follow up after MSC administration was 22.0 days (range 14-60 days).

Primary outcome: mortality

Outcomes reported across studies are summarized in Table 3. All nine studies reported mortality. The mortality rate at endpoint for all patients administered MSCs or MSC-EVs was 17 of 136 patients (12.5%). In the controlled studies, the mortality rate at endpoint for the combined control groups was 11 of 53 patients (20.7%), whereas the mortality rate for the combined MSC groups was 1 of 40 patients (2.5%). In meta-analysis of the controlled studies (n=3), MSCs were

associated with a decreased risk of death at study endpoint (RR: 0.18 [0.04-0.74, 95% CI, p=0.02, I2=0%]) compared to the control group (Figure 4).

Secondary outcomes

Time to Clinical Improvement: Five studies (3 controlled) reported on the median time from MSC administration to improvement of COVID-19 clinical symptoms (6.3, range 1.7-20.0 days for all patients who received MSCs). In the three controlled studies, time from MSC administration to improvement of COVID-19 clinical symptoms was 23.0 days in control groups (range not defined as patients did not improve in control groups of two studies) and 10.9 days (range 1.7-20.0 days) in MSC groups.

Hospitalization and Intensive Care Unit Metrics: Six of the nine studies (3 controlled) reported on the number of patients hospitalized for COVID-19 at the beginning and end of their study periods. All of the 97 patients (100%) who received MSCs or MSC-EVs were hospitalized at time of enrollment and only 32 of 97 patients (33.0%) were still in hospital at the end of the respective study periods. In controlled studies, patients who received MSCs were less likely to remain hospitalized at the end of the study period compared to controls (OR: 0.34 [0.12-0.91, 95% CI, p=0.03]).

Immune Biomarkers: All nine studies reported pro-inflammatory cytokines at baseline and study endpoint. Three of the controlled studies reported serum IL-6 levels at study endpoint in a format which could be combined in meta-analysis which revealed that MSCs significantly decreased serum IL-6 levels compared to controls (SMD: -0.69; 95% CI: -1.15 to -0.22, p=0.004, I2=0%) (Figure 5). Trends in several other pro-inflammatory cytokines from baseline to study endpoint were also observed among patients administered MSCs, however, none were

consistently reported in enough of the controlled studies to perform meta-analysis. C-reactive protein (CRP) changes were reported in seven studies (2 controlled) and levels decreased in patients administered MSCs in all studies and had greater reductions in treated patients compared with controls. D-dimer levels decreased in three studies and increased in one study in patients administered MSCs. Interferon-gamma (IFN-γ) declined from baseline to endpoint in patients given MSCs in all 4 studies where it was reported. TNF-α decreased from baseline to endpoint in patients administered MSCs in 5 studies.

Seven of the nine studies (2 controlled) reported on changes in circulating levels of immune cells and/or other immune biomarkers. Lymphocyte count changes increased from baseline to endpoint in all seven studies (2 controlled) that reported this outcome for patients who were administered MSCs.

Radiological Outcomes: Six of the nine studies (3 controlled) examined radiological improvement in patients following MSC administration. Five studies did so in a descriptive manner, with these studies reporting the disappearance of ground glass opacities, linear opacities and pleural effusions. One controlled study reported changes at 2 weeks in comparison to baseline and reported non-significant resolution of ground glass opacities (OR: 0.26 [0.06-1.09, 95% CI, p=0.07]), linear opacities (OR: 0.27 [0.07-1.11, 95%, p=0.07]), and pleural effusions OR: 1.23 [0.10-14.96. 95% CI, p=0.87]).

Virological Outcomes: Four studies (2 controlled) reported changes in viral load from baseline to study endpoint. At the beginning of these studies, all the patients administered MSCs (100%) were positive for SARS-CoV-2 viral RNA. At the respective endpoints of these studies, none of the patients administered MSCs (0%) were positive for SARS-CoV-2 viral RNA. Two studies (1 controlled) reported on changes in SARS-CoV-2 antibody titers in patients

administered MSCs. Both studies displayed increasing antibody titers from baseline to endpoint in patients treated with MSCs compared to controls.

Adverse events: Adverse events are summarized in Table 4. Three studies reported adverse events associated with MSC infusion. These adverse events included facial flushing, transient fever, and shivering. However, these symptoms resolved in all patients spontaneously or with minimal supportive treatment between 1 and 24 hours after MSC administration. Six studies reported no adverse events associated with MSC infusion. None of the studies reported severe adverse events associated with MSC infusion.

Risk of bias (RoB), Publication bias and Study quality: Risk of bias (RoB) was assessed for the outcomes of mortality and IL-6 levels in RCTs. Regarding mortality, one study (43) was found to have low risk of bias while the other RCT (41) had a risk of bias of 'some concerns' (Table S1) as the method of randomization was unclear and it was unclear whether there were deviations from intended interventions or selection of reported results. Regarding changes in IL-6 levels, one RCT (43) had a low risk of bias while the other RCT (41) had "some concerns" regarding potential risk of bias (Table S2) as the method of randomization was unclear and it was unclear whether there were deviations from intended interventions, missing outcome data or selective reporting of results. For non-randomized studies (40,42), both were found to have a moderate risk of bias (Table S3). Both studies had potential bias due to confounding, measurement of outcomes (as studies did not mention blinding), and selection of reported results (as neither study pre-registered their protocol). Of the included case-series, three were found to be of good quality (45,47,48) and two of fair quality (44,46) (Table S4). Two of the case series did not present characterisation of their MSCs (44,46).

3.5 Discussion

Our systematic review and meta-analysis of clinical studies examining the use of MSCs and/or their secretome as a therapeutic intervention for COVID-19 demonstrated a positive therapeutic effect with minimal safety concerns, though the number of studies was limited. Meta-analysis revealed that MSCs decreased the risk of death and we noted a decrease in IL-6 levels at study endpoint compared to control groups. Although we could not perform meta-analysis of other secondary outcomes due to inconsistent reporting, MSCs also appeared promising with regard to decreasing pro-inflammatory cytokines and immune cells, increasing anti-inflammatory cytokines and immune cells, improving respiratory function and the oxygenation index, correcting abnormal radiological findings, ameliorating clinical symptoms and reducing time in hospital, time in ICU, and time on mechanical ventilation. The strength of our conclusions, however, is limited by the small sample size and by the limited number of studies in this first edition of our living systematic review. We also detected potential reporting bias in all studies examined in our review. Reporting of common outcomes at uniform time points across studies, and use of MSC-based products characterized according to published minimal criteria from the ISCT (35) and MISEV (36) are key issues that must be addressed to overcome some of these observed differences between studies and will likely be necessary to accelerate translation of MSC-based therapeutics to mainstream clinical use. This could be facilitated through the use of a master protocol.

Our primary analysis was mortality given its clear relevance to lessening the paralytic and global impact of the pandemic. In a recently reported systematic review examining the efficacy of MSCs for ARDS, overall mortality appeared reduced, although not statistically different in the MSC group compared to the control group (RR: 0.63 [0.21-1.93], 95% CI,

p=0.064, I2=35.8%). (49). While some of the patients in this review had COVID-19 induced ARDS, other patients had a broad range of underlying causes for ARDS (e.g. pneumonia due to H7N9). The most promising study in terms of mortality reduction in this previous analysis was the single COVID-19 study that was also included in our review. Given the favorable reduction in mortality observed in our meta-analysis, it is possible that MSCs are particularly well suited to treat ARDS caused by COVID-19. Although we were unable to perform subgroup analysis to examine the difference in mortality reduction between mild/moderate COVID-19 patients (not experiencing ARDS) and severe/critical COVID-19 patients (experiencing ARDS) in this edition of our living systematic review, we plan on performing a detailed analysis of this nature in future iterations of our review.

IL-6 levels have received significant attention as a mediator of damaging inflammation in COVID-19, particularly as part of the cytokine storm in the pathogenesis of severe and critical cases (50). Antagonists of IL-6 receptors such as tocilizumab have been investigated as treatment but have not yielded mortality benefits in studies reported so far ([51], [52], [53]). Moreover, IL-6 has been associated with prognostic significance for COVID-19 (54). Our analysis identified that MSCs lowered IL-6 levels. Whether lowering IL-6 levels contributed directly to the observed mortality benefits remains unclear. Other mediators of inflammation were also variably reported and were lowered by MSC treatment in several studies in our review, including TNF-a, IFN-γ and IL-12. Additionally, a number of studies reported that MSC administration supported the production of anti-inflammatory cytokines such as IL-10, TGF-β, and PGE-2. Production of these anti-inflammatory cytokines by MSCs may further antagonize the pathogenic effects of pro-inflammatory cytokines (55). Furthermore, MSCs and their secreted factors may also support the regeneration of tissues damaged by the cytokine storm through release of growth factors

including HGF, KGF and VEGF (56,57). Thus, the pleiotropic anti-inflammatory and regenerative effects of MSCs may point to several potential mechanisms by which MSCs exert their apparent beneficial effect.

Although MSCs likely ameliorate COVID-19 through immunomodulation, MSCs may have modest constitutive immune modulating properties ([58], [59], [60]). MSCs primed through exposure to pro-inflammatory mediators such as IFN-γ, TNF-α, and IL-1β have more potent immune modulating activity and secrete higher levels of soluble anti-inflammatory factors such as IDO and PGE2 (19). In patients with severe or critical COVID-19, high levels of pro-inflammatory cytokines in patients at the time of MSC treatment may induce a greater immune modulatory phenotype of administered MSCs, even without prior ex vivo priming (4,61) and in patients with mild or moderate COVID-19, induction of an immune modulatory phenotype in MSCs may be less complete (61). The role of ex vivo priming of MSCs in the treatment of COVID-19 was not identified in studies included in the first iteration of our systematic review and may be worth pursuing in future trials.

Only one study in our review examined the use of the MSC "exosomes" that are part of the secretome rather than MSCs themselves (44). Exosomes, also referred as small extracellular vesicles, are secreted from MSCs and range from 30-150 nm in size and are formed in multivesicular bodies (MVBs) within MSCs (62). Studies have demonstrated that the therapeutic mechanisms of MSCs are largely mediated through the release of paracrine factors like MSC-EVs (63). MSC-EVs may also be amenable to a broader range of delivery methods, such as aerosol inhalation, which may be particularly relevant in the context of COVID-19 (64). Although we were unable to perform subgroup analysis to compare the efficacy of MSC-EVs to

their parent MSCs in this first edition of our living systematic review, we anticipate that an analysis of this nature will be possible in future updates.

All the studies in our review used third party allogeneic MSCs. One of the reasons why allogeneic MSC therapy is favoured over autologous MSC therapy is that third party allogeneic MSCs can be used in an "off the shelf" manner when needed (65). In contrast, autologous MSC therapy may introduce marked delays in treatment given the time and resources required to manufacture small batches of personalized autologous MSC products (66,67). Furthermore, autologous MSCs from patients with advanced age or underlying health conditions have diminished therapeutic efficacy compared to allogeneic MSCs isolated from healthy donors (68,69,70).

The importance of tissue source for expanding MSCs has been addressed in previous reports of MSC treatment (71). Most studies comparing the immunomodulatory properties of MSCs from different tissues sources have been performed in vitro. One study demonstrated that MSCs derived from adipose tissue (AT-MSCs) displayed superior inhibitory effects towards Th1 CD4+T cells, CD8+ T cells and NK cells compared to UC-MSCs and BM-MSCs (72). Moreover, UC-MSCs showed no inhibitory effects on B cells. Another study demonstrated that AT-MSCs have more potent immunomodulatory properties and exhibit greater IDO production compared to BM-MSCs (73). For the treatment of COVID-19 it may be important to select tissue sources that yield MSCs with reduced expression or lack of the ACE2 receptor (74,75). This could allow MSCs to persist longer following administration in patients with COVID-19. In our review, there were an insufficient number of studies identified to perform subgroup analysis based on MSC tissue source. As more studies reach completion, future subgroup analyses of this nature may be possible.

Our study has limitations worthy of mention. The number of studies and patients included in this first iteration of our review remains small. This modest number of studies and patients limits the confidence in the observed effects. Although this initial number of published studies identified in our search is relatively small, many registered clinical trials dealing with the use of MSC based products as a therapeutic intervention for COVID-19 were identified in a scoping review performed by our group (31). Additionally, only two studies reported sufficient information that allowed us to confirm that MSC products met the minimal ISCT (35) or MISEV (36) criteria for characterization. Use of MSCs that vary in terms of product characterization may influence the observed effects and limits the ability to pool results from multiple studies. Significant heterogeneity was observed between studies in terms of outcome reporting. Mortality and levels of pro-inflammatory markers were the only outcomes reported by all nine studies included in our review. Inconsistent outcome reporting reduces the number of outcomes that can be combined in meta-analysis and limits interpretation of results. None of the studies in our review examined the use of MSCs along with other COVID-19 therapeutics. Administering MSCs along with other COVID-19 therapeutics may augment their beneficial effects. Potential reporting bias was also observed in all studies included in our review. Indeed, a framework for inclusion of studies that meet robust quality criteria could facilitate earlier regulatory review of MSC-derived products based on data from meta-analysis. Regulatory review for emergency use of new treatments retains more flexibility for approvals in many jurisdictions and this mechanism of approval could be appropriate for MSCs given the challenges of conducting large studies. A framework for inclusion of high-quality studies is provided that could accelerate future regulatory reviews (see Table 6).

Our systematic review and meta-analysis suggests that MSCs are a promising treatment for COVID-19, though the certainty of this effect is limited due to the small number of studies and modest numbers of patients enrolled, as well as substantial heterogeneity between studies in terms of study design, characterization of MSC products, and outcome reporting. Future studies should consider our proposed framework for the inclusion of high-quality studies in future iterations of this meta-analysis to improve the consistency of outcome reporting and reducing heterogeneity to refine our estimates of potential benefits and safety of MSCs to treat COVID-19. Demonstrating benefit of MSCs to treat COVID-19 using our proposed framework for identifying the highest quality evidence should accelerate regulatory approval of MSC-based therapies. With continued reporting of modest sized studies, we expect meta-analysis will remain critical for a timely understanding of the potential benefit of MSCs to treat COVID-19.

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3.7 Figures and Tables

Box 1. Cellular mechanisms implicated in MSC-based immune modulation.

Cell process or target	Description	Reference
Migration	MSCs migrate in response to inflammatory mediators (cytokines and chemokines) and chemotactic gradients (growth factors) produced by infection and/or tissue damage. MSC effects are mediated by release of soluble factors or via direct cell-cell contact.	(18)
Macrophage repolarization	MSCs induce polarization of M1 macrophages (pro-inflammatory) to M2 macrophages (anti-inflammatory) through secretion of IDO and PGE2	(19)
Dendritic cell (DC) inhibition	MSCs reduce pro-inflammatory cytokine release, decrease antigen presentation capabilities, and suppress differentiation and maturation of DCs through secretion of PGE2 and IL-10.	(19)
Natural Killer (NK) cell regulation	MSCs inhibit IFN-g secretion and cytotoxic capabilities of NK cells through secretion of TGF-b, PGE2, IDO, IL-10 and HGF. MSCs may promote the development of CD73+ regulatory NK cells	(19)
Neutrophil recruitment	MSCs suppress of NO secretion, inhibit respiratory bursts and decrease recruitment and infiltration of neutrophils through secretion of IL-2, IL-4, IL-10 CXCL2 and CXCR2	(18)
B cell proliferation & regulation	MSCs inhibit B cell proliferation by blocking the G0 and G1 phases of the cell cycle. MSCs also increase frequency and activity of regulatory B cells through secretion of IL-10, TGF-b and IDO.	(19)
T cell regulation	MSCs induce polarization of Th1 CD4+ T cells (pro-inflammatory) to Th2 (anti-inflammatory). MSCs may also reduce activation, proliferation and differentiation of CD4+ pro-inflammatory Th1, Th17 and CD8+ T cells through secretion of TGF-β1 and HGF. MSCs also reduce infiltration of CD3+ T cells into the injured tissues by up-regulating Foxp3+ regulatory T cells. MSCs may also induce T cell apoptosis.	(18,19)
Cell signaling	MSCs may down-regulate the STAT3 signaling pathway through secretion of IL-17A. MSCS may suppress NF-kB activation through secretion of NRF and IGFBP-3	(21,22)

IDO, indolamine 2,3-dioxygenase; PGE2, prostaglandin E2; IL, interleukin; IFN-γ, interferon gamma; TGF-β, transforming growth factor beta; HGF, human growth hormone; NO, nitric oxide; CXCL2, C-X-C chemokine ligand 2; CXCR2, C-X-C receptor 2; Th, T helper type; STAT3, signal transducer and activator of transcription 3; NF-kB, nuclear factor kappa-light-chain enhancer of activated B cells; NRF, nuclear receptor factor; IGFBP-3, insulin-like growth factor binding protein 3

Figure 1. Results of systematic search of the literature. MEDLINE and Embase and Cochrane Central Register of Controlled Trials, searched from 1947 up to February 3rd, 2021.

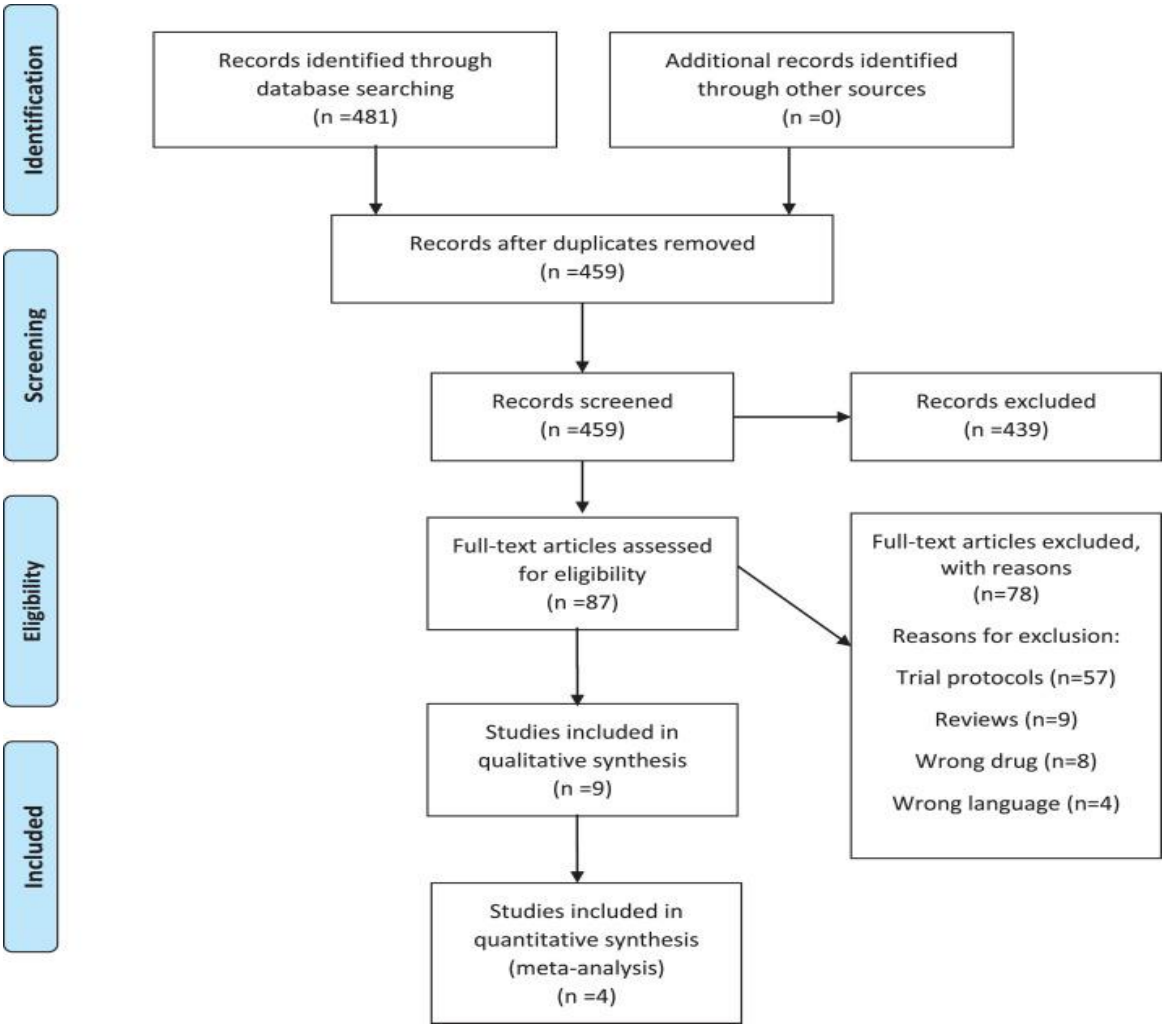

Table 1. Characteristics of patients enrolled in clinical studies of MSCs as a therapeutic intervention for COVID-19.

		Controlled studies (n=4)	
Patient characteristics	**All studies (n=9)**	**Control groups**	**MSC groups**
Total patients, n	189	53	40
Sex, %male	65.6	52.8	60.0
Mean age, years (SD)	58.3 (6.3)	57.9 (6.4)	55.5 (7.1)
Covid-19 severity, n (%)			
Mild	9 (4.8)	3 (5.7)	5 (12.5)
Moderate	30 (15.9)	5 (9.4)	5 (12.5)
Severe	123 (65.1)	45(84.9)	29 (72.5)
Critical	27 (14.3)	0 (0.0)	1 (2.5)
Co-morbidities, n (%)			
Hypertension	71 (37.6)	16 (30.2)	13 (32.5)
Diabetes	56 (29.6)	11 (20.8)	9 (22.5)
Obesity	16 (8.5)	5 (9.4)	11 (27.5)
Chronic obstructive pulmonary disease	8 (4.2)	0 (0.0)	0 (0.0)
Coronary artery disease	9 (4.7)	3 (5.7)	1 (2.5)
Hyperlipidemia	5 (2.6)	0 (0.0)	0 (0.0)
Chronic kidney failure	3 (1.6)	0 (0.0)	0 (0.0)
*Other**	20 (10.6)	3 (5.7)	0 (0.0)
Mean follow up, days (range)	22 (14-60)	21 (14-28)	21 (14-28)

SD, standard deviation
*includes ex-smoker, pre-diabetes, asthma

Table 2. Intervention characteristics for clinical studies of patients administered MSCs as a therapeutic intervention for COVID-19

Intervention	Total Studies, n	Controlled studies, n
MSC product		
MSCs	8	4
MSC-EVs ("exosomes")	1	0
Donor type		
Allogeneic	9	4
Autologous	0	0
MSC tissue source		
Umbilical cord / placenta	6	3
Adipose	1	0
Bone marrow	1	0
Not described	1	1
Product dose		
MSCs, cells/kg (n, studies)	1 - 2 x 10^6 (4)	1 - 2 x 10^6 (2)
MSCs, total cells(n, studies)	0.3 - 2.0 x 10^8 (4)	0.3-1.0 x 10^8 (2)
MSC-EVs, mL (ExoFlo™)(n, studies)	15 (1)	NA
No. MSC infusions, patients (%)		
1	57 (41.9)	19 (47.5)
2	31(22.2)	12 (30.0)
3	32 (23.5)	9 (22.5)
4	16 (11.8)	0 (0.0)
ISCT criteria		
fully met criteria (A-C below), n	2	1
(A) Plastic adherence	2	1
(B) Trilineage differentiation	3	2
(C) Positive/negative surface markers	5	3

EV – extracellular vesicles; NA – not applicable; ISCT – International Society of Cellular Therapy

Table 3. List of reported outcomes in clinical studies examining MSCs as a therapeutic intervention for COVID-19. Controlled studies are highlighted in grey. Outcomes reported in each study are indicated by (•) and outcomes not reported are indicated by (-).

