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Full-length article

Systematic review and meta-analysis of randomized controlled trials of mesenchymal stromal cells to treat coronavirus disease 2019: is it too late?

Aidan M. Kirkham[1,6], Adrian J.M. Bailey[6], Risa Shorr[8], Manoj M. Lalu[2,3,6,7,9], Dean A. Fergusson[4,5,6,10], David S. Allan[1,4,6,7,10,*]

[1] Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Canada
[2] Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Canada
[3] Department of Anesthesiology and Pain Medicine, University of Ottawa, Ottawa, Canada
[4] Department of Medicine, University of Ottawa, Ottawa, Canada
[5] Department of Epidemiology and Public Health, Faculty of Medicine, University of Ottawa, Ottawa, Canada
[6] Clinical Epidemiology, Ottawa Hospital Research Institute, Ottawa, Canada
[7] Regenerative Medicine, Ottawa Hospital Research Institute, Ottawa, Canada
[8] Medical Information and Learning Services, The Ottawa Hospital, Ottawa, Canada
[9] Department of Anesthesia, The Ottawa Hospital, Ottawa, Canada
[10] Department of Medicine, The Ottawa Hospital, Ottawa, Canada

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ABSTRACT

Background aims: Evidence regarding the extent that mesenchymal stromal cells (MSCs) may improve clinical outcomes in patients with coronavirus disease 2019 (COVID-19) has been limited by marked inter-study heterogeneity, inconsistent product characterization and appreciable risk of bias (RoB). Given the evolution of treatment options and trajectory of the pandemic, an updated analysis of high-quality evidence from randomized controlled trials is needed for a timely and conclusive understanding of the effectiveness of MSCs.
Methods: A systematic literature search through March 30, 2022, identified all English language, full-text randomized controlled trials examining the use of MSCs in the treatment of COVID-19.
Results: Eight studies were identified (316 patients, 165 administered MSCs and 151 controls). Controls evolved significantly over time with a broad range of comparison treatments. All studies reported mortality at study endpoint. Random effects meta-analysis revealed that MSCs decreased relative risk of death (risk ratio, 0.63, 95% confidence interval, 0.42–0.94, $P = 0.02$, $I^2 = 14\%$) with no significant difference in absolute risk of death. MSCs decreased length of hospital stay and C-reactive protein levels and increased odds of clinical improvement at study endpoint compared with controls. Rates of adverse events and severe adverse events were similar between MSC and control groups. Only two (25%) studies reported all four International Society for Cell & Gene Therapy criteria for MSC characterization. Included studies had low (n = 7) or some (n = 1) concerns regarding RoB.
Conclusions: MSCs may reduce risk of death in patients with severe or critical COVID-19 and improve secondary clinical outcomes. Variable outcome reporting, inconsistent product characterization and variable control group treatments remain barriers to higher-quality evidence and may constrain clinical usage. A master protocol is proposed and appears necessary for accelerated translation of higher-quality evidence for future applications of MSC therapy.

Introduction

Improved treatment options for coronavirus disease 2019 (COVID-19) remain relevant as the pandemic evolves given continued waves of hospitalization, incomplete vaccination coverage, variable patient responses to vaccines and the potential for new variants of the severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) virus to emerge [1–3]. Mesenchymal stromal cells (MSCs) have emerged as a promising therapy given their immunomodulatory and tissue reparative capabilities [4–6]. At the start of the pandemic, many studies of MSCs for COVID-19 were launched [7]. Moreover, the two previous iterations of the authors' living

* Correspondence: David S. Allan, MD, Clinical Epidemiology, Ottawa Hospital Research Institute, 501 Smyth Rd, Box 704, Ottawa K1H 8L6, Canada.
E-mail address: daallan@toh.ca (D.S. Allan).