Study	Mortality rate	Diagnosis to intervention (time)	Intervention to recovery (time)	No. pts hospitalized	No. pts on supplemental O_2	No. pts on ventilator	Time in hospital	Progression of symptoms	Improvement of symptoms	Time to clinical improvement	Oxygenation levels	Immune cell levels	Pro-inflammatory cytokines	Anti-inflammatory cytokine s	Viral load	Radiological outcomes
(40)	•	•	•	•	-	-	•	•	•	•	•	•	•	•	-	•
(41)	•	•	-	•	•	•	•	•	•	•	•	•	•	-	-	•
(42)	•	•	•	•	-	•	•	•	-	•	•	-	•	-	•	•
(43)	•	•	•	-	•	•	-	•	-	-	-	-	•	•	•	-
(44)	•	•	•	•	-	-	•	•	-	-	•	•	•		-	-
(45)	•	-	-	-	-	-	-	-	-	-	•	•	•	•	•	•
(46)	•	•	•	•	-	-	-	-	-	-	•	•	•	-	•	-
(47)	•	•	•	•	•	•	-	•	•	•	-	•	•	-	-	•
(48)	•	•	•	-	•	•	•	•	•	•	•	•	•	•	-	•
Total	9	8	7	6	4	5	5	7	4	5	7	7	9	4	4	6

Pts – Patients

Figure 2. Forest plot demonstrating decreased risk of death at study endpoint in patients administered MSCs compared to control patients. Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

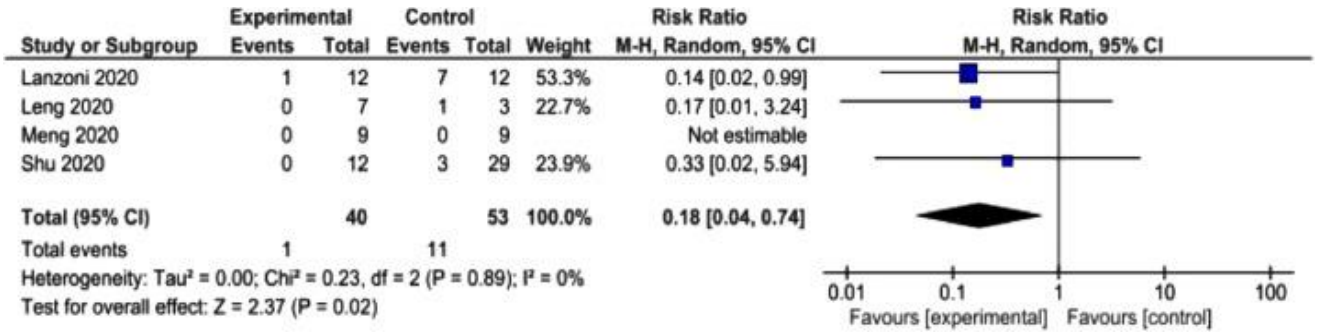

Figure 3. Forest plot demonstrating standardized mean difference in interleukin-6 (IL-6) levels between experimental (MSC) and control groups at study endpoint.

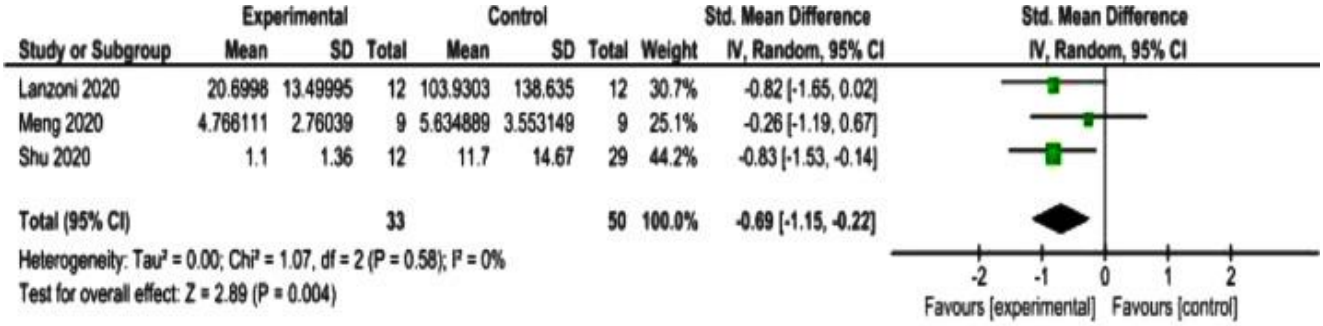

Table 4. Adverse events (AEs) and severe adverse events (SAEs) reported in clinical studies examining MSCs as a therapeutic intervention for COVID-19. Controlled studies are highlighted in grey.

Study	Safety lab values	Treatment related AEs	Non-treatment related AEs	Treatment related SAEs	Non-treatment related SAEs
(40)	-	-	-	-	-
(41)	-	-	-	-	-
(42)	●	●	-	-	●
(43)	-	●	●	-	●
(44)	-	-	-	-	●
(45)	-	-	-	-	●
(46)	-	-	-	-	-
(47)	●	-	-	-	-
(48)	●	●	-	-	-
Total	3	3	1	0	4

Table 5. Concomitant therapies reported in studies. Controlled studies are highlighted in grey.

Study (ref)	Antiviral agents	Antibiotic agents	Glucocorticoids	Transfusion based interventions
(40)	None	None	None	None
(41)	Abidor/Oseltamivir	Moxifloxacin	Systemic glucocorticoids	None
(42)	Lopinavir/Ritonavir	None	Glucocorticoids	None
(43)	"Best standard of care"	"Best standard of care"	"Best standard of care"	"Best standard of care"
(44)	None	Hydroxychloroquine, Azithromycin	None	None
(45)	"Concomitant medication"	"Concomitant medication"	"Concomitant medication"	"Concomitant medication"
(46)	Umifenovir, Interferon alfa-2b, Oseltamivir	Chloroquine	Methylprednisolone	Intravenous immunoglobulin, Intravenous albumin
(47)	"Supportive therapy at discretion of clinician"	"Supportive therapy at discretion of clinician"	"Supportive therapy at discretion of clinician"	"Supportive therapy at discretion of clinician"
(48)	Lopinavir/Ritonavir, Ribavirin, Favipiravir, Oseltamivir,	Hydroxychloroquine, Azithromycin, Meropenem, Vancomycin, Imipenem, Colistin	None	Intravenous immunoglobulin

Table 6. Recommended criteria for performing meta-analysis for purposes of potential regulatory approval of MSC-based therapy for COVID-19.

Number of studies	• Sufficient number and similar enough to perform meta-analysis that achieves the required power for determining efficacy. See sample size.
Study characteristics	• Controlled with contemporary and similar control groups. Randomized is preferable. Concomitant therapies should be controlled.
Sample size	• To reduce mortality from 10% to 5%, a total sample of size of 686 in the intervention group is needed (24)
Study populations	• Severe or critical COVID-19 in hospitalized patients is most commonly reported.
Outcome measurement	• Mortality at day 28 is most commonly reported. • WHO response criteria recommended but not commonly reported. • Secondary: IL6 levels, functional status, hospitalization, ICU admission, pulmonary function at 1, 6, 12 months. • Safety and adverse event reporting in accordance with best practices.
Product characterization	• MSCs produced and characterized according to GMP practices and ISCT criteria. • MSC-EVs characterized in accordance with MISEV criteria.
Risk of Bias	• Studies with high risk of potential bias should not be included in meta-analysis.

3.8 Appendix

Table S1. Risk of bias of randomized studies according to the Cochrane Risk of Bias tool 2.0 for risk of death at study endpoint

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Lanzoni 2020	+	+	+	+	+	+
Shu 2020	~	~	+	+	~	~

Legend: +=Low risk of bias, ~=Some concerns, -=High risk of bias

Table S2. Risk of bias of randomized studies according to the Cochrane Risk of Bias tool 2.0 for IL-6 levels at study endpoint

Bias						
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Lanzoni 2020	+	+	+	+	+	+
Shu 2020	~	~	~	+	~	~

Legend: +=Low risk of bias, ~=Some concerns, -=High risk of bias

Table S3. Risk of bias of non-randomized studies according to the ROBINS-I tool.

Study	**Bias due to confounding**	**Bias in the selection of participants into the study**	**Bias in the classification of interventions**	**Bias due to deviations from intended interventions**	**Bias due to missing data**	**Bias in measurement of the outcomes**	**Bias in selection of the reported result**	**Overall Risk of Bias**
Leng 2020	MR	LR	LR	LR	LR	MR	MR	MR
Meng 2020	MR	LR	LR	LR	LR	MR	MR	MR

Legend: LR=Low risk of bias, MR=Moderate risk of bias, SR=Serious risk of bias, CR=Critical risk of bias

Table S4. Risk of bias of case-series according to EBM tool.

Domain	Feng 2020	Guo 2020	Hameshian 2020	Sánchez-Guijo 2020	Sengupta 2020
1. Does the patient(s) represent(s) the whole experience of the investigator (centre) or is the selection method unclear to the extent that other patients with similar presentation may not have been reported?	Yes	Yes	Yes	Yes	Yes
2. Was the exposure adequately ascertained?	Yes	Yes	Yes	Yes	Yes
3. Was the outcome adequately ascertained?	Yes	Yes	Yes	Yes	Yes
4. Were other alternative causes that may explain the observation ruled out?	Attempted	Unclear	Attempted	Unclear	Unclear
5. Was there a challenge/rechallenge phenomenon?	N/A	N/A	N/A	N/A	N/A
6. Was there a dose–response effect?	N/A	N/A	N/A	Unclear due to low # of patients	N/A
7. Was follow-up long enough for outcomes to occur?	Yes	Yes	Yes	Yes	Yes
8. Is the case(s) described with sufficient details to allow other investigators to replicate the research or to allow practitioners make inferences related to their own practice?	Yes	No. MSC characterisation missing.	Yes	Yes	No. MSC characterisation missing.

Figure S1. Systematic Literature Search Strategy

Database: Embase Classic+Embase <1947 to 2021 February 03>, Ovid MEDLINE(R) ALL <1946 to February 03, 2021>, EBM Reviews - Cochrane Central Register of Controlled Trials <December 2020>
Search Strategy:
---
1 COVID-19/ (17886)
2 (exp coronavirus/ or coronavirus*.mp. or corona virus*.mp.) and (wuhan or beijing or shanghai).mp. (9261)
3 ((coronavirus or corona virus) adj3 "2019").tw. (41532)
4 (covid or covid2019).tw,kf. (175983)
5 covid19.tw,kw. or covid 19.kf. (42778)
6 sars cov 2.tw,kw. (62711)
7 (ncov or n cov).tw,kw. (3072)
8 novel coronavirus.tw,kw. (14551)
9 sars cov2.tw,kw. (3114)
10 Coronavirus Infections/ and Pandemics/ (39146)
11 (ncov19 or ncov-19 or 2019-novel CoV).tw,kf. (119)
12 or/1-11 (198342)
13 Mesenchymal Stromal Cells/ (49672)
14 Mesenchymal Stem Cell Transplantation/ (24831)
15 Multipotent Stem Cells/ (8889)
16 (MSC or MSCs or ADMSC or ADMSCs or BM-MSC or BM-MSCs or BMD-MSC or BMD-MSCs or BMDMSC or BMDMSCs).tw,kw. (88902)
17 (mesenchymal adj5 (cell* or stem or stromal or progenitor* or multipotent or bone marrow or adipose or placenta*)).tw. (199459)
18 (mesenchymal and (cell* or stem or stromal or progenitor or multipotent or bone marrow or adipose or placenta*)).kw. (33543)
19 ((multipotent or multi-potent) adj (stroma* cell* or stem cell*)).tw,kf. (4692)
20 (msc ev or msc cm).tw,kf. (1071)
21 (colony-forming adj2 fibroblast*).tw. (1675)
22 marrow stroma* cell*.tw. (17531)
23 Mesoderm/cy (5853)
24 Exosomes/ or Extracellular Vesicles/ (42505)
25 exosom*.tw,kw. (41425)
26 Cell-Derived Microparticles/ (8876)
27 (microvesicle* or micro vesicle*).tw,kw. (11432)
28 ((cell or cells or extracellular) adj2 vesicle*).tw. (29239)
29 microparticle*.tw,kw. (42112)
30 (extracellular and vesicle*).kf. (5278)
31 ((cell or cells) and vesicle*).kf. (2195)
32 or/13-31 (354252)
33 12 and 32 (743)
34 33 use medall (306) Medline

35 (Coronavirinae/ or coronavirus*.mp. or corona virus*.mp.) and (wuhan or beijing or shanghai or hubei).mp. (9894)
36 ((coronavirus* or corona virus* or coronavirus* or coronaviridae or coronaviridae or betacoronavirus*) adj3 ("19" or "2019")).tw. (52354)
37 (covid or covid19).tw. (173537)
38 sars cov 2.tw. (53939)
39 (ncov or n cov).tw. (2696)
40 (novel coronavirus* or novel corona virus*).tw. (14310)
41 (CoV 2 or CoV2 or sarscov2 or 2019nCoV or novel CoV or wuhan virus).tw. (56040)
42 (coronavirus infection/ or severe acute respiratory syndrome/) and (pandemic/ or pandemic*.tw.) (51355)

43 limit 42 to yr="2019 -Current" (50254)
44 35 or 36 or 37 or 38 or 39 or 40 or 41 or 43 (197531)
45 exp mesenchymal stem cell/ (102855)
46 exp mesenchymal stem cell transplantation/ (24831)
47 exp mesenchymal stroma cell/ (14237)
48 (MSC or MSCs or ADMSC or ADMSCs or BM-MSC or BM-MSCs or BMD-MSC or BMD-MSCs or BMDMSC or BMDMSCs or BMSC or BMSCs or HBMSC or HBMSCs or ABMSC or ABMSCs or MAPC or MAPCs).tw. (106309)
49 (((multipotent or multi-potent) adj3 (stem or stroma$1 or progenitor*)) and (cell$1 or cell-induced)).tw. (16261)
50 multipotent stem cell/ (9199)
51 (mesenchymal adj5 (cell* or stem or stroma* or progenitor* or multipotent or bone marrow or adipose or placenta*)).tw. (199944)
52 (colony-forming adj2 fibroblast*).tw. (1675)
53 (marrow stroma* adj2 cell*).tw. (18363)
54 exosome/ (31880)
55 exosom*.tw. (37895)
56 (microvesicle* or micro vesicle*).tw. (10182)
57 microparticle*.tw. (40494)
58 ((cell or cells or extracellular) adj2 vesicle*).tw. (29239)
59 exp membrane microparticle/ (8167)
60 (msc ev or msc cm).tw. (1070)
61 or/45-60 (359808)
62 44 and 61 (735)
63 62 use emczd (370) Embase
64 COVID-19/ (17886)
65 (exp coronavirus/ or coronavirus*.mp. or corona virus*.mp.) and (wuhan or beijing or shanghai).mp. (9261)
66 ((coronavirus or corona virus) adj3 "2019").tw. (41532)
67 (covid or covid2019).tw,kw. (175722)
68 covid19.tw,kw. or covid 19.kw. (75538)
69 sars cov 2.tw,kw. (62711)
70 (ncov or n cov).tw,kw. (3072)
71 novel coronavirus.tw,kw. (14551)
72 sars cov2.tw,kw. (3114)
73 Coronavirus Infections/ and Pandemics/ (39146)
74 (ncov19 or ncov-19 or 2019-novel CoV).tw,kw. (125)
75 or/64-74 (199082)
76 Mesenchymal Stromal Cells/ (49672)
77 Mesenchymal Stem Cell Transplantation/ (24831)
78 Multipotent Stem Cells/ (8889)
79 (MSC or MSCs or ADMSC or ADMSCs or BM-MSC or BM-MSCs or BMD-MSC or BMD-MSCs or BMDMSC or BMDMSCs).tw,kw. (88902)
80 (mesenchymal adj5 (cell* or stem or stromal or progenitor* or multipotent or bone marrow or adipose or placenta*)).tw. (199459)
81 (mesenchymal and (cell* or stem or stromal or progenitor or multipotent or bone marrow or adipose or placenta*)).kw. (33543)
82 ((multipotent or multi-potent) adj (stroma* cell* or stem cell*)).tw,kw. (4749)
83 (msc ev or msc cm).tw,kw. (1070)
84 (colony-forming adj2 fibroblast*).tw. (1675)
85 marrow stroma* cell*.tw. (17531)
86 Mesoderm/cy (5853)
87 Exosomes/ or Extracellular Vesicles/ (42505)
88 exosom*.tw,kw. (41425)
89 Cell-Derived Microparticles/ (8876)
90 (microvesicle* or micro vesicle*).tw,kw. (11432)

91 ((cell or cells or extracellular) adj2 vesicle*).tw. (29239)
92 microparticle*.tw,kw. (42112)
93 (extracellular and vesicle*).kw. (5106)
94 ((cell or cells) and vesicle*).kw. (2780)
95 or/76-94 (354775)
96 75 and 95 (739)
97 96 use cctr (79) Cochrane
98 34 or 63 or 97 (755)
99 remove duplicates from 98 (487)
100 99 use medall (295)
101 99 use emczd (115)
102 99 use cctr (77)

Table S5. PRISMA Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	5-6
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	6
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	7-8
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	7
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	7, Figure S1
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	9
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	9-10
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	8-9
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear	8-9

Section and Topic	Item #	Checklist item	Location where item is reported
		information.	
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	10
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	10
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	10
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	10
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	10
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	10
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	10-11
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	11
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	NA
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	12, Figure 1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	12
Study characteristics	17	Cite each included study and present its characteristics.	12
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	17-18, Tables S1, S2, S3, S4
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	12-17, Tables 1, 2, 3, 4, 5
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	12-18, Tables 1, 2, 3, 4, 5, S1, S2, S3
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the	14, 15, Figures 2, 3

Section and Topic	Item #	Checklist item	Location where item is reported
		effect.	
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	NA
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	NA
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	NA
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	NA
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	19-22
	23b	Discuss any limitations of the evidence included in the review.	22-23
	23c	Discuss any limitations of the review processes used.	22-23
	23d	Discuss implications of the results for practice, policy, and future research.	23-24, Table 6
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	7
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	7
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	NA
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	2
Competing interests	26	Declare any competing interests of review authors.	2
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	2

4. UPDATED LIVING SYSTEMATIC REVIEW AND META-ANALYSIS OF CONTROLLED TRIALS OF MESENCHYMAL STROMAL CELLS TO TREAT COVID-19: A FRAMEWORK FOR ACCELERATED SYNTHESIS OF TRIAL EVIDENCE FOR RAPID APPROVAL – FASTER APPROVAL

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Kirkham AM, Bailey AJM, Monaghan M, Shorr R, Lalu MM, Fergusson DA, Allan DS. Updated living systematic review and meta-analysis of controlled trials of mesenchymal stromal cells to treat COVID-19: A framework for accelerated synthesis of trial evidence for rapid approval – *FASTER Approval*

4.1 Abstract

Background: Mesenchymal stromal cells (MSCs) may reduce mortality in patients with COVID-19, however, early evidence is based on few studies with marked interstudy heterogeneity. A second iteration of our living systematic review and meta-analysis evaluates a framework needed for synthesizing evidence from high quality studies to accelerate consideration for approval.

Methods: A systematic search of the literature was conducted to November 15th, 2021 to identify all English-language, full-text, controlled clinical studies examining MSCs to treat COVID-19. (PROSPERO: CRD42021225431).

Findings: Eleven studies were identified (403 patients with severe and/or critical COVID-19, including 207 given MSCs and 196 controls). All eleven studies reported mortality and were pooled through random effects meta-analysis. MSCs decreased relative risk of death at study endpoint (RR: 0.50 [0.34 to 0.75, 95% CI]) and RR of death at 28 days after treatment (0.19 [0.05 to 0.78, 95% CI]) compared to controls. MSCs also decreased length of hospital stay (mean difference (MD: -3.97 days [-6.09 to -1.85, 95% CI], n=5 studies) and increased oxygenation levels at study endpoint compared to controls (MD: 105.62 mmHg O2 [73.9 to 137.3, 95% CI], n=3 studies). Only 2 of 11 studies reported on all International Society for Cellular Therapy (ISCT) criteria for MSC characterization. Included randomized controlled trials were found to have 'some concerns' (n=2) to low (n=4) risk of bias (RoB), while all non-randomized studies were found to have moderate (n=5) RoB.

Interpretation: Our updated living systematic review concludes that MSCs can likely reduce mortality in patients with severe or critical COVID-19. A master protocol based on our Faster Approval framework appears necessary to facilitate more accelerated accumulation of high-

quality evidence that would reduce RoB, improve consistency in product characterization and standardize outcome reporting.

Funding: Faculty of Medicine, University of Ottawa and Canadian Blood Services.

4.2 Introduction

Since its initial emergence in December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19), has killed millions of people and infected many hundreds of millions more (1). Despite intensive measures implemented to contain its spread, SARS-CoV-2 continues to infect people across the globe at an unprecedented rate (2,3). Although safe and effective vaccines have been developed and administered to billions of people (4-6), many areas are struggling to vaccinate enough of their population (7, 8). Furthermore, COVID-19 vaccines display reduced efficacy in certain patient populations such as those with autoimmune conditions, blood cancers, or transplant recipients (9, 10). Moreover, SARS-CoV-2 variants of concern and potential future variants pose a continued risk to disease control (11, 12). Taken together, there remains a continued urgent need for safe and effective therapies to reduce the morbidity and mortality associated with COVID-19.

From the onset of the pandemic, mesenchymal stromal cells (MSCs) were quickly viewed as a promising therapy to treat COVID-19 due to their proven immunomodulatory and regenerative capabilities across a wide variety of related diseases and conditions (13, 14). Many clinical trials of MSC based therapy have been registered (15) but remain underpowered to determine safety and efficacy on their own due to small sample size and there is considerable

heterogeneity in terms of study design, intervention characteristics and planned primary and secondary outcomes.

To determine the preliminary safety and efficacy of MSCs in the context of COVID-19, we performed an initial systematic review and meta-analysis examining all clinical studies (controlled and uncontrolled) examining the use of MSCs for COVID-19 (16). Our initial search identified nine studies (4 controlled), and meta-analysis demonstrated that MSCs significantly decreased the risk of death at study endpoint compared to controls. Moreover, MSCs were found to be safe, with no severe adverse events reported in any of the patients. However, this initial analysis had several limitations including a small number of controlled studies, heterogeneity in study design and intervention characteristics, potential ROB and limited reporting of MSC product characterization in accordance with criteria from the International Society of Cellular Therapy (ISCT) (17). We proposed a framework for the selection of high-quality studies that would yield a more robust evidence base that could be used in support of requests for regulatory approval of MSC products and that could accelerate more widespread use and availability of MSCs to treat COVID-19. This update to our living systematic review leverages our proposed framework for the accelerated synthesis of trial evidence for rapid approval – or FASTER Approval – that will provide more clear guidance on the extent to which MSC-based therapy could be considered for approval to treat COVID-19. Moreover, we identify potential elements of a master protocol that would ensure the rapid generation of high-quality evidence to support the timely launch of future informative trials of MSC-based applications.

4.3 Methods

This systematic review is reported in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) guidelines (18) (Table S3). The study

protocol has been published in *Systematic Reviews* (19) and is registered at the International Prospective Registry of Systematic Reviews (PROSPERO; CRD42021225431).

Literature Search Strategy:

Our literature search strategy was performed as described previously (16) and was updated to November 15, 2021 (from 1947 to November 15, 2021) (Figure S20). In this second iteration, we only included controlled clinical studies examining the use of MSCs as a therapeutic intervention for COVID-19.

Eligibility Criteria:

Our eligibility criteria were the same as described previously, except we only included controlled clinical studies. Uncontrolled clinical studies, case series, reviews, commentaries, editorials, letters, case reports, conference abstracts, unpublished gray literature and other study types (*in vitro* studies, preclinical animal studies, etc.) were excluded.

Outcomes:

Mortality at study endpoint and at 28 days were our primary outcomes for analysis. Our secondary outcomes for analysis included number of patients requiring hospital admission, number of patients requiring intensive care unit (ICU) admission, number of patients requiring mechanical ventilation, length of time in hospital, length of time in ICU, length of time on mechanical ventilation, circulating levels of immune cells, pro-inflammatory cytokines and anti-inflammatory cytokines, and adverse events.

Study selection and Data extraction:

Study selection, data extraction, and data analysis steps were performed as described in our first edition of our living systematic review (16). ROB assessment for randomized controlled trials and non-randomized studies was performed using the ROB 2 (20) and ROBINS I tools (21), respectively.

Data Analysis:

The results from individual studies were pooled for meta-analysis using Review Manager (Version 5.4) Systematic Review Software (https://training.cochrane.org/online-learning/core-software-cochrane-reviews/revman/revman-5-download). For dichotomous outcomes, both Risk Ratios (RRs) and Risk Differences (RDs) between the control and experimental groups were calculated for each outcome. Both RDs and RRs were presented to account for studies that reported zero events in both control and experimental groups. For continuous outcomes, the mean difference (MD) or standardized mean difference (SMD) between control and experimental groups was calculated using random effects meta-analyses. The SMD was applied for analyses of inflammatory markers (IL-6, ferritin, C-RP) as it was anticipated these outcomes would vary substantially according to time of measurement. Moreover, the mean of medians (MoM) and the interquartile range (IQR) were reported along with MD and SMD for continuous outcomes to account for dispersion and help describe the shape of the distribution (normal, right skewed, left skewed, etc.). Pooled analysis was performed using the DerSimonian and Laird random effects model (22). All data is presented with 95% confidence intervals (CIs). Meta-analysis was only performed when two or more controlled studies reported on the same outcome. Outcomes that were reported in less than two controlled studies or where adequate data for inclusion in meta-analysis was not provided were analyzed in a descriptive manner using summary tables. Statistical heterogeneity was assessed using the I^2 statistic. The thresholds for

interpretation of I^2 were: 0–40% (low heterogeneity), 30–60% (moderate heterogeneity), 50–90% (substantial heterogeneity), and 75–100% (considerable heterogeneity) Potential subgroup analyses (MSCs isolated from different tissue sources, MSCs compared to their secretome, MSCs that have been treated and/or genetic modified before administration or isolation of paracrine factors, according to COVID-19 severity, presence of co-morbidities, patient age, patient sex, based on geographic location and by type of funding support for the study) were determined *a priori* in our study protocol (19) and were described in the first edition of our living systematic review (16). Publication bias was assessed using funnel plots for outcomes reported in ten or more studies by plotting the effect measure (RD, SMD, etc.) against the standard error.

Role of the Funding Source

The funding sources had no role in the study design, analysis, interpretation of data, writing of the manuscript, or decision to submit the manuscript for publication.

4.4 Results

Literature search:

Our systematic search of the literature yielded 602 unique citations. After full text screening, eleven studies (23-33) met all criteria for inclusion in our review. Reasons for study exclusion at the full-text stage were related to publication type (n=25), outcomes reported (n=18), non-MSC-based therapy (n=6), did not involve patients with COVID-19 (n=2), uncontrolled study design (n=3) and language other than English (Chinese n=2, Russian n=2) (Figure 1).

Study characteristics:

The characteristics of the eleven included studies are summarized in Table 1. Five of the studies were RCTs (24, 26, 30, 31, 33) and six were non-randomized controlled trials (23, 25, 27, 28, 29, 32). Study publication date ranged from March 9, 2020, to October 26, 2021. Seven of the studies were conducted in China, (23-29), one in the USA (30), one in Indonesia (31), one in Germany (32) and one in Turkey (33).

Participant characteristics:

In total, there were 403 patients (mean age 58 ±7; 229 male) enrolled across all study groups, with 207 patients (mean age 57 ±9; 115 male) being administered MSCs and 196 patients (mean age 60 ± 5; 114 male) serving as controls. The overall distribution of COVID-19 disease severity was similar for patients in the intervention and control groups, with 62% and 23% of all patients having severe and critical disease severity, respectively (Table 1).

In terms of patient co-morbidities, there was a greater proportion of patients with hypertension in the control group compared to the intervention group (31% vs 24%), a greater proportion of obese patients in the MSC group compared to the control group (5% vs 3%), and a greater proportion of patients with other co-morbidities in the control group compared to the MSC group (13% vs 9%). However, all other co-morbidities including diabetes, COPD, coronary artery disease and chronic kidney failure were well balanced between control and intervention groups. (Table 1).

Intervention characteristics:

Intervention characteristics are summarized in Table 2. All eleven studies used MSCs, with no studies using MSC secreted factors (MSC-Extracellular Vesicles (MSC-EVs), MSC-Conditioned Medium (MSC-CM)). All MSCs were derived from allogeneic human tissues,

including umbilical cord blood and/or tissue (n=8) (24-26, 28-31, 33), menstrual blood (n=1) (27) and bone marrow mononuclear cells (n=1) (32). One study did not report the specific tissue source from which its MSCs were derived (23). The passage number in *ex vivo* culture of the MSCs varied widely between studies (passage 3 to passage 6) (see Table 2), with four of the studies (28, 29, 30, 32) not reporting how many passages were performed before harvesting MSCs from *ex vivo* culture. In terms of the extent to which studies reported on specific ISCT criteria (17) for MSC characterization, only two of the eleven studies reported sufficient information regarding all four minimal criteria established by the ISCT (17). Specific details regarding the number of studies reporting on each of the four individual ISCT criteria (17) can be found in Table 2.

MSC dosing and administration were reported differently between studies. The two formats in which MSC dosage were reported included cells/kg of body weight (n=7; 1 - 3 x 10^6 cells/kg) and total cells/injection (n=4; 30 - 100 x 10^6 cells). In four studies, MSCs were cryopreserved prior to administration and in one other study, the MSCs were either fresh or cryopreserved. The remaining studies (n=7, 67%) did not report whether the MSCs they administered were fresh or cryopreserved. All eleven studies administered their MSCs intravenously. Most patients (n=112, 54.1%) received three infusions of MSCs, although other studies reported administering one or two MSC infusions (see Table 2). The reported time from COVID-19 diagnosis to intervention was similar between control groups (median 9.8, range 1 – 47 days) and intervention groups (median 10.4, range 1 –45) in the controlled studies.

Patients were administered other therapeutic agents in addition to MSCs in ten of the eleven studies (91%). The specific therapeutic agents administered to patients varied considerably between studies and is summarized in Table 5. Two of the studies stated that they

used other therapeutics in addition to MSCs but did not specify exactly which therapeutic agents were used (25, 28). The mean follow-up period was 27.7 days (range 14-60), with mean follow-ups of 26.9 (range 14-60) and 28.8 (range 14-60) for the control and experimental groups respectively (Table 1)

Outcome reporting

Outcome reporting across studies was variable, with some outcomes like mortality and pro-inflammatory cytokines being reported in all eleven studies, while other outcomes including progression of clinical symptoms and viral load were reported in less than half of the studies. An overview of outcomes reported across studies is summarized in Table 3.

Primary outcome analysis: Mortality

All eleven studies reported mortality at study endpoint, which ranged from 14 to 60 days. The mortality rate at study endpoint for patients in the control groups was 51 of 196 patients (26%), compared to 18 of 207 patients (8.7%) in the groups administered MSCs. In meta-analysis (n=11 studies), patients administered MSCs had a significant decrease in relative risk of mortality (RR: 0.50 [0.34 to 0.75, 95% CI, p=0.0006, I^2=0%]) (Figure 2a) and absolute risk of death (RD: -0.15 [-0.29 to -0.02, 95% CI, p=0.03, I^2=84%]) at study endpoint compared to controls (Figure 2b). Meta-analysis of studies reporting risk of death at 28 days (n=5 studies) also demonstrated a significant decrease in relative risk of death in patients administered MSCs compared to controls (RR: 0.19 [0.05-0.78, 95% CI, p=0.02, I^2=0%]) (Figure S1a) whereas no significant difference in absolute risk of death at 28 days was observed (RD: -0.09 [-0.23 to 0.04, 95% CI, p=0.19, I^2=82%]) (Figure S1b).

We addressed several potential factors in subgroup analysis to assess their impact on mortality. In all cases, the relative risk of death was reduced to a similar extent compared to controls (See Figures S2-S6). Specifically, we examined differences in the relative risk of death in subgroups of studies where the study endpoint was <28 or ≥ 28d, whether patients had ARDS or did not have ARDS, whether studies included patients with any severity of disease or just severe or critical disease, and whether cord blood tissue versus other cell sources were used to generate and expand MSCs, and whether a single infusion of MSCs compared to multiple infusions were administered.

Secondary outcomes:

Hospitalization

Four of the eleven studies reported on the number of patients still requiring hospital admission at study endpoint. No difference in relative risk (RR: 0.90 [0.65-1.26, 95% CI, p=0.55, I2=46%]) (Figure S7.) of requiring hospital admission at study endpoint for patients administered MSCs compared to controls was demonstrated in meta-analysis. However, length of stay in hospital (reported in 5 studies) was reduced in MSC groups compared to controls (MD: -3.97 days [-6.09 to -1.85, 95% CI, p=0.0002, I^2=0%]; MSC: MoM=25.7 days (IQR=10.6); Control: MoM= 28.4 days (IQR=11.9))) (Figure S8).

Oxygenation Levels

Eight studies reported changes in oxygenation levels from baseline to endpoint in their patients. Many measures were used to quantify the change in oxygenation, including PaO2/FiO2, FiO2 (%), SpO_2 (%), maximum forced vital capacity (Vcmax) and diffusion lung capacity for carbon monoxide. Three of the studies reported PaO_2/FiO_2 and could be combined in meta-analysis

which demonstrated that patients administered MSCs had a significantly higher oxygenation index at endpoint compared to controls (MD: 105.62 mmHg [73.9-137.3, 95% CI, p<0.00001, I^2=0%]; MSC: MoM= 372.0 mmHg (IQR= 19.4); Control: MoM= 278.3 mmHg (IQR=32.1)) (Figure 3).

Pro-inflammatory and Anti-inflammatory cytokine levels

All eleven studies reported changes in a range of pro-inflammatory cytokines from baseline to the end of the study period. Seven of the studies reported Interleukin-6 (IL-6) levels, and meta-analysis revealed no significant decrease in IL-6 levels at study endpoint compared to controls (SMD: -0.22 [-0.60-0.16, 95% CI, p=0.25, I^2=43%]; MSC: MoM=73.8 (IQR=51.9); Control: MoM=50.8 (IQR=99.7)) (Figure S12). However, in a subset of studies, MSCs significantly reduced IL-6 levels compared to controls when measured 3 – 7 days following MSC administration (SMD: -0.62 [-1.03- -0.21, 95% CI, p=0.003, I^2=0%]; MSC: MoM=29.5 (IQR=34.5); Control: MoM=59.6 (IQR=97.1)) (Figure S13). Five of the studies reported C-reactive protein (CRP) levels at study endpoint, and meta-analysis revealed that patients administered MSCs had significantly lower C-reactive protein (C-RP) levels at study endpoint compared to controls. (SMD: -0.41 [-0.77- -0.05, 95% CI, p=0.02, I^2=23%]; MSC: MoM=36.8 (IQR= 61.8); Control= 58.2 (IQR=95.9)). (Figure S14).

Other outcomes

Other outcomes measured across studies including admission to intensive care unit, need for mechanical ventilation and/or ventilation parameters, immune cell levels, radiological parameters, virological and/or antibody responses and clinical scale scores are summarized in the supplementary materials (pages 1-4).

Adverse events

Adverse event reporting was described for all studies and is summarized in Table 4. Adverse events occurred in four studies in association with MSC infusion. Meta-analysis demonstrated no significant difference in the relative risk of adverse events between treated and control groups (RR: 0.78 [0.58-1.06, 95% CI, p=0.11, I2=43%]) (Figure 4.). Specific adverse events occurring in patients administered MSCs included facial flushing, transient fever, hypoxemia, palpitations, and dizziness. However, these symptoms resolved in all patients spontaneously or with minimal supportive treatment following MSC administration. Only one of the studies reported the occurrence of treatment related severe adverse events. However, this study did not specify which or how many severe adverse events occurred, only that "more serious adverse events were recorded for the placebo group than for the MSC group; however, the difference was not statistically significant" (29).

Risk of bias (RoB)

RoB was assessed for each outcome reported in RCTs using the Cochrane Risk of Bias 2 tool (ROB 2) (20). The RoB analysis for our primary outcome of risk of death at study endpoint can be seen in Table S1. Four studies (26, 29, 30, 32) were found to have low RoB and two studies had a RoB of 'some concerns' (24, 29), as the method of randomization was unclear for both studies, and it was unclear whether there were deviations from intended interventions or selection of reported results in one of the studies. With regards to other outcomes that were subject to meta-analysis (e.g. risk of death at 28 days, levels of pro-inflammatory cytokines, length of stay in hospital, risk of requiring mechanical ventilation at study endpoint, etc.), each study had the same RoB classification for both individual RoB domains and overall RoB. (n=4 low RoB, n=2 'some concerns' RoB; Table S1). RoB was assessed for the non-randomized

controlled studies using the ROBINS I tool (21). All studies (23, 25, 27, 28, 31) were found to have a moderate RoB overall (Table S2). All studies had potential bias due to confounding, measurement of outcomes (as studies did not mention blinding), and selection of reported results (as none of the studies reported a pre-registered protocol). Funnel plots for the primary analysis of mortality yielded asymmetrical distributions for both RR (Figure S19 a) and RD (Figure S19 b) effect measures, suggesting possible publication bias.

FASTER Approval criteria evaluation

In terms of the extent to which the overall evidence meets the criteria for high quality evidence outlined in our proposed framework described in our first iteration of the living systematic review (16), referred to as the *FASTER Approval* criteria (Table 6), two of the domains were considered satisfactory (sample size, study populations), four of the domains were considered unclear (number of studies, study characteristics, outcome measurement, RoB) and one of the domains was considered unsatisfactory (product characterization).

4.5 Discussion

This second update to our living systematic review and meta-analysis suggests MSCs are safe and may be effective for the treatment of COVID-19. The number of studies in our analysis remains limited (n=11), however, and most studies did not report sufficient characterization of MSC products in accordance with published ISCT minimal criteria (17). In particular, plastic adherence, cell viability and tri-lineage differentiation potential were not reported for most studies. Moreover, outcome reporting varied between studies which limits the ability to perform meaningful meta-analyses regarding measures of clinical benefit. Some aspects of study design can be further addressed to reduce the potential risk of bias and improve confidence in the

evidence. Enhancing the likelihood of MSC-based therapy achieving regulatory approval and more widespread clinical use will require greater consistency in product characterization, outcome reporting, and overall study design, which could be facilitated with the design and implementation of a master protocol that could be shared by investigators working towards a common goal.

MSCs decreased both the relative and absolute risk of death at study endpoint compared to controls in our meta-analysis and MSCs decreased relative risk of death at 28 days. Although a limited number of other therapeutics including remdesivir (34) and bamlanivimab (35) have also demonstrated promise in terms of decreasing mortality in patients suffering from COVID-19, their mechanisms of action appear more limited in scope. Although the specific mechanisms and pathways interrogated by MSCs in patients with COVID-19 have yet to be confirmed, work in other related diseases and conditions indicates that MSCs ameliorate COVID-19 through a combination of immunomodulatory, antibacterial, tissue regenerative, and anti-apoptotic mechanisms (36, 37). The pleiotropic effects of MSCs could explain the significant reduction in mortality observed for COVID-19 patients. More insight regarding specific mechanisms could allow further optimization of MSC therapy by leveraging key therapeutic mechanisms (38).

Oxygenation at study endpoint was improved in patients administered MSCs compared to controls and subgroup analysis indicated that MSCs were effective in reducing the risk of death at study endpoint for patients experiencing ARDS. Mechanisms of how MSCs could improve oxygenation and ameliorate COVID-19-induced ARDS remain unknown but may involve attenuation of the cytokine storm and/or healing of damaged and dysfunctional lung tissue (36-38). Optimal management of COVID-19-induced ARDS is an area of active research (39, 40) and further studies that involve MSCs appear warranted.

MSCs did not decrease the levels of pro-inflammatory cytokines and immune biomarkers including IL-6, D-dimer, ferritin, and IFN-g when measured at various time points after treatment. Given the highly dynamic nature of these biomarkers at various points before, during and after the height of the cytokine storm that can be influenced by IL-6 amplifier (IL-AMP) and by activated pro-inflammatory immune cells (41-44), we observed that patients administered MSCs had significantly reduced IL-6 levels compared to controls at early time points (<7 days), but not at later periods after MSC administration.

Interestingly, patients administered MSCs had significantly reduced CRP levels at study endpoint compared to controls. CRP is an acute phase protein that is produced by the liver and increases rapidly in response to inflammation, cell injury and tissue necrosis (45-47). Binding of CRP to molecules on microorganisms activates the complement system, which plays an important role in innate host immunity. CRP levels are prognostic in patients with COVID-19 with higher CRP levels associated with significantly worse disease severity, worse clinical outcomes and higher mortality rates (49-54). Moreover, patients with elevated CRP levels have been associated with higher rates of extrapulmonary COVID-19 complications including venous thromboembolism (VTE) and acute kidney injury (AKI) (55).

Subgroup analysis from our review suggested MSCs from umbilical cord tissue were inferior to MSCs from other tissue sources at reducing the risk of death at study endpoint. Interestingly, previous reports have suggested that MSCs derived from umbilical cord tissue could be optimal for the treatment of COVID-19 given the ease of expansion and rapid doubling times along with low levels of surface ACE-R which should mitigate against infection by SARS-CoV-2 (56, 57). Moreover, many publications also suggest that cord tissue-derived MSCs are rich in anti-

inflammatory cytokines (58). Given this contrast in perspectives, more work appears necessaary to determine the optimal tissue source for MSCs to treat COVID-19 (59, 60).

All patients received allogeneic MSCs. Allogeneic MSCs have advantages over autologous MSCs, including greater ease of acquiring the source material for product manufacturing and improved therapeutic efficacy compared to autologous MSCs, especially if isolated from individuals with advanced age or pre-existing medical conditions like diabetes or autoimmune diseases (61-64). Most importantly, third party allogeneic MSCs may be stored and used when needed (65). This avoids the long process of manufacturing small personalized autologous MSC products – paramount for rapidly progressing diseases like COVID-19, where delayed treatment leads to increased morbidity and mortality (66, 67). Although allogeneic MSCs appear to be effective in the context of COVID-19, the therapeutic implications of HLA-matching of MSCs between donors and recipients is currently less well understood and may be an important direction for future study to optimize MSC therapy (68). HLA matching of MSCs between donors and recipients may help avoid rejection of MSCs by the immune system and/or the occurrence of adverse events (68).

Adherence to ISCT criteria (17) remains low amongst studies included in our analysis, with only two studies reporting on all four criteria. Incomplete adherence and reporting of ISCT criteria is not restricted to the studies identified in our review, with one recent analysis demonstrating that 33% of MSC-based clinical trials included no ISCT characterization data (69). Poor adherence to ISCT criteria has been hypothesized to be one of the reasons why the success of MSC therapies in preclinical studies fails to translate to clinical settings (70, 71). Of note, six of the eleven studies reported MSC viability, a criterion added to the most recent update to the ISCT guidelines (17). Administering viable MSCs increases the likelihood that cells will

persist *in vivo* upon administration, which is likely critical to ensuring therapeutic success (72). Greater adherence and reporting of MSC product characterization according to all four ISCT criteria (17) would augment confidence in the consistency of MSC products across studies.

Optimal timing of MSC administration to patients with COVID-19 remains uncertain (41, 73). With their potential to attenuate the cytokine storm early in COVID-19 disease progression, some suggest administering MSCs at the onset of hypoxia to avoid intubation or mechanical ventilation. (41, 73). Concern regarding potential exacerbation of a hypercoaguable state, however, has been expressed (74) given that MSCs from certain tissue sources are rich in tissue factor (TF/CD142) (74). Future subgroup analysis examining the clinical outcomes and occurrence of adverse events in patients administered MSCs at different stages of COVID-19 disease would be informative and may help in the design of an optimized dosing schedule.