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systematic review and meta-analysis indicated a potential reduction in mortality in severe and critical cases of COVID-19 [8,9]. However, because of modest enrollment targets, variable study methodology and inconsistent MSC product characterization, individual studies are insufficiently powered to determine safety and efficacy on their own [10,11]. A timely assessment of evidence would require combining the results of individual studies through meta-analysis, which is strengthened when common outcomes are reported and studies are sufficiently similar [12,13]. Product characterization, risk of bias (RoB) in published reports and variable outcome reporting were identified as key barriers in previous iterations of the authors' living systematic review [8,9]. To augment the quality of evidence synthesized from published studies at this juncture in the pandemic, a focus on randomized controlled trials (RCTs) now appears most appropriate [14]. Without sound evidence now, it seems highly unlikely that clinical trials of MSCs for COVID-19 will recruit enough patients to have an impact on the pandemic [15,16]. However, preparing for future threats or considering MSCs in the treatment of related conditions seems worthwhile [17] and warrants a proposed strategy for coordinated research using MSCs that could yield improved quality of evidence on a faster timeframe.

MSCs are multipotent stem-like cells that can be isolated from a variety of adult and neonatal tissues, including adipose, bone marrow, umbilical cord and placenta [18]. The therapeutic mechanisms of MSCs may be mediated through the release of paracrine factors, which contain the same therapeutic molecules (microRNAs, proteins, lipids, etc.) as their parent cells [19]. This allows MSCs to exert their therapeutic effects without cell engraftment. Moreover, the therapeutic efficacy of MSCs is independent of the SARS-CoV-2 viral strain under consideration [6,20], which may offer a solution to reduced efficacy in the context of SARS-CoV-2 escape variants, which may evolve to evade targeted treatments such as antiviral drugs and monoclonal antibodies over time [21,22].

Previous iterations of the authors' living systematic review suggested that MSCs could reduce COVID-19-related mortality and improve a number of important secondary clinical outcomes (length of hospitalization, oxygenation index, levels of pro-inflammatory mediators, etc.) [8,9]. Moreover, treatment-related adverse events were generally mild, and rates of severe adverse events were low and not increased between MSC and control groups. However, confidence in the conclusions drawn from the authors' meta-analyses continues to be limited by a lack of definitive estimates of safety and efficacy, variability in study design and outcome reporting and inconsistent MSC product characterization that infrequently aligned with published International Society for Cell & Gene Therapy (ISCT) guidelines. Moreover, considerable RoB was detected. By including only high-quality studies such as RCTs, the authors will provide more robust estimates of the safety and efficacy of MSCs as a therapeutic intervention for COVID-19. The authors' analysis will also provide the rationale and basis for a master protocol that could guide future studies of MSC-based therapy to accelerate the timelines for obtaining high-quality evidence.

Methods

This systematic review is reported in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (see supplementary Table 3) [23]. The study protocol has been registered at the International Prospective Register of Systematic Reviews (CRD42021225431) and published in *Systematic Reviews* [24]. The first and second editions of this living systematic review were published in *Cytotherapy* [8] and *Stem Cells Translational Medicine* [9], respectively.

Literature search strategy

The literature search strategy was performed as described in the first edition of the authors' living systematic review [8] and updated to March 30, 2022 (i.e., 1947 to March 30, 2022) (see supplementary Figure 15). In this third iteration of the living systematic review, the authors included only RCTs examining the use of MSCs as a therapeutic intervention for COVID-19.

Eligibility criteria

The authors' eligibility criteria were similar to those described in previous editions of the living systematic review [8,9]. However, the authors included only RCTs to minimize bias and strengthen the conclusions. Non-RCTs, uncontrolled clinical trials, case series, reviews, commentaries, editorials, letters, case reports, conference abstracts, unpublished gray literature and other study types (*in vitro* studies, pre-clinical animal studies, etc.) were excluded.

Outcomes

Mortality at study endpoint and mortality at 28 days were the authors' primary outcomes for meta-analysis. The authors' secondary outcomes 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 the hospital, length of time in the ICU, length of time on mechanical ventilation, circulating levels of immune cells, pro-inflammatory cytokines and anti-inflammatory cytokines and occurrence of adverse events.

Study selection and data extraction

Study selection, data extraction and data analysis steps were performed as described in previous editions of the authors' living systematic review [8,9]. As all included studies were RCTs, RoB was assessed using the Cochrane Risk of Bias 2 tool [25].

Data analysis

Results from individual studies were pooled for meta-analysis using RevMan 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), risk differences (RDs) and odds ratios between control and experimental groups were calculated for each relevant outcome. For certain outcomes, both RRs and RDs 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-analysis. SMD was applied for analyses of inflammatory markers (IL-6, ferritin, C-reactive protein [CRP]), as it was anticipated that these outcomes would vary substantially according to time of measurement. Moreover, mean of the medians and 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 [26]. All data are presented with 95% confidence intervals (CIs). Meta-analysis was performed only for quantitative outcomes that were reported in three or more studies in a format amenable to pooling. Outcomes were analyzed descriptively if they were reported in less than three studies or where quantitative data were reported in a format in which pooling of individual study results was not possible. 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 with their secretome, MSCs

treated and/or genetically modified before administration or isolation of paracrine factors, COVID-19 severity, presence of comorbidities, patient age, patient sex, geographic location and type of funding support for the study) were determined *a priori* in the authors' published protocol article [24]. Publication bias was not assessed, as less than 10 studies were included in this edition of the living systematic review.

Results

Literature search

The authors' systematic literature search yielded 712 unique citations. After title/abstract and full text screening, eight studies [27–34] met all criteria for inclusion in the authors' review. Reasons for study exclusion at the full-text stage included wrong publication type (n = 39), different outcome (n = 20), uncontrolled design (n = 9), MSCs not studied (n = 6) and language and wrong population studied (n = 3) (Figure 1).

Study characteristics

Characteristics of the eight included studies are summarized in Table 1. All eight studies were RCTs [27–34]. Study publication dates ranged from August 18, 2020 [27], to March 21, 2022 [33]. Two of the studies were conducted in China [27,30], one was conducted in the USA [28], one was conducted in Indonesia [29], one was conducted in