Our study has limitations worthy of mention. First, the number of studies and patients included in our analysis remains relatively small. This modest number of studies and patients limits our confidence in the observed effects. Slow accrual and delays in the completion and publication of studies remains an issue that impedes more rapid clinical translation of results. Indeed, a large number of registered controlled clinical trials were identified in a scoping review of the literature but remain unpublished (15). As more of these registered trials reach completion and are published, more refined estimates should be possible with regards to the safety and efficacy of MSC-based products as a therapeutic intervention for COVID-19. Given the limited study size of future RCTs, it appears meta-analysis will remain central to understanding the benefit of MSC therapy. Variable outcome reporting across the studies included in our review was also a major limitation and limits meta-analysis. The only outcome reported across all studies was mortality but standard time points for assessing mortality were not uniformly

reported. Future studies should strive towards consensus on outcome reporting. Additional outcomes of interest could include the number of days free from mechanical ventilation, although we acknowledge that practices in ventilator support of critically ill patients with COVID continues to evolve. Moreover, only two studies reported on whether MSC products met all ISCT criteria (17), further limiting the confidence in our pooled results. Lastly, asymmetry was detected in the funnel plots for mortality which suggests possible publication bias.

In conclusion, this second iteration of our living systematic review and meta-analysis demonstrates continued promise with regards to the use of MSCs as a therapeutic intervention for COVID-19. Challenges persist, however, which limit the certainty of our conclusions, including a limited number of published studies, modest patient enrollment, and substantial interstudy heterogeneity with regards to study design, patient characteristics, outcome reporting, and adherence to ISCT criteria. Future iterations of our systematic review will analyze data only from studies that meet the criteria for high quality studies outlined in our *FASTER Approval* framework. The development of a master protocol for MSC therapy should leverage aspects of our framework to ensure future studies report high quality evidence. A strategy that ensures higher quality evidence for knowledge synthesis appears necessary to leverage the results from modest-sized trials and where evidence from meta-analysis could be used to support applications for regulatory approval and to accelerate more widespread clinical application of MSC therapy for COVID-19.

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3.7 Figures and Tables

Figure 1. Results of systematic search of the literature. MEDLINE and Embase and Cochrane Central Register of Controlled Trials, searched from 1947 up to November 15th, 2021.

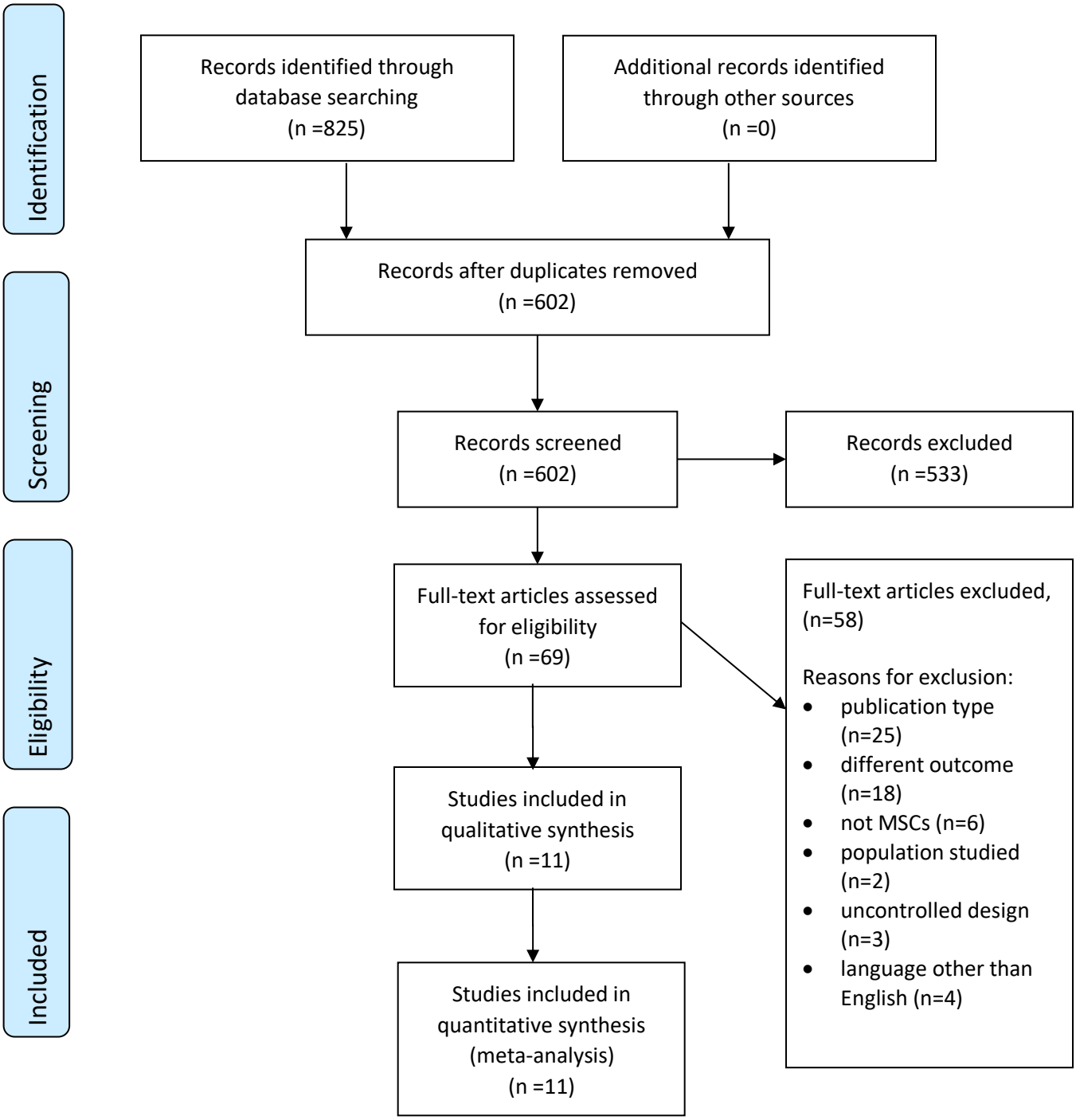
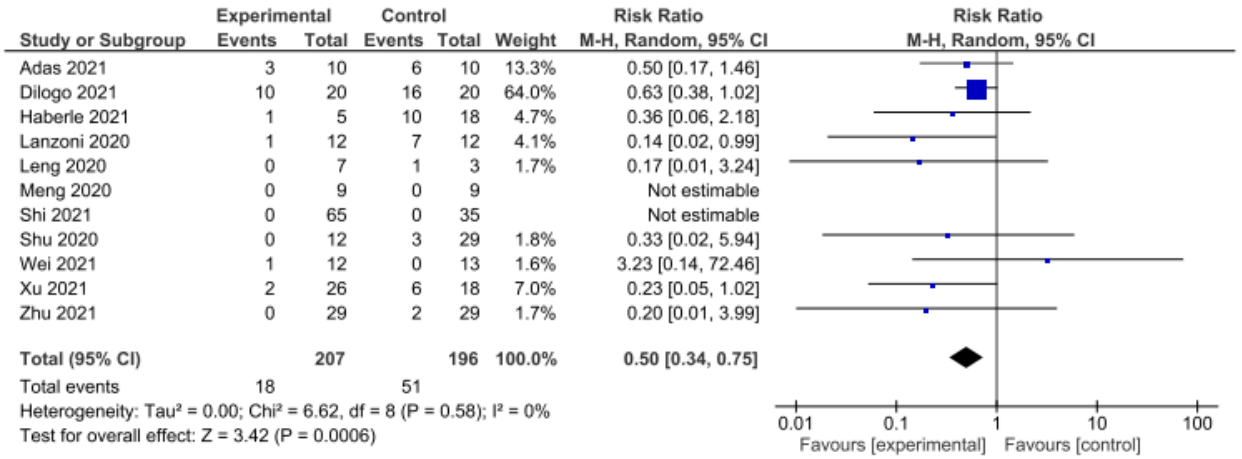


Figure 2. Forest plot demonstrating significantly decreased relative (a) and absolute (b) risk of death at study endpoint in patients administered MSCs (experimental) compared to patients not administered MSCs (control). Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

a.



b.

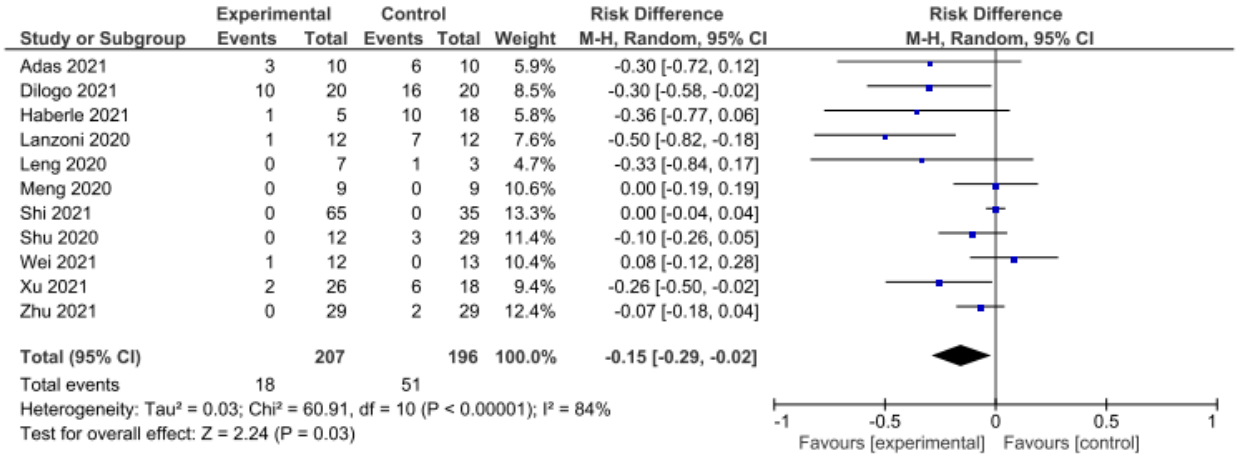


Figure 3. Forest plot demonstrating increased oxygenation index at study endpoint in patients administered MSCs (experimental) and patients not administered MSCs (control). Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

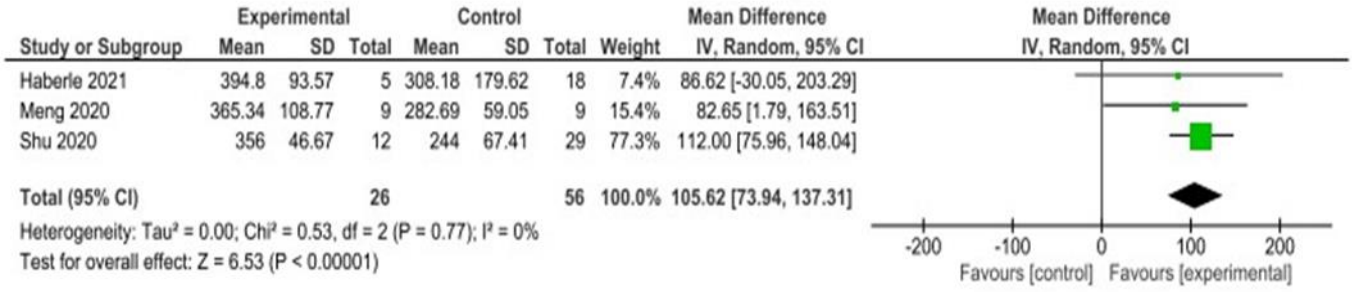
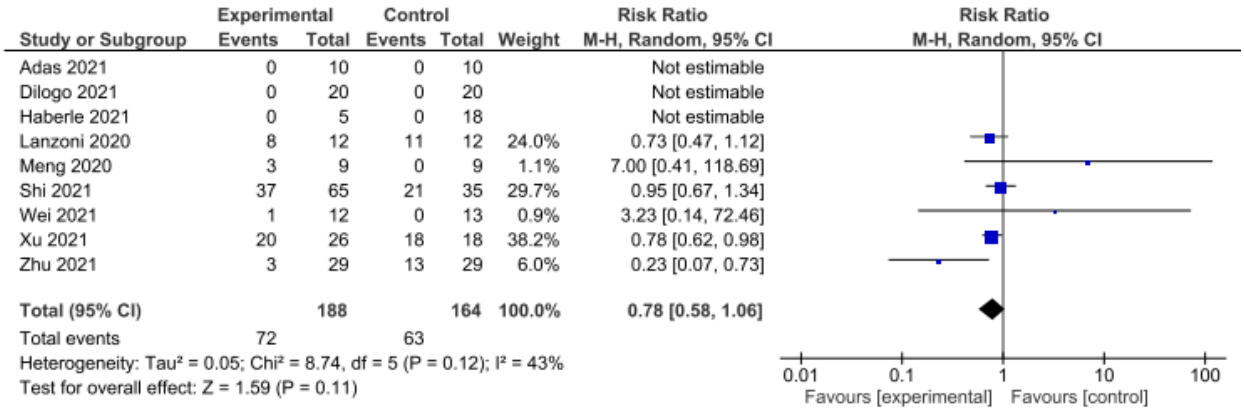


Figure 4. Forest plot demonstrating no significant difference in relative (a) and absolute (b) risk of experiencing adverse events in patients administered MSCs (experimental) compared to controls. Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

a.



b.

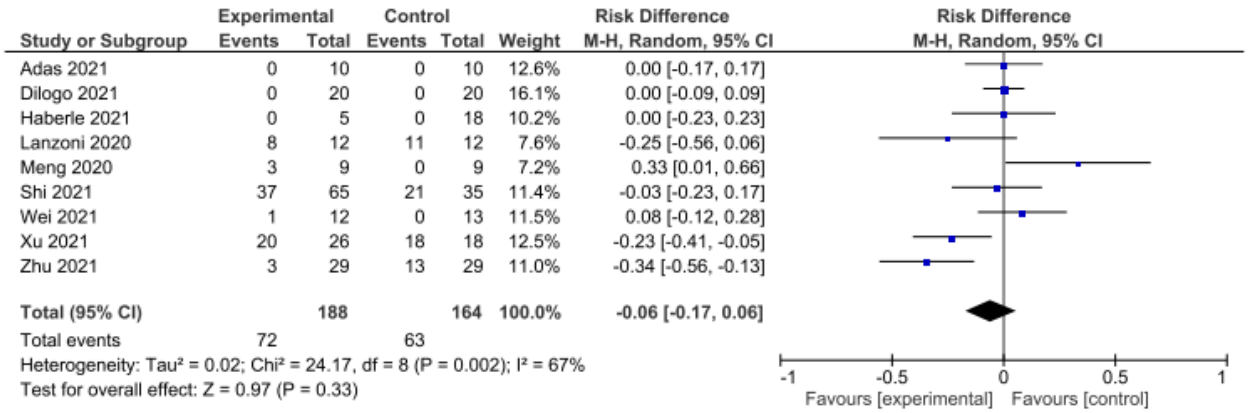


Table 1. Characteristics of patients enrolled in clinical studies of MSCs as a therapeutic intervention for COVID-19.

Patient characteristics	**All groups**	**Control groups**	**MSC groups**
Total patients, n	403	196	207
Sex, %male	56.9	55.4	58.4
Mean age, years (SD)	58.4 (7.1)	60.1 (5.3)	56.7 (8.5)
Covid-19 severity, n (%)			
Mild	39 (9.7)	19 (9.7)	20 (9.7)
Moderate	20 (5.0)	10 (5.1)	10 (4.8)
Severe	251 (62.3)	124 (63.3)	127 (61.4)
Critical	93 (23.1)	43 (21.9)	50 (24.2)
Co-morbidities, n (%)			
Hypertension	109 (27.0)	60 (30.6)	49 (23.7)
Diabetes	70 (17.4)	34 (17.3)	36 (17.4)
Obesity	16 (4.0)	5 (2.6)	11 (5.3)
Chronic obstructive pulmonary disease	4 (1.0)	1 (0.5)	3 (1.4)
Coronary artery disease	9 (2.2)	6 (3.1)	3 (1.4)
Congestive heart failure	10 (2.5)	6 (3.1)	4 (1.9)
Chronic kidney failure	7 (1.7)	5 (2.6)	2 (1.0)
*Other**	45 (11.2)	26 (13.3)	19 (9.2)
Mean follow up, days (range)	27.7 (14-60)	26.9 (14-60)	28.8 (14-60)

SD, standard deviation

*includes current smoker, ex-smoker, pre-diabetes, asthma, tuberculosis, chronic bronchitis, haemorrhagic cerebral infarction, coronary heart disease and chronic atrial fibrillation

Table 2. Intervention characteristics for clinical studies of patients administered MSCs as a therapeutic intervention for COVID-19.

Intervention	Total Studies, n
MSC tissue source	
Umbilical cord blood or tissue	8
Menstrual blood	1
Bone marrow mononuclear cells	1
Not described	1
MSCs fresh or frozen/cryopreserved	
Fresh only	0
Fresh or cryopreserved	1
Cryopreserved	4
Not stated	6
Product dose	
MSCs, cells/kg (n=7)	1 - 3 x 10^6
MSCs, total cells (n=4)	30 - 100 x 10^6
Route of administration	
Intravenous	11
No. MSC infusions, patients (%)	
1	80 (38.6)
2	15 (7.2)
3	112 (54.1)
MSC passage number	
P3 or P4	2
P5	3
P3-P5	1
P5-P6	1
Not stated	4
ISCT criteria	
fully met criteria (A-D below), n	2
(A) Plastic adherence	3
(B) Trilineage differentiation	5
(C) Positive/negative surface markers	8
(D) MSC viability	6

NA – not applicable; ISCT – International Society of Cellular Therapy

Table 3. List of reported outcomes in clinical studies examining MSCs as a therapeutic intervention for COVID-19. Outcomes reported in each study are indicated by (•) and outcomes not reported are indicated by (-).

Study	Mortality rate	Diagnosis to intervention (time)	Intervention to recovery (time)	No. pts hospitalized	No. pts on supplemental O_2	No. pts on ventilator	Time in hospital	Progression of symptoms	Improvement of symptoms	Time to clinical improvement	Oxygenation levels	Immune cell levels	Pro-inflammatory cytokines	Anti-inflammatory cytokine s	Viral load	Radiological outcomes
(Leng)	•	•	•	•	-	-	•	•	•	•	•	•	•	•	-	•
(Shu)	•	•	-	•	•	•	•	•	•	•	•	•	•	-	-	•
(Meng)	•	•	•	•	•	•	•	•	-	•	•	-	•	-	•	•
(Shi)	•	•	-	•	•	•	-	-	-	-	•	•	•	•	-	•
(Xu)	•	-	•	-	-	•	•	-	•	•	•	-	•	-	•	•
(Wei)	•	•	-	-	•	•	-	-	-	-	•	•	•	•	-	•
(Zhu)	•	•	•	-	-	-	•	-	•	•	-	-	•	•	-	•
(Lanzoni)	•	•	•	-	•	•	-	•	-	-	-	-	•	•	•	-
(Dilogo)	•	•	-	-	-	-	-	-	•	-	-	•	•	•	-	-
(Haberle)	•	-	-	-	-	•	-	-	-	-	•	•	•	-	-	-
(Adas)	•	-	-	•	-	•	•	-	•	-	•	•	•	•	•	-
Total	11	8	5	5	5	8	6	4	6	5	8	7	11	7	4	7

Pts – Patients

Table 4. Adverse events (AEs) and severe adverse events (SAEs) reported in clinical studies examining MSCs as a therapeutic intervention for COVID-19.

Study	Safety lab values	Treatment related AEs	Non-treatment related AEs	Treatment related SAEs	Non-treatment related SAEs
(Leng)	•	•	•	•	•
(Shu)	•	•	•	•	•
(Meng)	-	-	•	•	-
(Shi)	-	•	-	•	•
(Xu)	-	-	-	•	•
(Wei)	-	•	•	•	-
Zhu	-	-	-	-	-
(Lanzoni)	•	-	-	•	-
(Dilogo)	•	•	•	•	•
(Haberle)	•	•	•	•	•
(Adas)	•	•	•	•	•
Total	5	4	4	1	4

Table 5. Concomitant therapies reported in studies. Italics indicates the wording used in the reports.

Study (ref)	Antiviral agents	Antibiotic agents	Glucocorticoids	Transfusion based interventions	Other interventions
(Leng)	None	None	None	None	None
(Shu)	Abidor/ Oseltamivir	Moxifloxacin	Yes	None	None
(Meng)	Lopinavir/ Ritonavir	None	Yes	None	None
(Shi)	*Antiviral drugs*	*Antibiotics*	Yes	None	None
(Xu)	*Antiviral therapy*	*Antibacterial treatment*	None	*Extracorporeal blood purification system*	Multiple *
(Wei)	Arbidol, Lopinavir–Ritonavir	None	Yes	None	None
(Zhu)	α-Interferon, Ribavirin, Ganciclovir	Moxifloxacin, Piperacillin tazobactam, Levofloxacin	Yes	None	None
(Lanzoni)	BAT	BAT	BAT	BAT	BAT
(Dilogo)	Oseltamivir	Azithromycin	None	None	None
(Haberle)	None	None	Yes	None	Multiple**
(Adas)	Favipiravir, HCQ	Piperacillin-tazobactam,	Yes	None	Enoxaparin

BAT, best available therapy; HCQ, hydroxychloroquine

* includes *hormone, gut microflora modulator, Chinese medicine treatment, basic disease medication*

**includes *immunosuppressive medication, ACE inhibitor, AT1 receptor blocker, beta-blocker, other antihypertensive drugs, Calcium antagonists, antiplatelet drugs*

Table 6. Assessment of proposed criteria in *FASTER Approval* framework for performing meta-analysis of high-quality studies of MSC-based therapy for COVID-19. Check marks indicate criteria satisfied, dark circle indicates uncertainty if criteria satisfied and "x" indicates criteria not met.