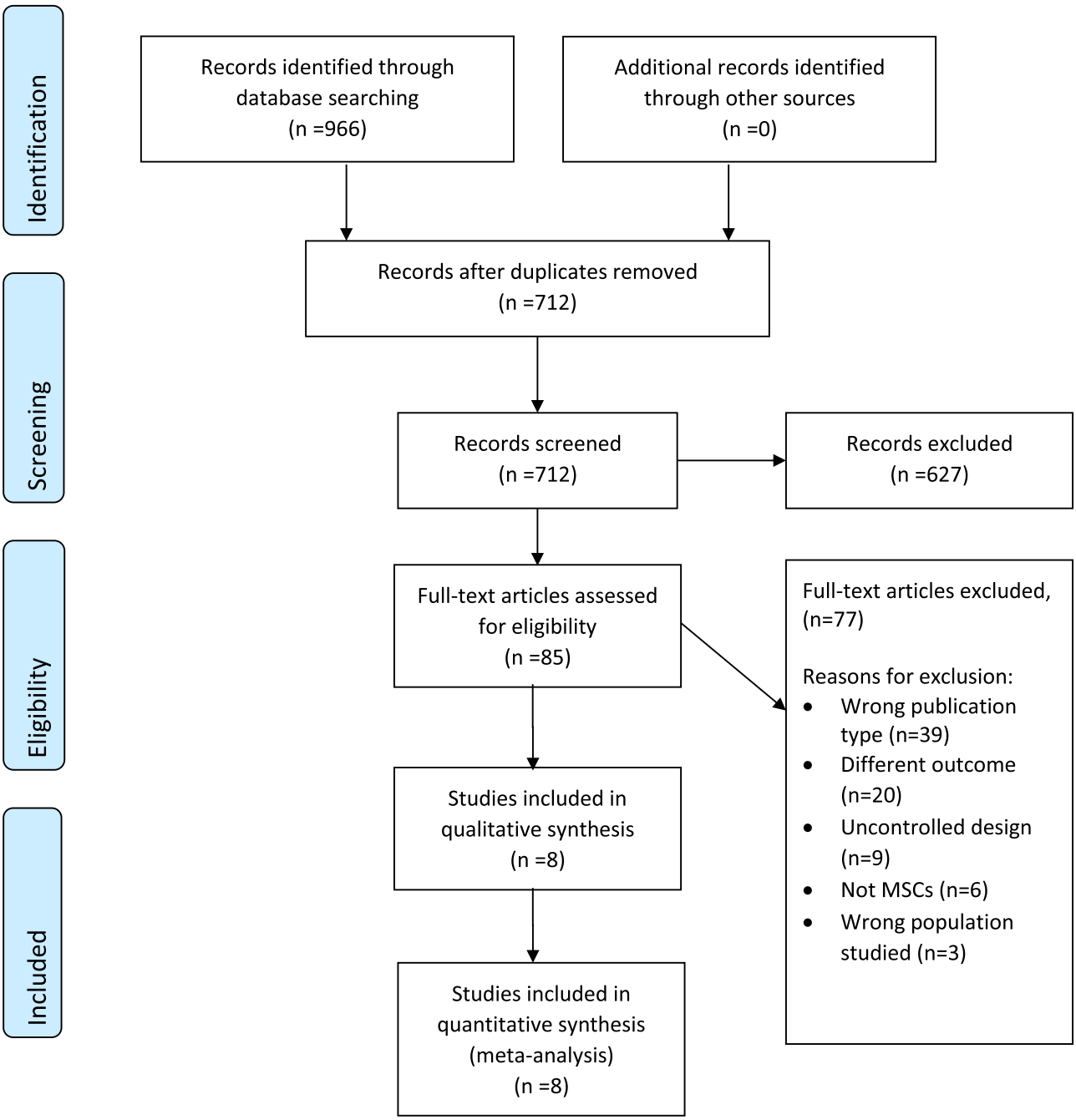


Fig. 1. Results of third systematic search of the literature. MEDLINE, Embase and Cochrane Central Register of Controlled Trials searched from 1947 through March 30, 2022.

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	316	151	165
Sex, male, %	64.2	64.9	63.6
Age, years, mean (SD)	58.0 (4.7)	58.8 (3.3)	57.2 (5.9)
COVID-19 severity, n (%)			
Mild	20 (6.3)	10 (6.6)	10 (6.1)
Moderate	22 (7.0)	12 (7.9)	10 (6.1)
Severe	197 (62.3)	93 (61.6)	104 (63.0)
Critical	77 (24.4)	36 (23.8)	41 (24.8)
Comorbidities, n (%)			
Hypertension	107 (33.9)	53 (35.1)	54 (32.7)
Diabetes	70 (22.2)	35 (23.2)	35 (21.2)
Obesity	25 (7.9)	8 (5.3)	17 (10.3)
Chronic obstructive pulmonary disease	4 (1.3)	2 (1.3)	2 (1.2)
Coronary artery disease	9 (2.8)	5 (3.3)	4 (2.4)
Congestive heart failure	2 (0.6)	1 (0.7)	1 (0.6)
Chronic kidney failure	8 (2.5)	5 (3.3)	3 (1.8)
Other[a]	37 (11.7)	16 (10.6)	21 (12.7)
Median follow-up, days (range)	28 (14–122)	28 (14–122)	28.8 (14–122)

SD, standard deviation.
[a] Includes heart disease, tuberculosis, chronic bronchitis, atrial fibrillation, stroke, schizophrenia and cancer.

Turkey [31], one was conducted in Iran [32], one was conducted in Brazil [33] and one was conducted in France [34].

Participant characteristics

A total of 316 patients (mean age, 58.0 ± 4.7, male, 203) were enrolled across all study groups, with 165 (mean age, 57.2 ± 5.9, male, 105) being administered MSCs and 151 (mean age, 58.8 ± 3.3, male, 98) serving as controls. The overall distribution of disease severity was similar for patients in the intervention and control groups, with 62.3% and 24.4% of all patients having severe and critical COVID-19, respectively (Table 1). In terms of patient comorbidities, those associated with worse COVID-19 outcomes, such as hypertension, obesity, diabetes, chronic kidney failure, chronic obstructive pulmonary disease and coronary artery disease, were well balanced between the control and MSC groups.

Intervention characteristics

Intervention characteristics are summarized in Table 2. Seven of the eight studies used MSCs [27–31,33,34], and one used MSC-derived secretome [32]. All MSCs were derived from allogeneic human tissues, including umbilical cord tissue (n = 7) [27–31,33,34] and menstrual blood (n = 1) [32]. The passage number in *ex vivo* culture of MSCs varied widely between studies (passage two to passage six) (Table 2), with one of the studies [28] 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 [35] for MSC characterization, only two of the eight included studies reported sufficient information to satisfy all four minimal criteria laid out in the latest guidance document published by the ISCT. Details regarding the number of studies reporting each of the four ISCT criteria can be found in Table 2.

MSC dosing and administration strategies varied between studies. Three formats for MSC dosing were reported: cells/kg of body weight ($1–3 \times 10^6$ cells/kg, n = 5), total cells/injection ($1.2–2.0 \times 10^8$ cells, n = 2) and milliliters of MSC secretome (25 mL, n = 1). In five of the studies, MSCs were cryopreserved prior to administration. The remaining three studies did not report whether their MSC-derived products were administered fresh or were cryopreserved. All eight studies used MSC-derived products administered intravenously.

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

Intervention	Studies, n
MSC tissue source	
Umbilical cord blood or tissue	7
Menstrual blood	1
MSCs, fresh or frozen/cryopreserved	
Fresh	0
Cryopreserved	5
Not stated	3
Product dose	
MSCs, cells/kg (n = 5)	$1–3 \times 10^6$
MSCs, total cells (n = 2)	$1.2–2.0 \times 10^8$
MSC secretome, mL (n = 1)	25
Route of administration	
Intravenous	8
No. patient MSC infusions (%)	
1	32 (19.4)
2	12 (7.3)
3	107 (64.8)
5	14 (8.5)
MSC passage number	
2	1
4	2
5	1
3–5	2
5–6	1
Not stated	1
ISCT criteria	
Fully met criteria, n	2
Plastic adherence	3
Trilineage differentiation	2
Positive/negative surface markers	7
	6
MSC viability	

Most (64.8%, n = 107) patients received three infusions of MSC-derived products, although other studies reported administering one, two or five MSC product infusions (Table 2). The reported time from COVID-19 diagnosis to intervention was similar between control (median, 12.4 days, range, 1–47) and MSC (median, 11.1 days, range, 1–45) groups.

Patients were administered other therapeutic agents in addition to MSCs in all eight included studies. The specific therapeutic agents administered to patients varied considerably between studies and are summarized in Table 5. However, the specific concomitant therapies administered to MSC and control patients in each individual study were well balanced. Two of the studies indicated that other therapeutics were used in addition to MSCs, but the researchers did not specify exactly which therapeutic agents were used [28,32]. The mean follow-up period was 28 days for both the control and MSC groups (Table 1).

Outcome reporting

Outcome reporting across studies was variable, with only a few outcomes, including mortality and levels of pro-inflammatory cytokines, being reported in all eight studies. Outcomes such as number of patients on mechanical ventilation, time to improvement of clinical symptoms and improvement in radiological parameters and viral load were reported in fewer than half of the studies. An overview of outcomes reported across studies is shown in Table 3.

Primary outcome analysis: mortality

All eight studies reported mortality at study endpoint, which ranged from 14 days to 122 days. Mortality rate at the study endpoint for patients in the control groups was 49 of 151 patients (32.5%)

Table 3
Reported outcomes in clinical studies examining MSCs as a therapeutic intervention for COVID-19.

Study	Mortality rate	Diagnosis to intervention, time	Intervention to recovery, time	No. patients hospitalized	No. patients on supplemental oxygen	No. patients on ventilator	Time in hospital	Progression of symptoms	Improvement in symptoms	Time to clinical improvement	Oxygenation level	Immune cell level	Pro-inflammatory cytokines	Anti-inflammatory cytokine s	Viral load	Radiological outcome
Shu [27]	•	•	–	•	•	•	•	•	•	•	•	•	•	–	–	•
Lanzoni [28]	•	•	•	–	•	–	–	–	–	–	–	–	•	•	•	–
Dilogo [29]	•	•	–	–	–	–	–	–	•	–	–	•	•	•	–	–
Shi [30]	•	•	–	•	•	•	–	–	–	–	•	•	•	•	–	•
Adas [31]	•	–	–	•	–	•	•	–	•	–	•	•	•	•	–	–
Fathi-Kazerooni [32]	•	•	•	•	–	–	•	–	•	•	•	–	•	–	–	–
Rebelatto [33]	•	•	•	–	–	–	–	–	–	–	–	•	•	•	•	•
Monsel [34]	•	–	–	–	•	•	–	•	–	–	•	–	•	•	•	–
Total	8	6	3	4	4	3	6	4	4	2	5	5	8	6	3	3

Outcomes reported for each study are indicated by • and outcomes not reported are indicated by –.