Number of studies	• Sufficient number and similar enough to perform meta-analysis that achieves the required power for determining efficacy. See sample size.	✓ 11 controlled studies identified
Study characteristics	• Controlled with contemporary and similar control groups. Randomized is preferable. Concomitant therapies should be controlled.	✓ RCTs: n=6 ? Concomitant therapies not always controlled
Sample size	• To reduce mortality from 20% to 10%, 199 subjects in intervention group(s) needed (24).	✓ Sample size = 403 total, with 207 patients in treatment (MSC) arm
Study populations	• Severe or critical COVID-19 in hospitalized patients (most common)	✓ Most patients presented with severe (62.3%) or critical (23.1%) COVID-19
Outcome measurement	• Mortality at day 28 • WHO response criteria • Secondary: IL6 levels, hospitalization, ICU admission, pulmonary function at 1, 6, 12 months. • Safety and adverse event reporting	? Mortality at 28 days (n=5) × WHO response criteria (n=0) ? IL-6 levels (n=7), hospitalization (n=5), ICU admission (n=4), pulmonary function at 1 (n=0), 6 (n=0) and 12 (n=0) months ✓ Safety laboratory values (n=5) and adverse event reporting (n=9)
Product characterization	• MSCs produced and characterized according to ISCT criteria.	× Full criteria (n=2) ✓ Positive/negative surface markers (n=8), ? MSC viability (n=6) ? Trilineage differentiation potential (n=5), ? Plastic adherence (n=3)
Risk of Bias	• Studies with high risk of potential bias should not be included in meta-analysis.	?'Some concerns' or Moderate RoB: n=7 (~64%)

4.8 Appendix

Supplementary outcomes

Intensive Care Unit and Mechanical Ventilation

Only one study reported the number of patients requiring ICU admission at study endpoint. In this study, there was no difference in the number of patients requiring ICU admission at study endpoint as all patients in both the control and MSC groups had been discharged from the ICU by study endpoint. Four studies reported the length of stay in the ICU for their patients. Meta-analysis demonstrated no significant difference in the length of ICU stay for patients administered MSCs compared to the control group (MD: 5.41 [-5.02-15.84, 95% CI, p=0.31, I^2=55%]) (Figure S9). Five patients reported the number of patients requiring supplemental oxygen at study endpoint. Meta-analysis demonstrated no significant difference in the relative risk of requiring supplemental oxygen at study endpoint for patients administered MSCs compared to controls. (RR: 1.02 [0.80-1.31, 95% CI, p=0.86, I2=0%]) (Figure S10) Only two studies reported the length of time that their patients were on supplemental oxygen. In both studies, the length of time on supplemental oxygen was greater for the control group compared to the MSC group. Eight studies reported the number of patients requiring mechanical ventilation at study endpoint. Meta-analysis demonstrated no significant difference in the relative risk of requiring mechanical ventilation at study endpoint in patients administered MSCs compared to controls (RR: 0.36 [0.13-1.03, 95% CI, p=0.06, I2=0%]) (Figure S11). Only two studies reported the length of time that their patients were on mechanical ventilation. For both studies, the length of time on mechanical ventilation was greater for control group compared to the MSC group.

Pro-inflammatory and Anti-inflammatory cytokine levels

Three of the studies reported D-dimer levels at study endpoint. Meta-analysis revealed that MSCs did not significantly decrease D-dimer levels at study endpoint compared to controls (SMD: 0.06 [-0.61-0.72, 95% CI, p=0.87, I^2=32%]) (Figure S15). Three studies reported Ferritin levels at study endpoint. Meta-analysis demonstrated that MSCs did not significantly alter ferritin levels at study endpoint compared to controls (SMD: -0.33 [-0.90-0.24, 95% CI, p=0.25, I^2=7%]) (Figure S16). Three studies reported IFN-g levels at study endpoint. Meta-analysis demonstrated that MSCs did not significantly alter IFN-g levels at study endpoint compared to controls (SMD: -0.72 [-1.77-0.33, 95% CI, p=0.18]) (Figure S17). The levels of several other pro-inflammatory cytokines including IL-2, IL-8 and TNF-a at study endpoint were also reported. MSCs were found to increase the levels of all these pro-inflammatory cytokines from baseline to study endpoint. However, these pro-inflammatory cytokines were not present in enough studies to perform meta-analysis. Six of the studies reported changes in levels of anti-inflammatory cytokines from baseline to the study endpoint. The levels of several anti-inflammatory cytokines were reported including IL-10, TNF-b, IL-13, and IL-1Ra. MSCs were found to increase the levels of all these anti-inflammatory cytokines from baseline to study endpoint. However, none of these anti-inflammatory cytokines were reported in enough studies to perform meta-analysis.

Immune Cell Levels

Seven of the ten studies reported on changes in circulating levels of immune cells. In terms of specific immune cell subsets. Patients administered MSCs demonstrated increased lymphocyte counts and decreased leukocyte counts at study endpoint compared to controls. In terms of specific immune cell subsets, patients who were administered MSCs displayed

increased CD4+ T cell, CD8+ T cell, B cell and NK cell counts at study endpoint compared to controls. Moreover, patients who were administered MSCs displayed decreased neutrophil counts at study endpoints compared to controls.

Radiological Parameters

Six of the ten studies examined radiological improvement in patients following MSC administration. The methods employed to assess radiological improvement varied between studies, with pre-MSC and post-MSC scores (n=1), change in the total lesion proportion of the whole lung volume (n=1), change in solid component lesion proportion of whole lung volume (n=1) and rate of chest imaging changes (n=1) all being used to quantify the magnitude and extent of radiological improvement. For all studies that examined radiological improvement, patients administered MSCs showed superior radiological improvement compared to control patients. Certain studies also detailed the improvement of specific pulmonary radiological findings. Two studies reported changes in ground glass opacities, with one of the studies reporting the number of patients with ground glass opacities pre and post MSC treatment and the other reporting change in ground-glass lesion proportion of whole lung volume. In both studies, MSCs were associated with improvements in ground glass opacity size and occurrence compared to controls. Two studies reported changes in pleural effusion. Both studies reported pleural effusion improvement as the number of patients with pleural effusions at study endpoint.

Virological and Antibody Outcomes

Four studies reported changes in viral load from baseline to endpoint. Changes in viral load were quantified using a variety of methods in different studies including viral load at study endpoint and mean time until patients became virus negative. Two studies reported on changes in SARS-CoV-2 antibody titers in patients administered MSCs. In both studies, patients

administered MSCs displayed more pronounced increases in antibody titers from baseline to endpoint for both IgG and IgM antibodies compared to control patients.

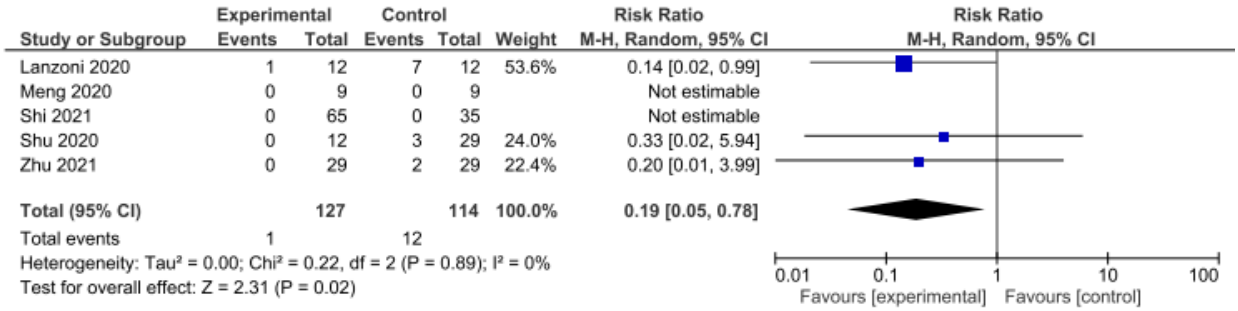
Time to Clinical Improvement and Clinical Scale Scores

Three studies reported on the time to improvement of clinical symptoms of their patients. Meta-analysis revealed that patients administered MSCs had significantly reduced time to improvement of clinical symptoms compared to controls (MD: -5.75 [-7.76 - -3.74, 95% CI, p=<0.00001, I2=0%]) (Figure S18). Four studies quantified the extent of clinical improvement of their patients using various clinical scales. One study used the 7-Category Ordinal scale, one study used the Six-category scale, one study used Apache-2 scores and one study used the Murray score. In three of the studies, patients administered MSCs displayed superior clinical scores for the 7-category ordinal scale, Apache-2 score and Murray score at study endpoint compared to control patients. However, in the study that used the six-category ordinal scale, the MSC group had a higher score (score=2) at study endpoint compared to the control group (score=0), indicating worse clinical function.

Supplementary figures

Figure S1. Forest plot demonstrating significantly reduced relative risk of death at 28 days in patients administered MSCs (experimental) compared to controls (a). However, there was no significant decrease in the absolute risk of death at 28 days in patients administered MSCs (experimental) compared to controls (b). Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

a.



b.

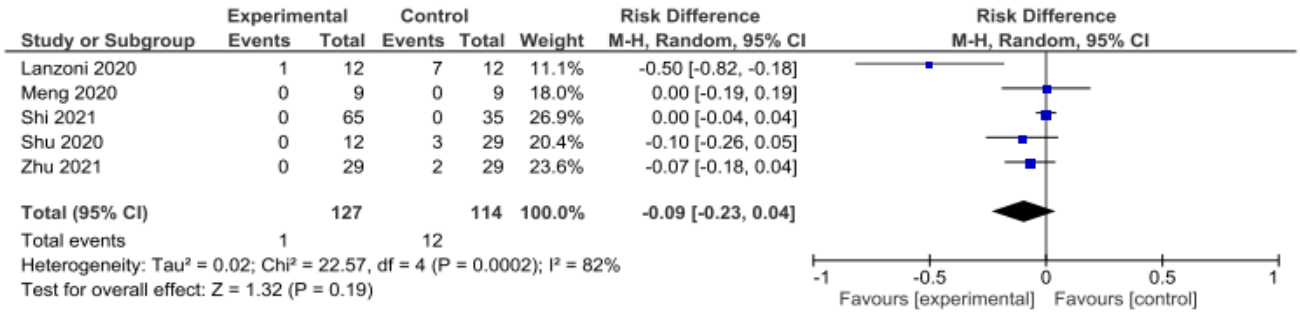
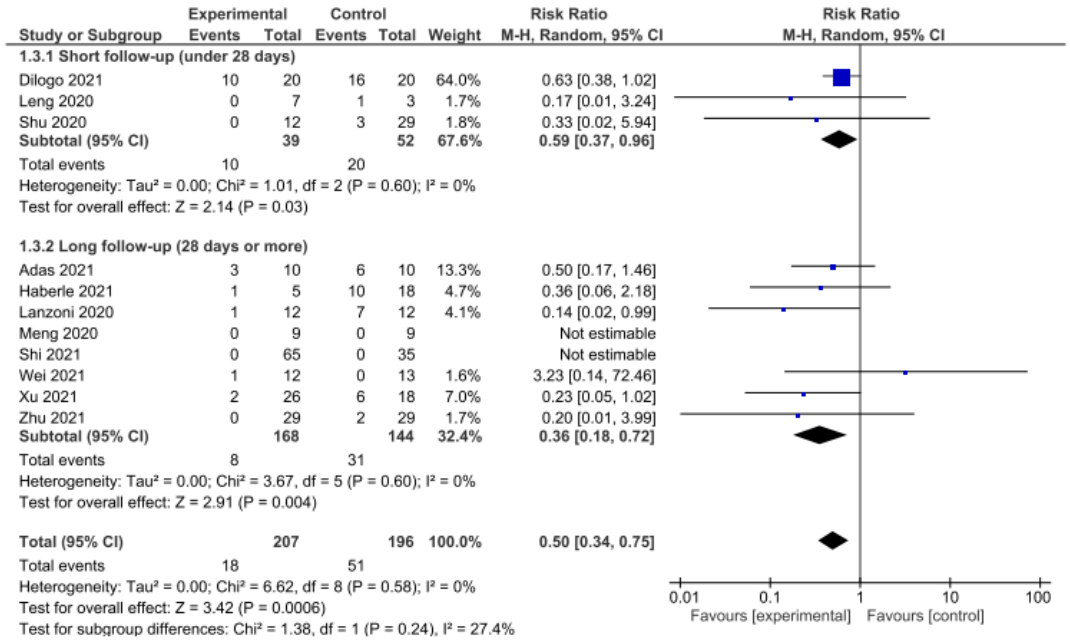


Figure S2. Subgroup analysis demonstrating significantly decreased relative risk of death at study endpoint for patients administered MSCs (experimental) compared to controls in studies with both short (less than 28 days) and long (more than or equal to 28 days) follow-up periods (a). However, when absolute risk was examined, patients administered MSCs were only found to have a significantly reduced absolute risk of death at study endpoint compared to controls in studies with short follow-up (b). Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

a.



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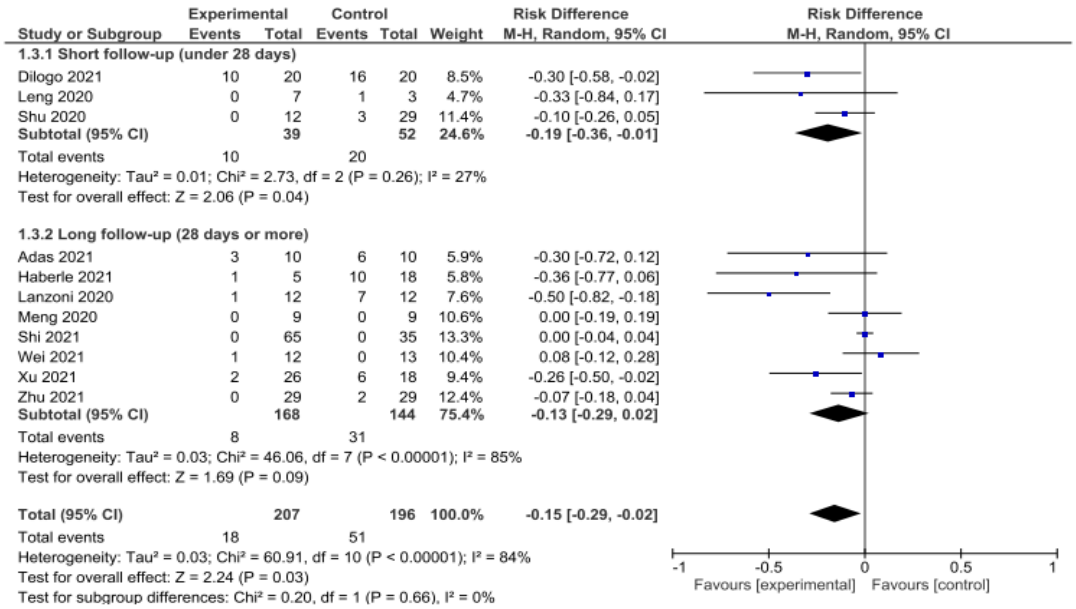
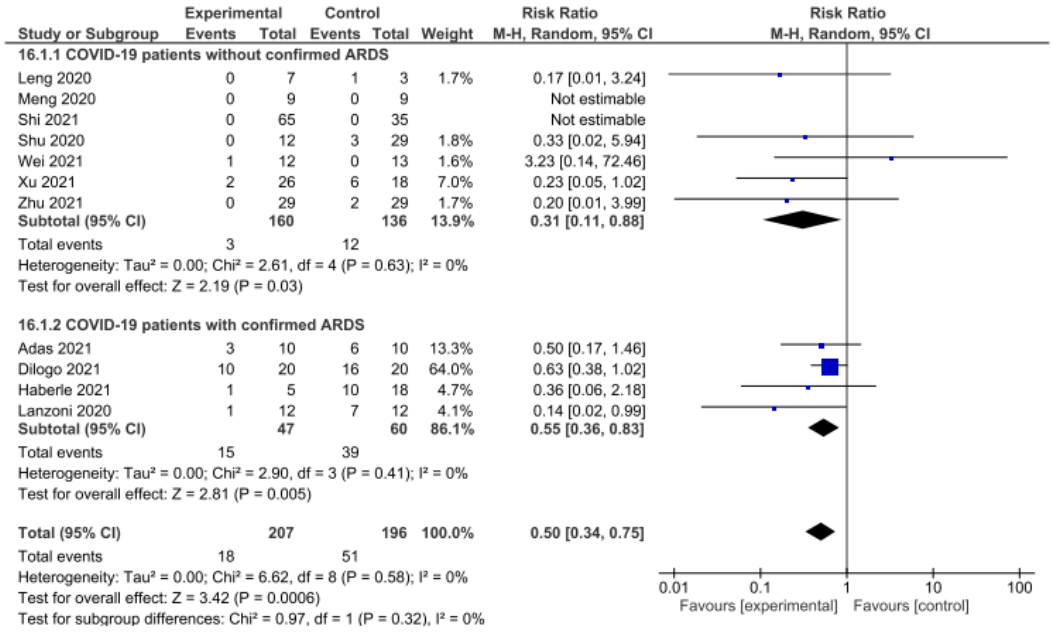


Figure S3. Subgroup analysis demonstrating significantly reduced relative risk of death at study endpoint for patients administered MSCs (experimental) compared to controls in patients with confirmed ARDS and patients without confirmed ARDS (a). However, when absolute risk was examined, only patients with confirmed ARDS that were administered MSCs had a reduced absolute risk of death at study endpoint compared to controls (b). Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

a.



b.

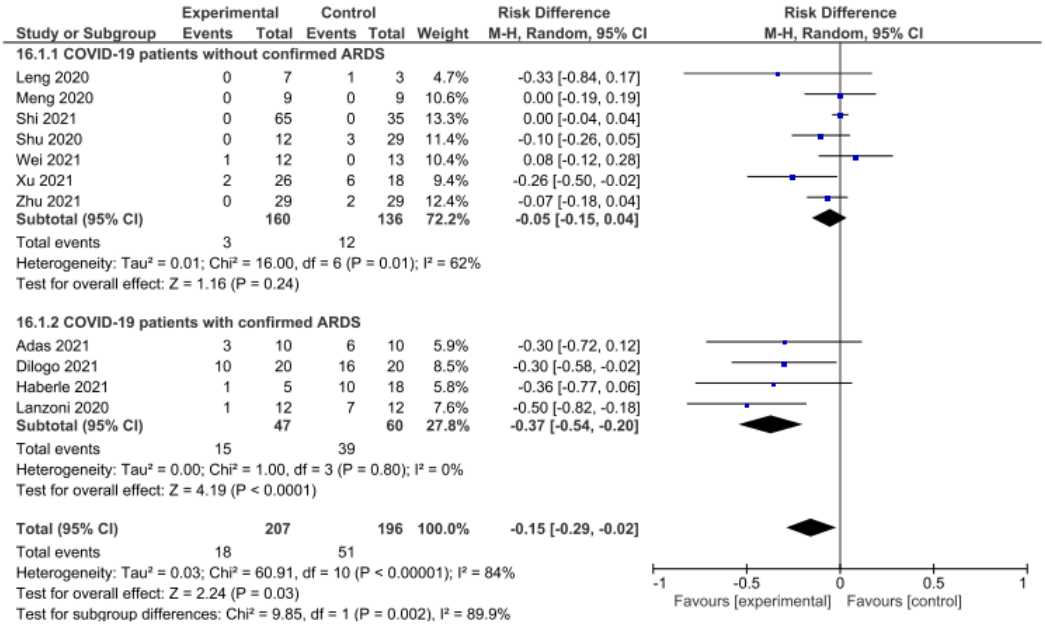
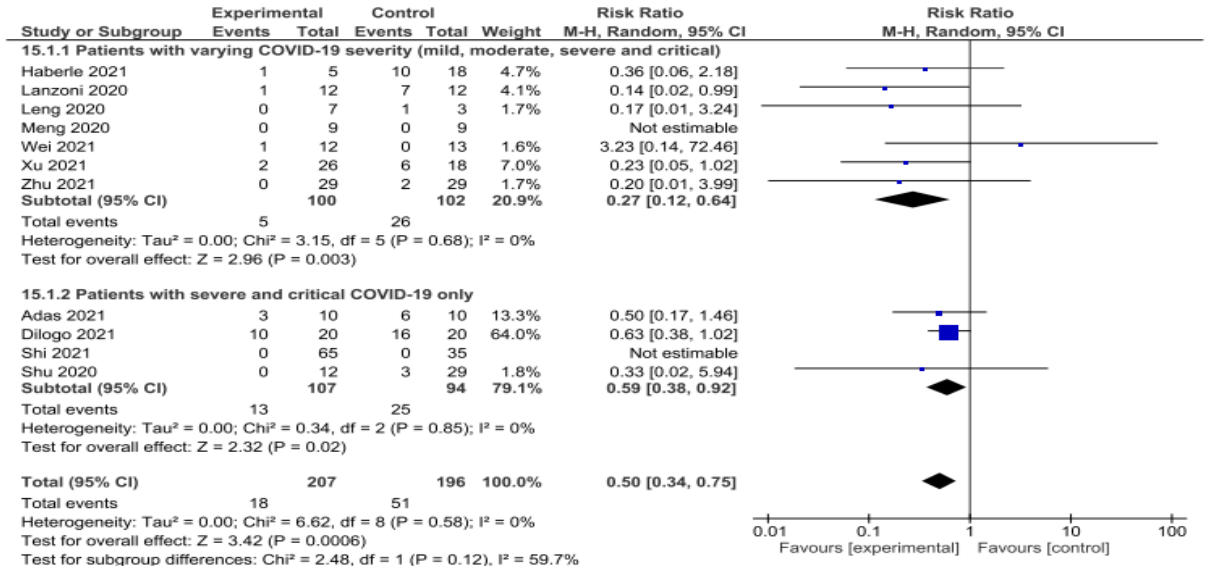


Figure S4. Subgroup analysis demonstrating significantly reduced relative risk of death at study endpoint in patients administered MSCs (experimental) compared to controls in studies reporting a wide variety of COVID-19 severities (mild, moderate, severe, critical) and studies with only severe and critical COVID-19 patients (a). However, when absolute risk was examined, only patients with a wide variety of COVID-19 severities that were administered MSCs were found to have a significantly reduced absolute risk of death at study endpoint compared to controls (b). Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

a.



b.

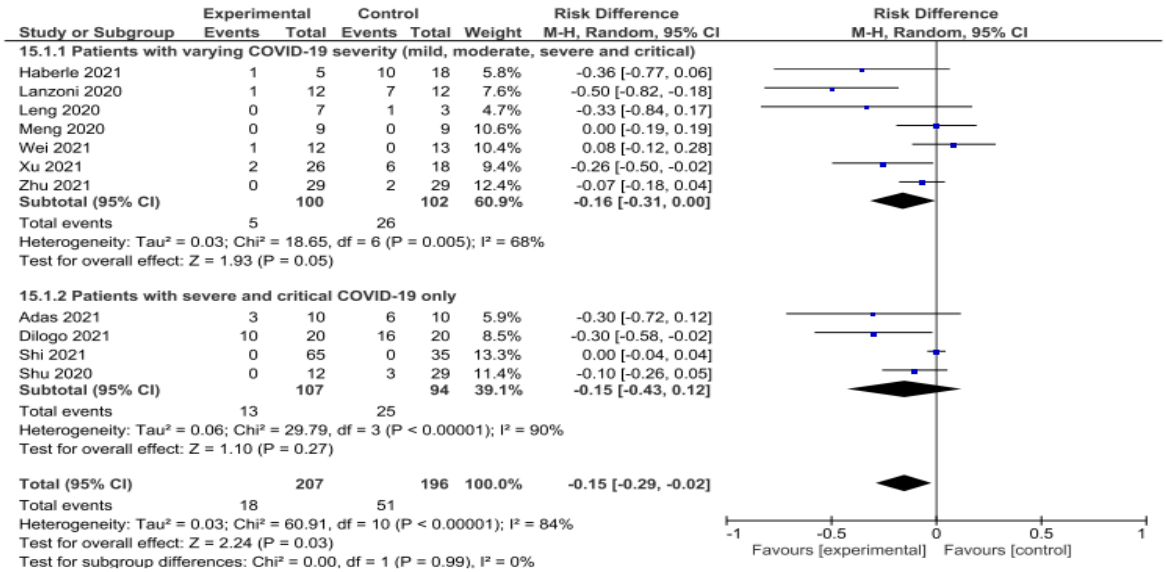
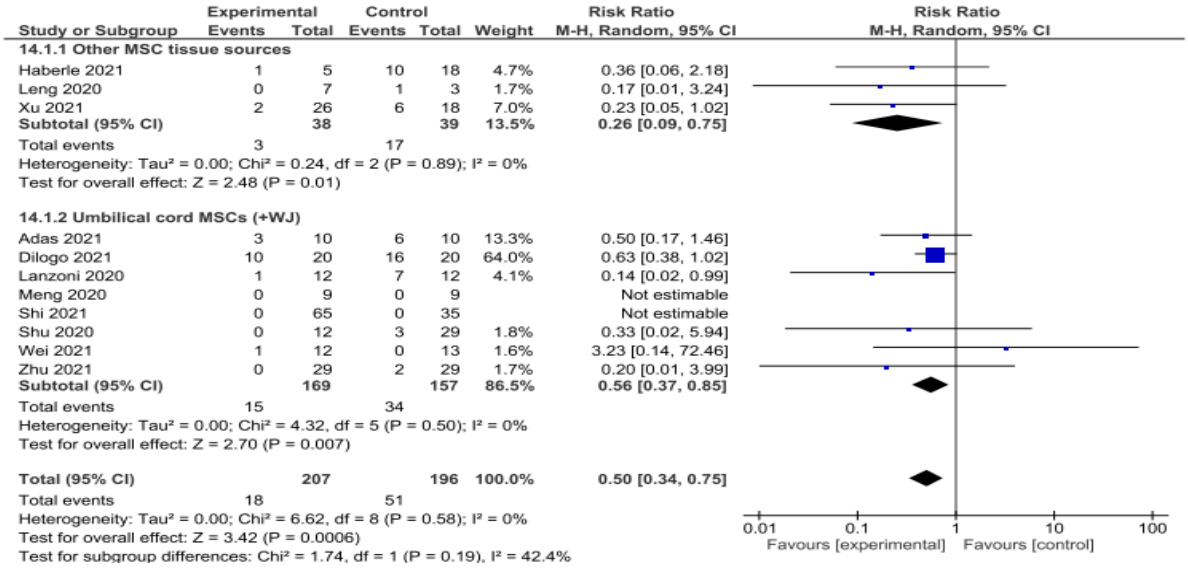


Figure S5. Subgroup analysis demonstrating significantly reduced relative risk of death at study endpoint in patients administered MSCs (experimental) compared to controls in both studies that used MSCs from umbilical cord tissue and studies that used MSCs from other tissue sources (a). However, when absolute risk was examined, only patients administered MSCs from other tissues sources were found to have a significantly reduced absolute risk of death at study endpoint compared to controls (b). Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

a.



b.

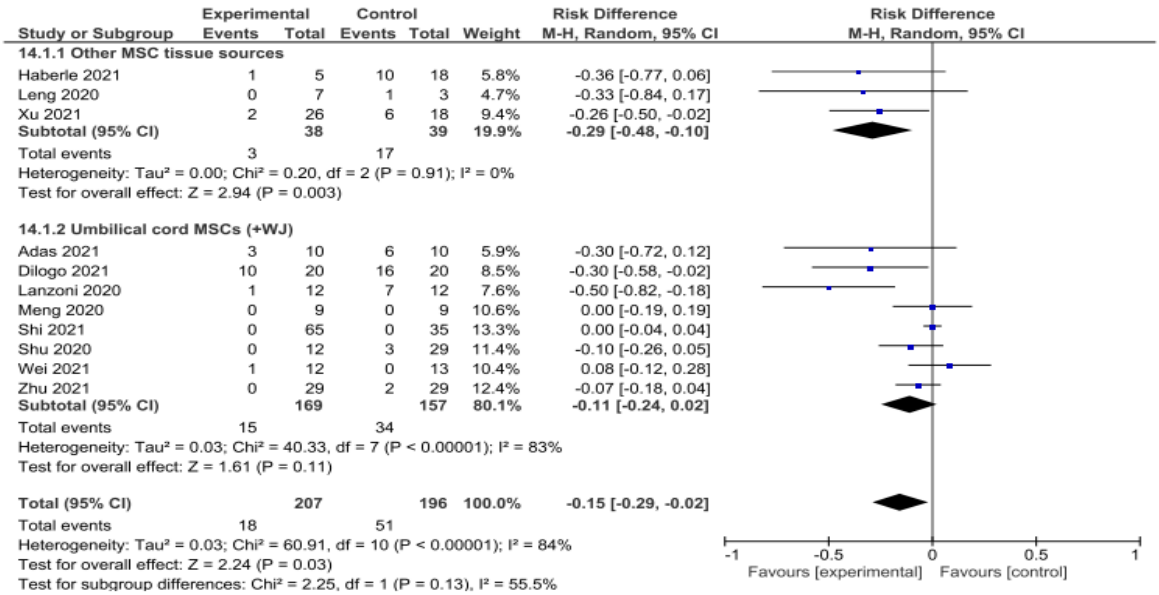
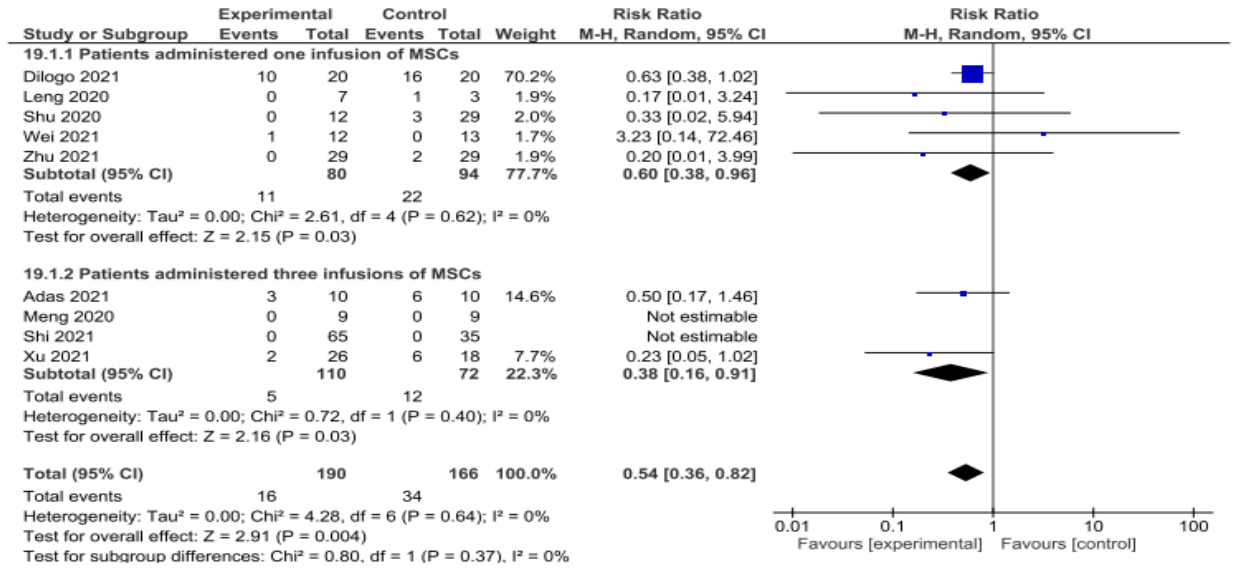


Figure S6. Subgroup analysis demonstrating significantly reduced relative risk of death at study endpoint in patients administered MSCs (experimental) compared to controls in both studies that administered a single infusion of MSCs and studies that administered three infusions of MSCs (a). However, when absolute risk was examined, no significant reduction in absolute risk of death at study endpoint was observed in both patients administered one and three infusions of MSCs compared to controls (b). Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

a.



b.

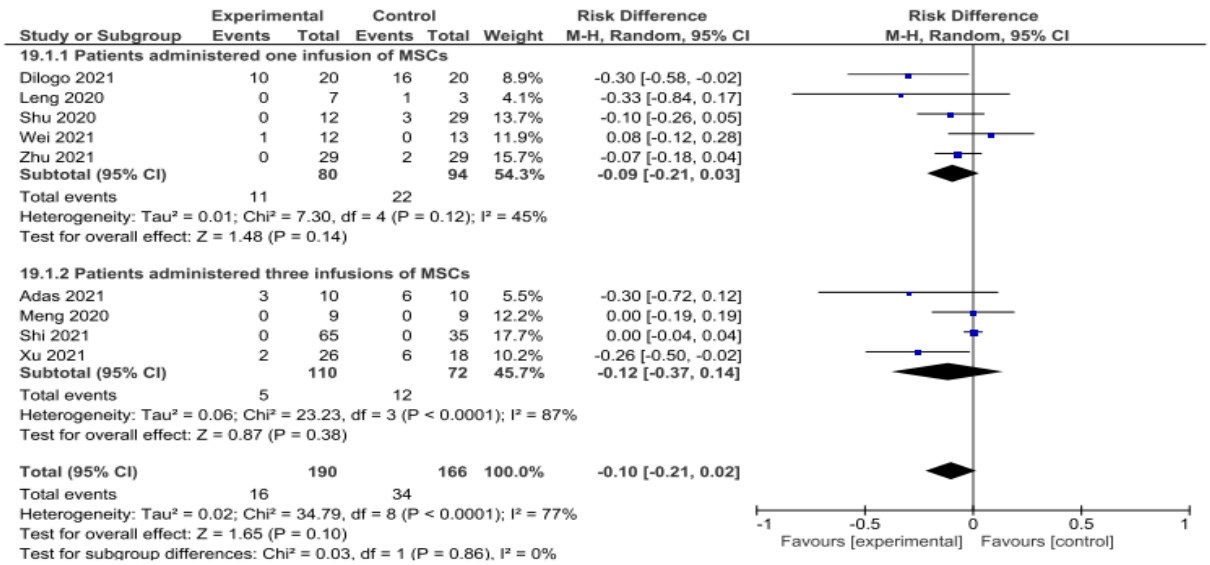
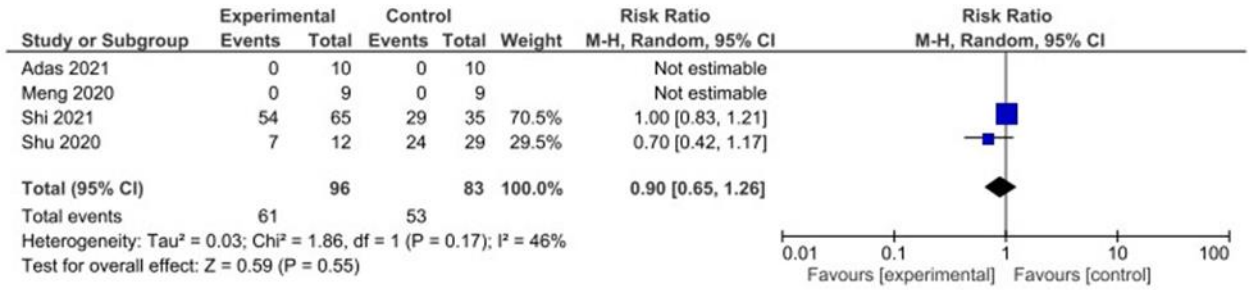


Figure S7. Forest plot demonstrating no significant difference in relative (a) or absolute (b) risk of requiring hospital admission at study endpoint in patients administered MSCs (experimental) compared to controls. Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

a.



b.

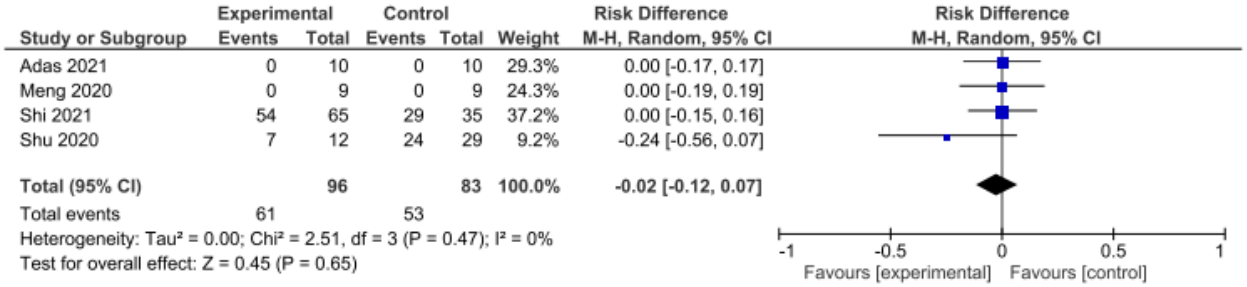


Figure S8. Forest plot demonstrating decreased length of stay in hospital for patients administered MSCs (experimental) and patients not administered MSCs (control). Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

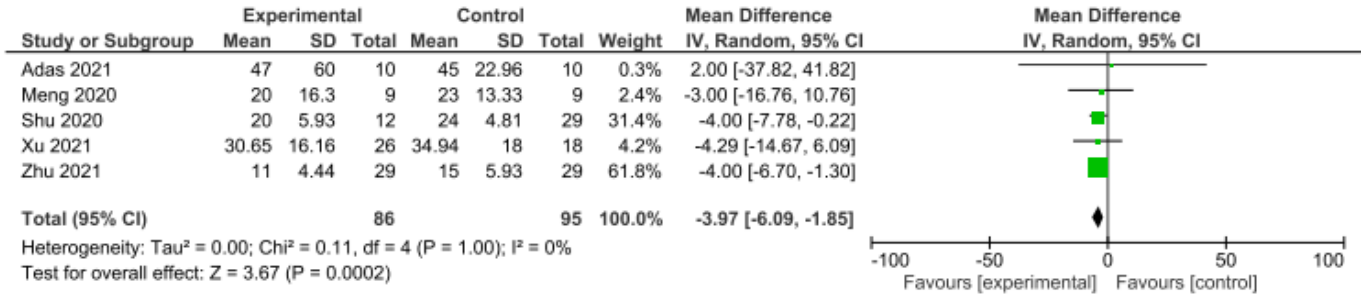


Figure S9. Forest plot demonstrating no significant difference in length of stay in intensive care unit (ICU) in patients administered MSCs (experimental) compared to controls. Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

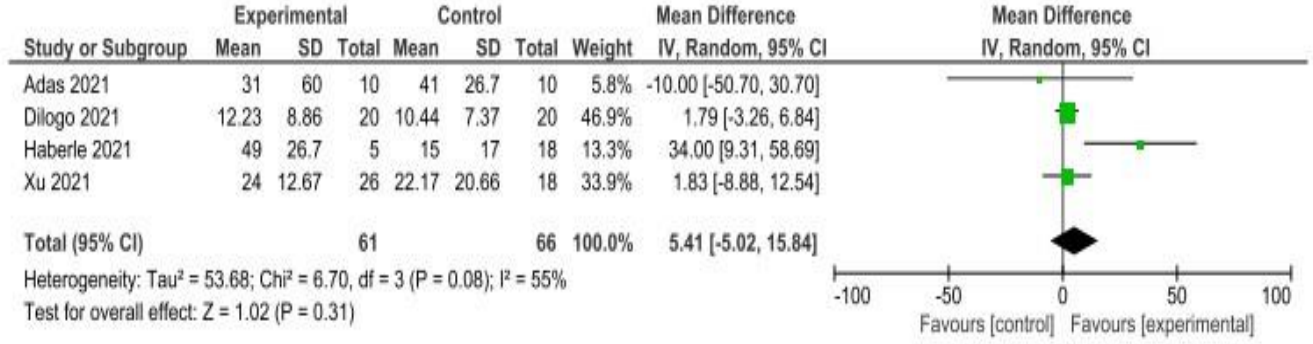
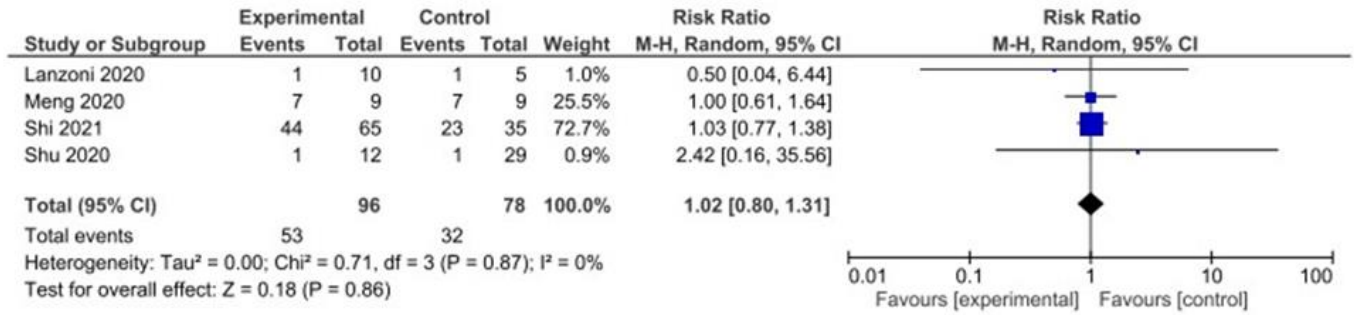


Figure S10. Forest plot demonstrating no significant difference in relative (a) or absolute (b) risk of requiring supplemental oxygen at study endpoint in patients administered MSCs (experimental) compared to controls. Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

a.



b.

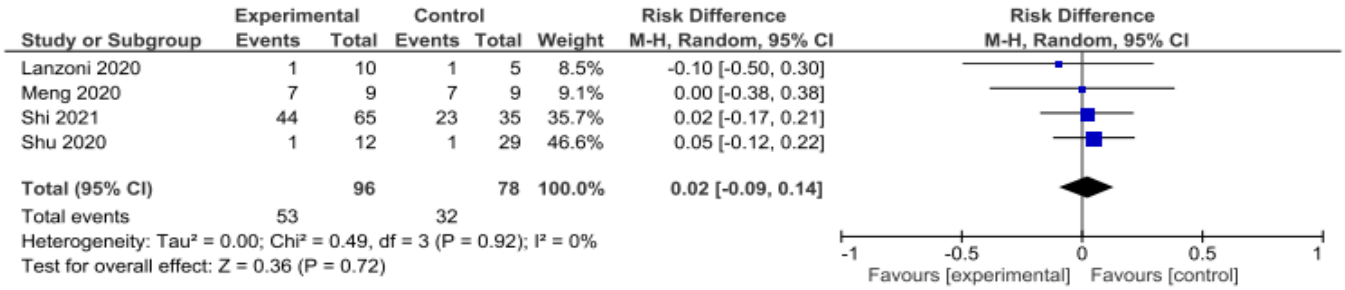
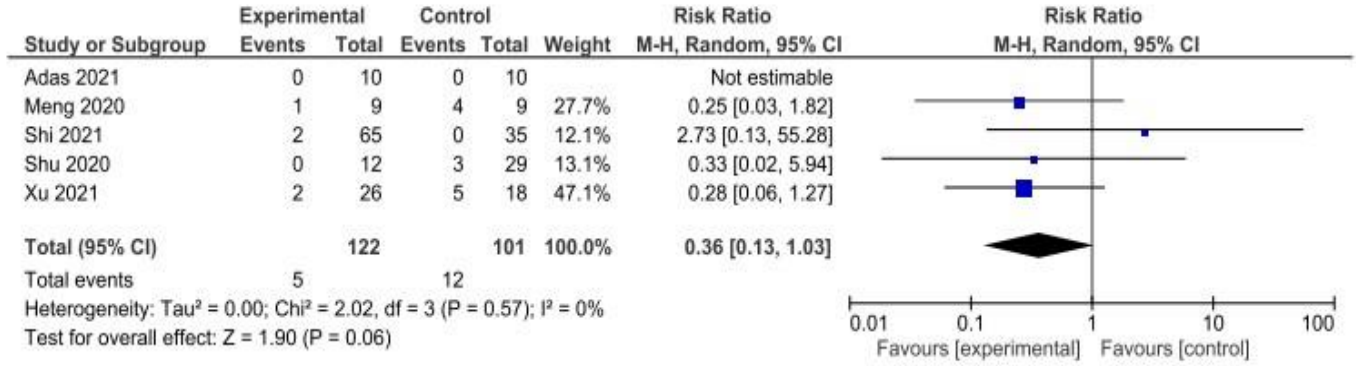


Figure S11. Forest plot demonstrating significantly reduced relative risk of requiring mechanical ventilation at study endpoint for patients administered MSCs (experimental) compared to controls (a). However, no significant reduction in absolute risk of requiring mechanical ventilation was observed in patients administered MSCs compared to controls (b). Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

a.



b.

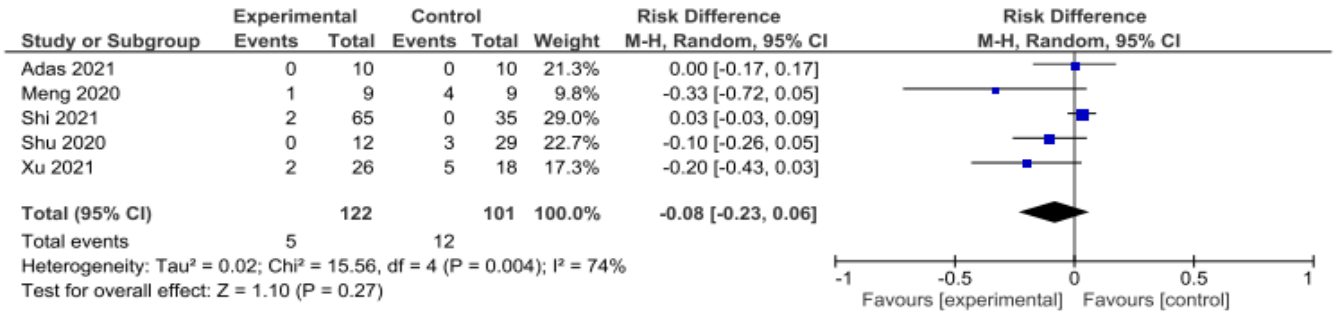


Figure S12. Forest plot demonstrating no significant difference in Interleukin-6 (IL-6) levels (pg/mL) at study endpoint in patients administered MSCs (experimental) compared to controls. Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

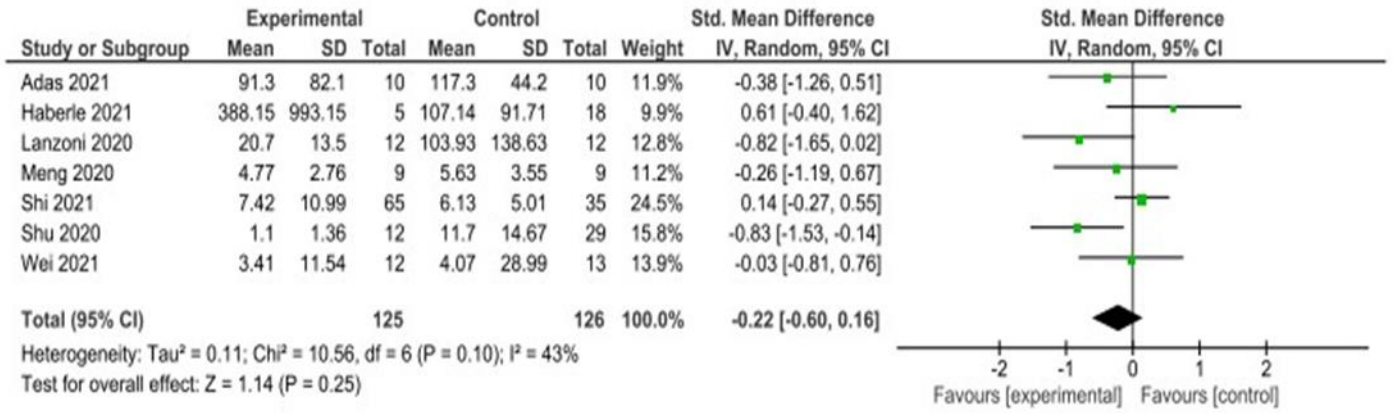


Figure S13. Subgroup analysis demonstrating significantly reduced Interleukin-6 (IL-6) levels (pg/mL) in patients administered MSCs (experimental) compared to controls in studies that measured IL-6 levels at short time points (3-7 days) following MSC administration. However, there was no significant difference in IL-6 levels between patients administered MSCs (experimental) compared to controls in studies that measured IL-6 levels at long time points following MSC administration (14-16 days). Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

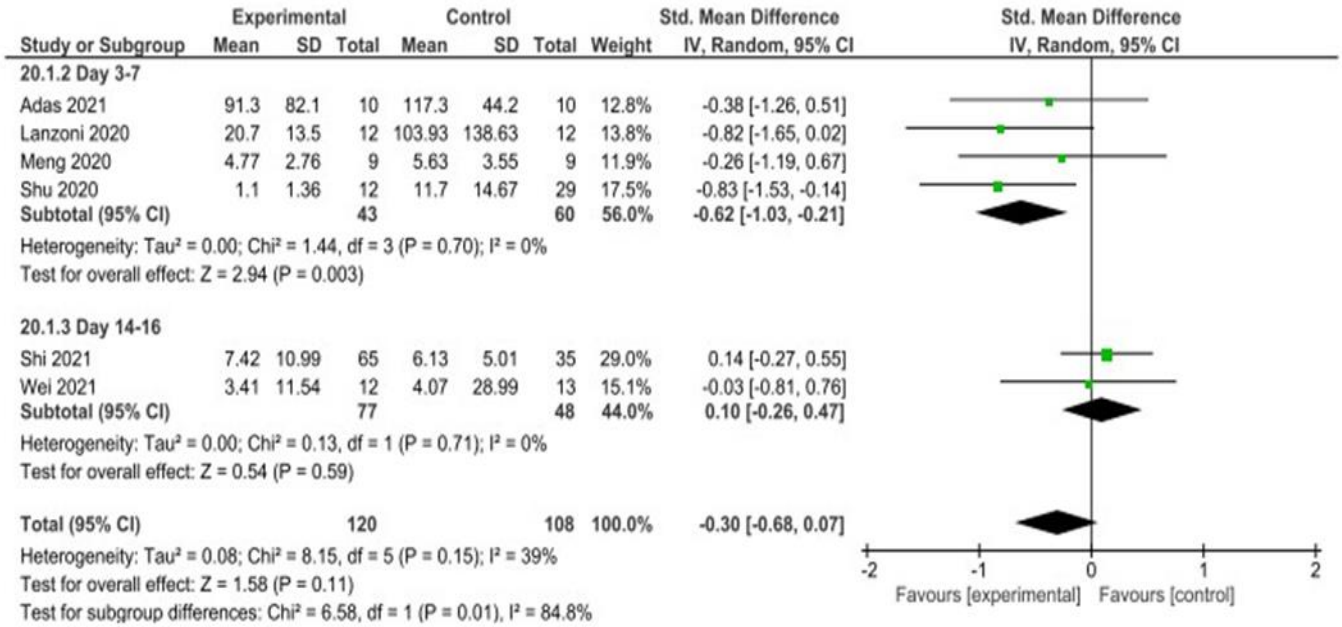


Figure S14 Forest plot demonstrating significantly lower C-reactive protein (C-RP) levels (mg/L) at study endpoint in patients administered MSCs (experimental) compared to controls. Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

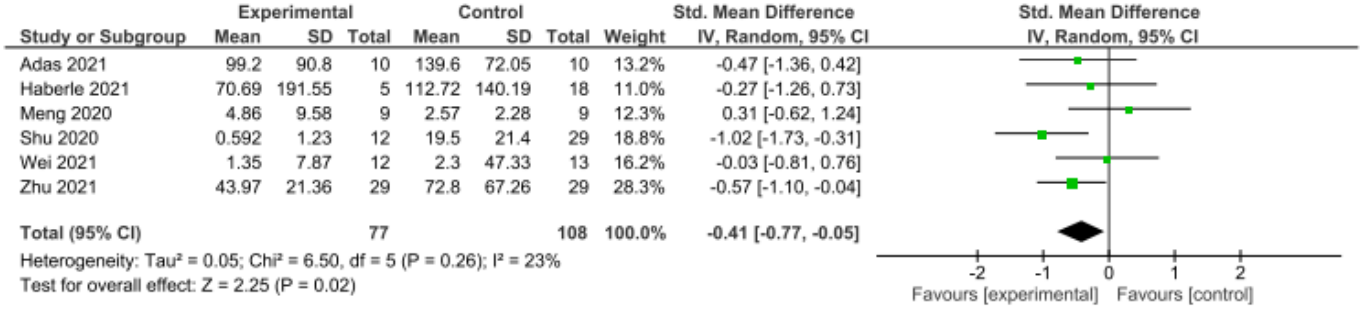


Figure S15. Forest plot demonstrating no significant difference in D-dimer levels (mg/L) at study endpoint in patients administered MSCs (experimental) compared to controls. Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

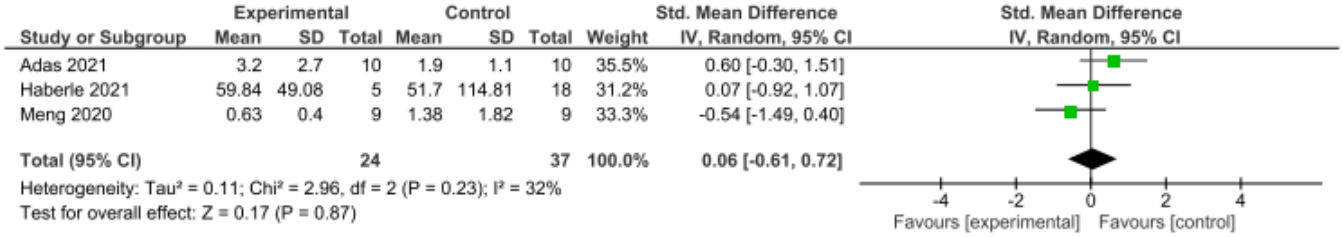


Figure S16. Forest plot demonstrating no significant difference in Ferritin levels (ug/L) at study endpoint in patients administered MSCs (experimental) compared to controls. Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

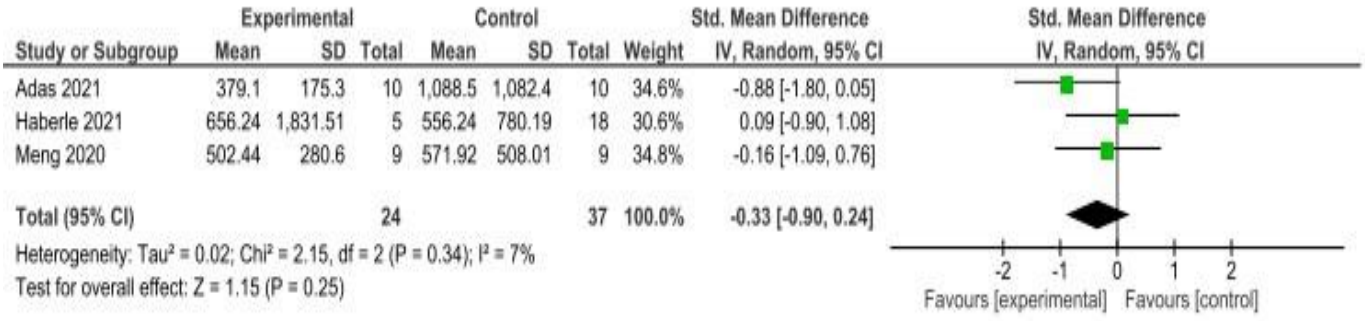


Figure S17. Forest plot demonstrating no significant difference in IFN-g levels (pg/mL) at study endpoint in patients administered MSCs (experimental) compared to controls. Substantial heterogeneity was also observed (83%). Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

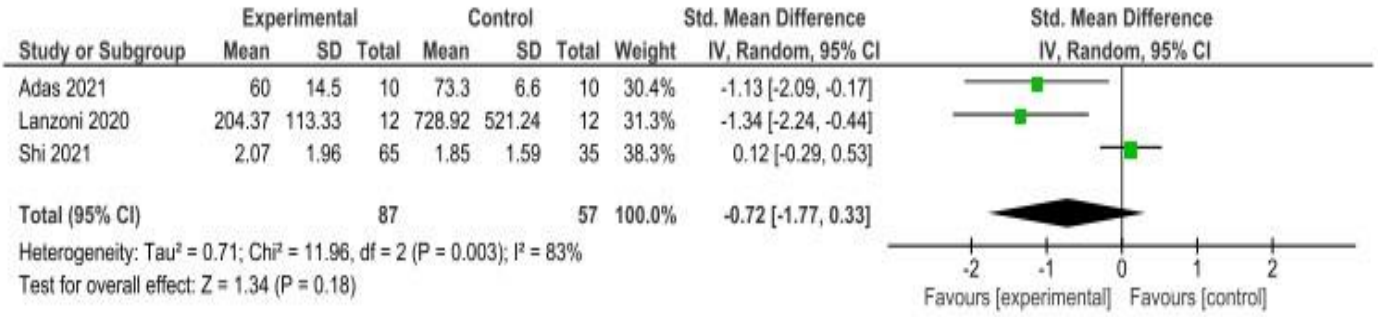


Figure S18. Forest plot demonstrating significntly reduced time (days) to improvement of clinical symptoms in patients administered MSCs (experimental) compared to controls. Control groups received standard of care for COVID-19 at the time of hospital admission, which varied depending on the institution.

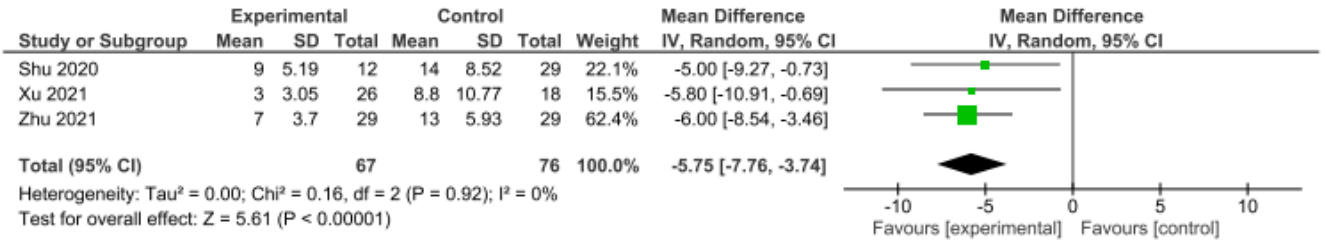
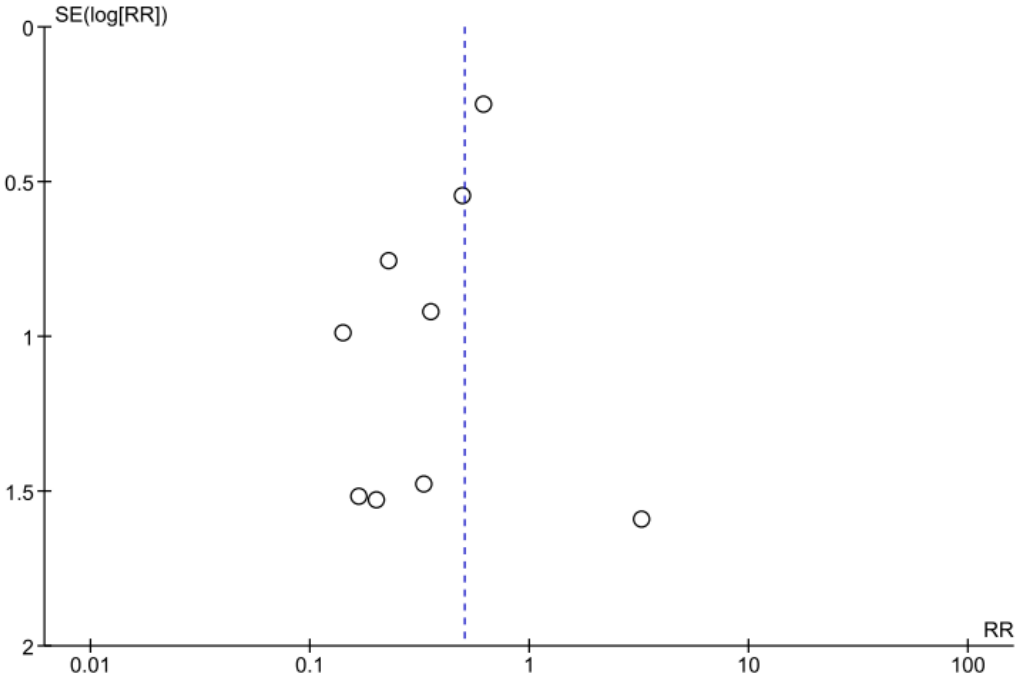


Figure S19. Funnel plot analysis assessing publication bias for relative (a) and absolute (b) risk of mortality at study endpoint. The asymmetrical nature of both funnel plots indicates the presence of publication bias.

a.



b.

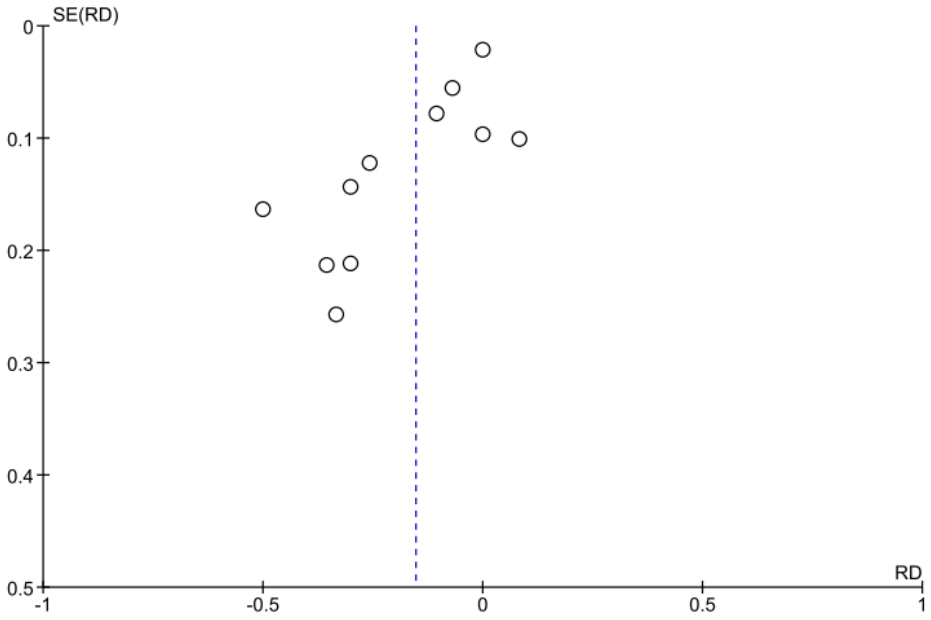


Figure S20. Systematic Literature Search Strategy (November 15th 2021)

Ovid MEDLINE(R) ALL <1946 to November 15, 2021>

1 COVID-19/ 118687
2 (exp coronavirus/ or coronavirus*.mp. or corona virus*.mp.) and (wuhan or beijing or shanghai).mp. 6006
3 ((coronavirus or corona virus) adj3 "2019").tw. 38689
4 (covid or covid2019).tw,kf. 175238
5 covid19.tw,kw. or covid 19.kf. 86882
6 sars cov 2.tw,kw. 63667
7 (ncov or n cov).tw,kw. 1887
8 novel coronavirus.tw,kw. 9959
9 sars cov2.tw,kw. 2595
10 Coronavirus Infections/ and Pandemics/ 39089
11 (ncov19 or ncov-19 or 2019-novel CoV).tw,kf. 376
12 or/1-11 197587
13 Mesenchymal Stromal Cells/ 44068
14 Mesenchymal Stem Cell Transplantation/ 13580
15 Multipotent Stem Cells/ 3185
16 (MSC or MSCs or ADMSC or ADMSCs or BM-MSC or BM-MSCs or BMD-MSC or BMD-MSCs or BMDMSC or BMDMSCs).tw,kw. 37763
17 (mesenchymal adj5 (cell* or stem or stromal or progenitor* or multipotent or bone marrow or adipose or placenta*)).tw. 88005
18 (mesenchymal and (cell* or stem or stromal or progenitor or multipotent or bone marrow or adipose or placenta*)).kw. 44
19 ((multipotent or multi-potent) adj (stroma* cell* or stem cell*)).tw,kf. 2132
20 (msc ev or msc cm).tw,kf. 454
21 (colony-forming adj2 fibroblast*).tw. 736
22 marrow stroma* cell*.tw.7522
23 Mesoderm/cy 5940
24 Exosomes/ or Extracellular Vesicles/ 14944
25 exosom*.tw,kw. 19843
26 Cell-Derived Microparticles/ 3401
27 (microvesicle* or micro vesicle*).tw,kw. 4768
28 ((cell or cells or extracellular) adj2 vesicle*).tw. 14955
29 microparticle*.tw,kw. 18557
30 (extracellular and vesicle*).kf. 7081
31 ((cell or cells) and vesicle*).kf. 2840
32 or/13-31 159275
33 12 and 32 666
34 ("2021073*" or 202108* or 202109* or 20211*).dt. 447043
35 33 and 34 125

Embase Classic+Embase <1947 to 2021 November 15>

1 (Coronavirinae/ or coronavirus*.mp. or corona virus*.mp.) and (wuhan or beijing or shanghai or hubei).mp. 7268
2 ((coronavirus* or corona virus* or coronavirus* or coronaviridae or coronaviridae or betacoronavirus*) adj3 ("19" or "2019")).tw. 45827
3 (covid or covid19).tw. 172037
4 sars cov 2.tw. 55447
5 (ncov or n cov).tw. 1986
6 (novel coronavirus* or novel corona virus*).tw. 9964
7 (CoV 2 or CoV2 or sarscov2 or 2019nCoV or novel CoV or wuhan virus).tw. 57606

8 (coronavirus infection/ or severe acute respiratory syndrome/) and (pandemic/ or pandemic*.tw.) 11154
9 limit 8 to yr="2019 -Current" 10384
10 1 or 2 or 3 or 4 or 5 or 6 or 7 or 9 192732
11 exp mesenchymal stem cell/ 59863
12 exp mesenchymal stem cell transplantation/ 13309
13 exp mesenchymal stroma cell/ 15336
14 (MSC or MSCs or ADMSC or ADMSCs or BM-MSC or BM-MSCs or BMD-MSC or BMD-MSCs or BMDMSC or BMDMSCs or BMSC or BMSCs or HBMSC or HBMSCs or ABMSC or ABMSCs or MAPC or MAPCs).tw. 66967
15 (((multipotent or multi-potent) adj3 (stem or stroma$1 or progenitor*)) and (cell$1 or cell-induced)).tw. 9787
16 multipotent stem cell/ 6292
17 (mesenchymal adj5 (cell* or stem or stroma* or progenitor* or multipotent or bone marrow or adipose or placenta*)).tw. 123035
18 (colony-forming adj2 fibroblast*).tw. 974
19 (marrow stroma* adj2 cell*).tw. 10808
20 exosome/ 38411
21 exosom*.tw. 25934
22 (microvesicle* or micro vesicle*).tw. 6556
23 microparticle*.tw. 24000
24 ((cell or cells or extracellular) adj2 vesicle*).tw. 20268
25 exp membrane microparticle/ 8782
26 (msc ev or msc cm).tw. 768
27 or/11-26 228504
28 10 and 27 846
29 ("2021073*" or 202108* or 202109* or 20211*).dc. 883461
30 28 and 29 262

EBM Reviews - Cochrane Central Register of Controlled Trials <October 2021>

1 COVID-19/ 737
2 (exp coronavirus/ or coronavirus*.mp. or corona virus*.mp.) and (wuhan or beijing or shanghai).mp. 213
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4 (covid or covid2019).tw,kw. 7791
5 covid19.tw,kw. or covid 19.kw. 457
6 sars cov 2.tw,kw. 2707
7 (ncov or n cov).tw,kw. 211
8 novel coronavirus.tw,kw. 465
9 sars cov2.tw,kw. 282
10 Coronavirus Infections/ and Pandemics/ 128
11 (ncov19 or ncov-19 or 2019-novel CoV).tw,kw. 43
12 or/1-11 8193
13 Mesenchymal Stromal Cells/ 2
14 Mesenchymal Stem Cell Transplantation/ 244
15 Multipotent Stem Cells/ 5
16 (MSC or MSCs or ADMSC or ADMSCs or BM-MSC or BM-MSCs or BMD-MSC or BMD-MSCs or BMDMSC or BMDMSCs).tw,kw. 1438
17 (mesenchymal adj5 (cell* or stem or stromal or progenitor* or multipotent or bone marrow or adipose or placenta*)).tw. 2149
18 (mesenchymal and (cell* or stem or stromal or progenitor or multipotent or bone marrow or adipose or placenta*)).kw. 804
19 ((multipotent or multi-potent) adj (stroma* cell* or stem cell*)).tw,kw. 21
20 (msc ev or msc cm).tw,kw. 6

21 (colony-forming adj2 fibroblast*).tw. 15
22 marrow stroma* cell*.tw.35
23 Mesoderm/cy 3
24 Exosomes/ or Extracellular Vesicles/ 33
25 exosom*.tw,kw. 279
26 Cell-Derived Microparticles/ 82
27 (microvesicle* or micro vesicle*).tw,kw. 71
28 ((cell or cells or extracellular) adj2 vesicle*).tw. 165
29 microparticle*.tw,kw. 612
30 (extracellular and vesicle*).kw. 1
31 ((cell or cells) and vesicle*).kw. 44
32 or/13-31 3712
33 12 and 32 124
34 limit 33 to yr="2021 -Current" 42

Supplementary Tables

Table S1. Risk of bias of randomized studies according to the Cochrane Risk of Bias tool 2.0

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Lanzoni 2020	+	+	+	+	+	+
Shu 2020	~	~	+	+	~	~
Adas 2021	+	+	+	+	+	+
Dilogo 2021	+	+	+	+	+	+
Shi 2021	+	+	+	+	+	+
Zhu 2021	~	+	+	+	+	~

Legend: +=Low risk of bias, ~=Some concerns, -=High risk of bias

Table S2. Risk of bias of non-randomized studies according to the ROBINS-I tool.

Study	Bias due to confounding	Bias in the selection of participants into the study	Bias in the classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of the outcomes	Bias in selection of the reported result	Overall Risk of Bias
Leng 2020	MR	LR	LR	LR	LR	MR	MR	MR
Meng 2020	MR	LR	LR	LR	LR	MR	MR	MR
Wei 2021	MR	LR	LR	LR	LR	MR	MR	MR
Haberle 2021	MR	LR	LR	LR	LR	MR	MR	MR
Xu 2021	MR	LR	LR	LR	LR	MR	MR	MR

Legend: LR=Low risk of bias, MR=Moderate risk of bias, SR=Serious risk of bias, CR=Critical risk of bias

Table S3. PRISMA Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	5-6
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	6
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	7
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	7, Fig S20
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Fig S20
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	7-8
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	7-8
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	7-8
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	7-8
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	8
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	8
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	8
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data	8

Section and Topic	Item #	Checklist item	Location where item is reported
		conversions.	
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	8
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	8-9
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	9
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	9
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	9
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	9
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	10, Fig 1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	10
Study characteristics	17	Cite each included study and present its characteristics.	10-12, Tables 1-5
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	16, Tables S1-S2
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	12-15, S1-S4, Figures 2-4, Figures S1-S18
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	10-16, Tables 1-5, Tables S1-S2
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	12-15, S1-S4, Figures 2-4, Figures S1-S18
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	13, Figures S1-S6
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	16, Figure S19
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	16, Figure S19
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	16-17, Table 6
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	18-21
	23b	Discuss any limitations of the evidence included in the review.	21-22

Section and Topic	Item #	Checklist item	Location where item is reported
	23c	Discuss any limitations of the review processes used.	21-22
	23d	Discuss implications of the results for practice, policy, and future research.	22-23
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	4,7
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	4,7
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	NA
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	4, 9
Competing interests	26	Declare any competing interests of review authors.	2
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	2

5. CONCLUSIONS

Summary of findings

Our published protocol for our living systematic review and meta-analysis[69] was paramount in establishing transparency in the methods we would follow when performing our living systematic review and included the eligibility criteria we would use in determining study inclusion, strategies we would employ to manage study records, techniques and tools we would use to extract and analyze data, and a tentative schedule for when the review would be updated. Moreover, this protocol paper gave us a platform to provide a rationale for why a living systematic review and meta-analysis was needed for this topic. Lastly, this protocol paper allowed us to communicate the importance of rigorous product characterization in the field of MSC-based regenerative medicine. We set an aggressive timeline for updating the review to match the rapid evolution of the pandemic and the expected rate of study publication based in the large number of registered trials identified in a scoping review of the literature[55].

Our first literature search (performed on February 3rd, 2021) yielded 9 studies (5 controlled, 4 uncontrolled) that met all criteria for inclusion in our review[70]. Pooling of study data demonstrated that MSCs had positive therapeutic effects in patients suffering from COVID-19. More specifically, meta-analysis revealed that MSCs significantly decreased the risk of death and IL-6 levels at study endpoint compared to controls. Other secondary outcomes could not be pooled through meta-analysis due to variable outcome reporting. Administration of MSCs to patients with COVID-19 was also found to be safe, with treatment related adverse events being mild and occurring less frequently in the MSC group compared to the control group. Most importantly no treatment-related SAEs were observed in patients administered MSCs. However,

the limited number of studies, considerable inter-study heterogeneity, inconsistent MSC characterization and appreciable RoB precluded any definitive estimates of efficacy. Although the first edition of our living systematic review found that the use of MSCs as a therapeutic intervention for COVID-19 appears to be safe and may improve a number of clinically relevant outcomes, several barriers were identified that prevented us from drawing more definitive conclusions. To overcome these barriers in future updates to this living systematic review, we drafted a preliminary framework consisting of seven criteria that we believe must be satisfied by future systematic reviews and meta-analyses for them to be considered as sufficient bodies of evidence to seek regulatory approval and clinical translation of MSCs as a therapeutic intervention for COVID-19. We postulated that satisfaction of all seven domains of this preliminary framework would indicate that the body of evidence presented in a meta-analysis is of high quality and relatively free of bias, which may in turn streamline the process of regulatory approval and mainstream clinical translation if MSCs were found to be safe and effective. We reasoned that this accelerated timeline was of critical importance if MSCs were to ever achieve widespread regulatory approval and mainstream clinical translation given the rapidly progressing nature of the COVID-19 pandemic.

To strengthen our conclusions and reduce the risk of bias, we only included controlled clinical studies in this second edition of our living systematic review. Our second literature search was conducted on November 15th, 2021 and identified eleven controlled studies that met all inclusion criteria. All eleven studies reported mortality as the primary outcome. As with the first iteration of our living systematic review, patients who were administered MSCs were found to have a decreased risk of death at study endpoint compared to controls. Furthermore, subgroup analysis revealed that MSCs decreased the risk of death at 28 days after treatment compared to

controls. In terms of secondary outcomes, patients that were administered MSCs had significantly shorter length of hospital stay and increased oxygenation levels at study endpoint compared to controls. Despite these promising results, only two of the eleven studies reported on all minimal ISCT criteria[53], and seven of the eleven studies had a rating of either "some concerns" or moderate with regards to risk of bias. Thus, although this second edition of our living systematic review continued to demonstrate promising trends regarding the safety and efficacy of MSCs as a therapeutic intervention for COVID-19, inter-study heterogeneity, inadequate MSC product characterization and risk of bias continue to hamper the strength of our conclusions. To promote improved quality of future studies in this field and strengthen conclusions of subsequent systematic reviews and meta-analyses, we proposed a framework to guide regulatory approval and clinical translation of MSCs for COVID-19, building off our framework that was designed to enhance the quality of evidence reviews. We termed this framework the Framework for the Accelerated Synthesis of Trial Evidence for Rapid Approval "*FASTER Approval*" criteria. This framework consists of seven domains that should be satisfied in order for a meta-analysis of MSCs as a therapeutic intervention for COVID-19 to be considered as a sufficient body of evidence to support applications for regulatory approval and clinical translation. We then evaluated the extent to which the evidence from the second iteration of our review addressed the criteria in our framework and concluded that the current body of evidence surrounding the use of MSCs as a therapeutic intervention for COVID-19 is not sufficient to seek regulatory approval. Until the barriers to providing more definitive conclusions are addressed, the findings of future systematic reviews and meta-analysis in this area remain unlikely to be definitive enough to draw firm conclusions regarding the safety and efficacy of MSCs as a therapeutic intervention for COVID-19. To overcome these barriers, a master

protocol for the treatment of COVID-19 using MSCs should be designed and implemented for future clinical studies in this area. Such a master protocol could be based off the seven key domains identified in our *FASTER Approval* criteria, which would provide clear study enrollment targets, standardized study protocols, common outcome measures, risk of bias reduction strategies and ensure that MSC products used are characterized according to minimal ISCT criteria. Regulation of such factors in future studies would lead to more effective pooling of study results and strengthen the conclusions of future systematic reviews, which may in turn speed up regulatory approval and mainstream clinical translation of MSCs for COVID-19. Funding for future MSC-based trials could be tied to adherence to a master protocol to encourage more responsible use of public research funding.

Discussion of collective significance

COVID-19 is a heterogeneous disease with regards to presenting symptoms, severity and organs affected[15]. Although various non-vaccine therapeutics such as antivirals, monoclonal antibodies and antibody cocktails have demonstrated benefits in the context of COVID-19[71, 72], they have one key limitation. Antivirals and antibody-based therapies work by targeting SARS-CoV-2 viral replication and cell entry[73,74]. Blockage of viral replication and cell entry keeps the viral titer at levels that can be managed by the body's own immune system, which has some capacity to prevent the spread of SARS-CoV-2 viral particles on its own[75]. However, these therapeutics are only effective early in the course of SARS-CoV-2 infection when patients have low viral titers[76,77]. Thus, these therapeutics are ineffective for patients presenting to hospital with severe or critical COVID-19 who are in the later stages of viral infection as the viral titers are too high for these drugs to effectively control viral replication and spread[76,77]. As most of the patients in our review presented with severe or critical COVID-19, the efficacy we observed in

the two updates of our living systematic review may indicate that MSCs could fill an unmet therapeutic need by helping improve outcomes in patients suffering from severe or critical COVID-19.

Vaccines are by far the most effective means to prevent COVID-19 related morbidity, hospitalization and mortality[78]. Briefly, vaccines work by introducing an antigen (protein, nucleic acid, attenuated viral particle, etc.) into the body, which is recognized as foreign by both innate (macrophages, neutrophils, etc.) and adaptive (B cells and T cells) immune cells[79,80]. Resolution of the immune response is followed by the development of a repertoire of memory adaptive immune cells (i.e. memory B cells and T cells)[79,80]. Upon antigen rechallenge, these memory cells can respond and carry out their anti-pathogenic actions much more rapidly than when they encounter a novel pathogen for the first time[79,80]. In the case of SARS-CoV-2 infection, this involves the production of antibodies directed against various structures of the SARS-COV-2 virion (S protein, nucleocapsid, etc.) or cytotoxic activity directed towards SARS-CoV-2 infected cells[79,80]. In contrast, the therapeutic actions of MSCs in the context of COVID-19 have been shown to be immunomodulatory and tissue reparative in nature[81,82]. As MSCs do not exert their therapeutic effects through direct targeting of viral pathogens, their exact role in the context of widespread SARS-CoV-2 vaccination remains unclear. One possibility is that MSCs may be a useful adjuvant therapy to be used in conjunction with vaccination[83]. Although vaccination should be the primary strategy to prevent COVID-19 related morbidity, hospitalization, and mortality, MSCs could serve as a useful therapeutic for patients that display decreased efficacy in potentiating vaccine induced cellular and/or humoral immune responses. These include patients with hematological malignancies patients on immunosuppressive drugs and/or patients of advanced age[84-86]. These patients are more susceptible to SARS-CoV-2

reinfection and adverse COVID-19 outcomes following vaccination than the general population[84-86]. Thus, having MSCs available as an adjuvant therapy to stabilize patients in these categories who present to hospital with severe or critical COVID-19 would be extremely beneficial.

Various anti-inflammatory drugs (glucocorticoids, tocilizumab, etc.) have been used to lessen morbidity and mortality in acutely ill COVID-19 patients with some success[87,88]. However, although these anti-inflammatory drugs effectively downregulate inflammation, they lack tissue reparative capabilities[87,88]. Moreover, many of these anti-inflammatory agents only target one unique inflammatory medicator out of the many which contribute to the development of the cytokine storm[87,88]. MSCs have been shown to possess both immunomodulatory and tissue reparative capabilities[82,83]. Moreover, the anti-inflammatory actions of MSCs are directed towards nearly all cytokine storm contributors[89]. Thus, MSCs serve as an ideal all in one therapeutic to target both the acute inflammatory cytokine storm while also repairing any tissues that have been damaged through hyperinflammation.

Supplementary oxygen and mechanical ventilation have become mainstay treatments for patients deteriorating rapidly due to COVID-19 induced ARDS[90,91]. These interventions were used frequently during the early stages of the pandemic when a significant portion of patients were presenting to hospital with life threatening COVID-19 symptoms[92,93]. Despite their lifesaving potential in certain patients, these interventions simply provide additional oxygen or ventilatory capacity without targeting the underlying pathways and mechanisms contributing to ARDS pathology[94,95]. Although little is known regarding the specific pathophysiological mechanisms of COVID-19 induced ARDS, studies of ARDS caused by other viral pathogens has revealed that ARDS is largely the result of the non-specific cytotoxic actions of infiltrating

immune cells (neutrophils, NK cells, macrophages, etc.) on lung epithelial cells[96,97]. MSCs may help dampen the pro-inflammatory and cytotoxic actions of these infiltrating immune cells by polarizing them towards and anti-inflammatory phenotypes[98,99]. Moreover, MSCs may also help repair any tissue damage contributing to ARDS, such as restoring the integrity of the alveolar-capillary membrane[100,101]. Taken together, MSCs are an attractive treatment for patients with COVID-19 induced ARDS due to their ability to attenuate hyperinflammation and repair damaged tissues. Furthermore, MSCs may be a particularly useful treatment for COVID-19 induced ARDS in situations where the number of ventilators is limited, or in cases where there are more patients requiring ventilation than there are ventilators available. Such conditions were encountered in the early stages of the pandemic[93] and may become a problem again with SARS-CoV-2 escape variants raising the possibility of future waves of severe COVID-19[102].

Unanswered questions and future directions

Given the current trajectory of the COVID-19 pandemic, it appears highly unlikely that MSCs will ever achieve regulatory approval and mainstream clinical translation as a therapeutic intervention for COVID-19. Moreover, MSCs are not currently listed as a recommended treatment for COVID-19 in guidelines published by any of the major health policy organizations (WHO, NIH, NHS, Health Canada, etc.). This is a missed opportunity given the significant funds allocated to development of this therapy and immense promise that was displayed in successive updates of our living systematic review. Although the regulatory approval and mainstream clinical translation of MSCs for COVID-19 may never be realized, future investigators can learn from the pitfalls encountered in this avenue of MSC research and avoid them when conducting clinical trials of MSCs for other diseases and conditions.

One of the major hurdles encountered in performing this living systematic review was the presence of inter-study heterogeneity. Unfortunately, inter-study heterogeneity is a phenomenon that continues to plague the field of MSC research. A scoping review performed by Rizik *et al* in 2016[103] which examined the use of MSCs to treat or prevent graft-versus-host disease (GVHD) revealed the same common sources of inter-study heterogeneity encountered in the studies we included in our living systematic review, such as inconsistent patient populations, diverse MSC tissue sources, variable administration strategies, failure to characterize MSCs according to minimal ISCT criteria[53] and lack of common outcome reporting[103]. Reducing these sources of inter-study heterogeneity will be paramount if MSCs are ever to achieve regulatory approval and mainstream clinical use for both COVID-19 and the many other diseases and conditions for which they have demonstrated immense promise. One such way to ensure that these aspects are standardized across future clinical trials would be to develop master protocols[104]. Such master protocols would provide recommendations pertaining to sample size, patient populations, MSC product characteristics, outcome measures and procedures to reduce RoB (randomization, blinding, etc.) that should be followed for all future clinical trials of MSCs for a given disease or condition. Standardization of these aspects across future clinical studies would allow for more informative pooling of the results of individual studies through meta-analysis, which would in turn increase the strength of the conclusions of future systematic reviews and potentially accelerate regulatory approval and mainstream clinical translation.

Failure to standardize and characterize MSCs continues to be a persistent problem despite the publication of two successive ISCT guidelines[52,53]. Proper MSC characterization is paramount to predictable therapeutic success and anticipated safety profile in the patients to whom they are being administered[105]. Moreover, this lack of effort towards characterization and

standardization of therapeutic product may be one reason why so many MSC based therapies that have shown promise in preclinical studies fail to successfully translate to clinical trials and mainstream clinical use[106]. The FDA has even released a perspective paper highlighting the fact that as more proposals are being submitted to the FDA seeking regulatory approval of MSC-based products, the MSC products themselves are becoming increasingly diverse[107]. As regulatory approval and subsequent mainstream clinical use of MSCs requires rigorous product characterization in addition to a predictable safety and efficacy profile, the field appears to be going in the opposite direction by increasing heterogeneity amongst MSC products rather than striving to achieve consensus. If regulatory approval and mainstream clinical translation of MSC based therapies is ever to be realized, the field must coalesce around common MSC isolation and characterization methods as a most basic step in the journey of achieving regulatory approval and mainstream clinical translation of MSC based products. One way to achieve this would be to ensure that MSC products used report on all minimal criteria put forth in the latest edition of the ISCT criteria[53]. Although there is currently debate on whether the ISCT criteria are truly an accurate and effective way to characterize MSC products[108,109], no other consensus guideline currently exists. Until then, studies in the field should strive to use products characterized according to ISCT criteria guidelines[53] to achieve product uniformity, which will be a critical step to achieving regulatory approval and mainstream clinical translation.

Interestingly, this lack of adequate characterizing of cell products in regenerative medicine does not appear to be unique to the field of MSC research. A systematic review performed by Liao *et al* in 2020 examining the use of Endothelial colony-forming cells (ECFCs) as a therapeutic intervention for a wide range of diseases in preclinical animal models had similar findings of inter-study heterogeneity regarding ECFCs characterization[110]. Like our

living systematic review, this review noted that lack of standardized cell characterization severely limited the strength of its conclusions, which precluded any definitive estimate of safety and efficacy[110]. Of note, a similar consensus guideline exists for the characterization of ECFCs according to a set of minimal criteria, which is termed the Medina criteria[111]. Altogether, both the ISCT and Medina criteria allow for more robust and definitive inter-study comparisons and pooling of results from individual studies. This greatly reduces RoB and increases the strength of conclusions of systematic reviews and meta-analyses. We believe that robust meta-analysis may form the basis for accelerated regulatory approval and mainstream clinical translation of cell therapies as per the conditions laid out in our *FASTER Approval* criteria. Though the specific sub criteria laid out in our *FASTER Approval* criteria are specific to the use of MSC based therapies for COVID-19, we believe that the seven main domains that form the basis of this criteria are applicable to the use of MSC-based products for a variety of diseases and conditions. It has been suggested that cell and gene therapies which target underlying biological mechanisms in a holistic manner are the future of regenerative medicine[112,113]. However, the major difference between conventional classes of drugs (i.e. pharmacological agents pharmacological agents) and cell-based therapeutics (MSCs ECPCs, etc.) is that the safety, therapeutic efficacy and *in vivo* persistence of conventional drugs is much more predictable[114,115]. This predictable safety and efficacy profile likely led to these drugs receiving regulatory approval in relatively rapid fashion[114,115]. In contrast, cell-based therapies continue to present challenges in these key domains, preventing their regulatory approval and widespread clinical implementation[106,107]. Should we one day be able to enrich for cell populations with predictable efficacy and safety profiles, mainstream clinical translation of cell-based therapies for regenerative medicine may

one day become a reality. Until then, pharmacological agents and other classes of drugs with more predictable *in vivo* effects will continue to dominate the medical field.

The journey to regulatory approval for Darvadstrocel (Alofisel®) highlights a case where a standardized master protocol may have been beneficial to accelerate the process of regulatory approval[116]. Darvadstrocel is an expanded human allogeneic adipose-derived MSC product that was granted regulatory approval by the EMA for the treatment of complex perianal fistulas in adult patients with non-active/mildly active luminal Crohn's disease[117]. In terms of the timelines to achieve of regulatory approval, Darvadstrocel was first designated as an orphan medicine in October 2009[117]. Following several years of demonstrated safety and efficacy across a number of large-scale clinical trials[118-120], Darvadstrocel was finally granted regulatory approval by the European Medicines Agency (EMA) in March 2018[117]. Although regulatory approval was eventually achieved, this process may have been accelerated through the development of a master protocol[104]. Interestingly, such master protocols were published shortly following the approval of Darvadostrel[121,122]. However, the EMA also published its own master protocol for treatment of perianal fistulas with Davadrostel in conjunction with its regulatory approval[117], making such protocols redundant. Development of such protocols ahead of time could have saved time and resources by reducing inter-study heterogeneity and making studies more amenable to pooling through meta-analysis[104]. This would have allowed investigators to achieve satisfactory sample sizes to detect statistically meaningful differences on a shorter timescale, which may have in turn have allowed investigators to seek regulatory approval much sooner than the nine-year period between orphan medicine designation and mainstream clinical translation of Davadrostel. Thus, although the regulatory approval of Davadrostel is an immense success story in the field of translational MSC research, investigators seeking regulatory approval of MSCs for

different diseases and conditions in the future should employ a master protocol to accelerate the process of regulatory approval and mainstream clinical translation. These master protocols would be specific for each disease and condition and provide guidance regarding recommended sample sizes, patient populations, MSC product characteristics, administration strategies, outcome measures and blinding procedures to be followed[104]. This would allow for standardization of these aspects across individual clinical studies, which would reduce RoB and make future trials more amenable to pooling through meta-analysis.

Another future research direction would be to determine whether the observed therapeutic benefits and lack of adverse events observed in patients administered MSCs for COVID-19 are also observed in patients administered MSC secreted factors, such as MSC extracelllular vesicles (MSC-EVs) or MSC conditioned media (MSC-CM). It has been demonstrated that MSC secreted factors contain many of the same active biomolecules possessed by their parent cells (proteins, lipids, nucleic acids)[123-124]. Furthermore, preclinical studies have suggested that MSC secreted factors have comparable therapeutic benefits to their parent cells in a number of COVID-19 related diseases and conditions[125-126]. Moreover, several review articles and editorials have suggested that MSC secreted factors possess many benefits that may make them even more attractive for use in COVID-19 patients compared to their parent cells such as lower immunogenicity and ease of storage[127]. Of particular note, it has been suggested that MSC exosomes may be especially beneficial in the context of COVID-19 as they can be administered through aerosol inhalation[128]. Aerosol inhalation of exosomes would allow for the delivery of MSCs based products directly to the lungs[128], which is the organ most frequently damaged in COVID-19 patients[129]. Furthermore, aerosol administration of MSC exosomes could provide an option for use of MSC based products the outpatient setting. This could be useful in improving

therapeutic outcomes of patients in areas that lack adequate hospital facilities to provide conventional MSC therapy and might also help prevent nosocomial COVID-19 outbreaks as COVID-19 patients could receive MSC based care from home rather than an inpatient setting. Thus, future clinical trials of MSC secreted factors as a therapeutic intervention for COVID-19 should be performed to ascertain the safety and efficacy of MSC secreted factors for patients with COVID-19 compared to their parent cells.

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