compared with 30 of 165 patients (18.2%) in the MSC groups. In meta-analysis (n = 8 studies), patients administered MSCs had a significantly decreased relative risk of mortality at the study endpoint compared with controls (RR, 0.63, 95% CI, 0.42–0.94, P=0.02, I^2=14%) (Figure 2A). However, absolute risk of death at the study endpoint was not significantly different in meta-analysis between patients administered MSCs and controls (RD, −0.14, 95% CI, −0.34 to 0.05, P=0.14, I^2=85%) (Figure 2B). Meta-analysis of studies reporting risk of death at 28 days (n=5 studies) also demonstrated no significant reduction in relative (RR, 0.57, 95% CI, 0.25–1.29, P = 0.18, I^2 = 34%) (see supplementary Figure 1A) or absolute (RD, −0.15, 95% CI, −0.38 to 0.07, P=0.18, I^2=88%) (see supplementary Figure 1B) risk of death at study endpoint in patients administered MSCs compared with controls. Interestingly, when the study examining MSC-conditioned medium [32] was removed from the authors' analysis of mortality at study endpoint, the beneficial effects of significantly reduced relative risk of death at study endpoint in patients administered MSC-based products were not observed (RR, 0.68, 95% CI, 0.38–1.19, P=0.18, I^2 = 26%) (see supplementary Figure 2A). However, as with the authors' previous analyses of mortality, absolute risk of death at study endpoint (RD, −0.11, 95% CI, −0.30 to 0.07, P = 0.22, I^2=82%) (see supplementary Figure 2B), relative risk of death at 28 days (RR, 0.49, 95% CI, 0.09–2.55, P = 0.40, I^2 = 56%) (see supplementary Figure 3A) and absolute risk of death at 28 days (RD, −0.10, 95% CI, −0.29 to 0.09, P=0.31, I^2=82%) (see supplementary Figure 3B) were not significantly different between patients administered MSCs and controls when the study examining the use of MSC-conditioned medium was excluded.

Subgroup analysis was performed to determine whether the impact of MSCs on mortality at study endpoint varied based on the tissue source from which the MSCs were isolated. More specifically, the authors sought to determine whether there was a significant difference in mortality at study endpoint compared with controls in patients administered MSCs derived from umbilical cord Wharton jelly compared with MSCs derived from unspecified umbilical cord tissue. The authors observed no significant difference in relative and absolute risk of mortality at study endpoint compared with controls (see supplementary Figure 4A,B). Other potential subgroup analyses (e.g., whether patients had or did not have acute respiratory distress syndrome, whether studies included patients with any severity of disease or just severe or critical disease, whether a single infusion or multiple infusions of MSCs were administered) could not be conducted because of an insufficient number of studies for the various subgroups.

Secondary outcomes

Hospitalization

Four of the eight studies described the number of patients requiring hospital admission at study endpoint. No significant difference in relative (RR, 0.82, 95% CI, 0.59–1.16, P = 0.27, I^2 = 52%) or absolute (RD, −0.08, 95% CI, −0.23 to 0.06, P = 0.27, I^2 = 52%) risk of requiring hospital admission at study endpoint was demonstrated in meta-analysis for patients administered MSCs compared with controls (see supplementary Figure 5A,B). However, length of hospital stay (n = 3 studies) was reduced in MSC groups compared with controls (MD, −4.42 days, 95% CI, −6.73 to −2.10, P = 0.0002, I^2 = 0%, MSC mean of the median, 20.0 days, IQR, 17.4, control mean of the median, 24.0 days, IQR, 14.0) (Figure 3).

Pro-inflammatory and anti-inflammatory cytokine levels

All eight studies reported changes in a range of pro-inflammatory cytokines from baseline to the end of the study period. Four of the studies reported CRP levels at study endpoint. Meta-analysis revealed that patients administered MSCs had significantly lower CRP levels at the study endpoint compared with controls (SMD, −0.80, 95% CI, −1.27 to −0.34, P = 0.0007, I^2 = 19%, MSC mean of the median, 63.5, IQR, 58.5, control mean of the median, 98.9, IQR, 42.4) (Figure 4). Five of the studies reported IL-6 levels at the study endpoint. Meta-analysis revealed no significant decrease in IL-6 levels at the study endpoint compared with controls (SMD, −0.46, 95% CI, −0.96 to 0.03,

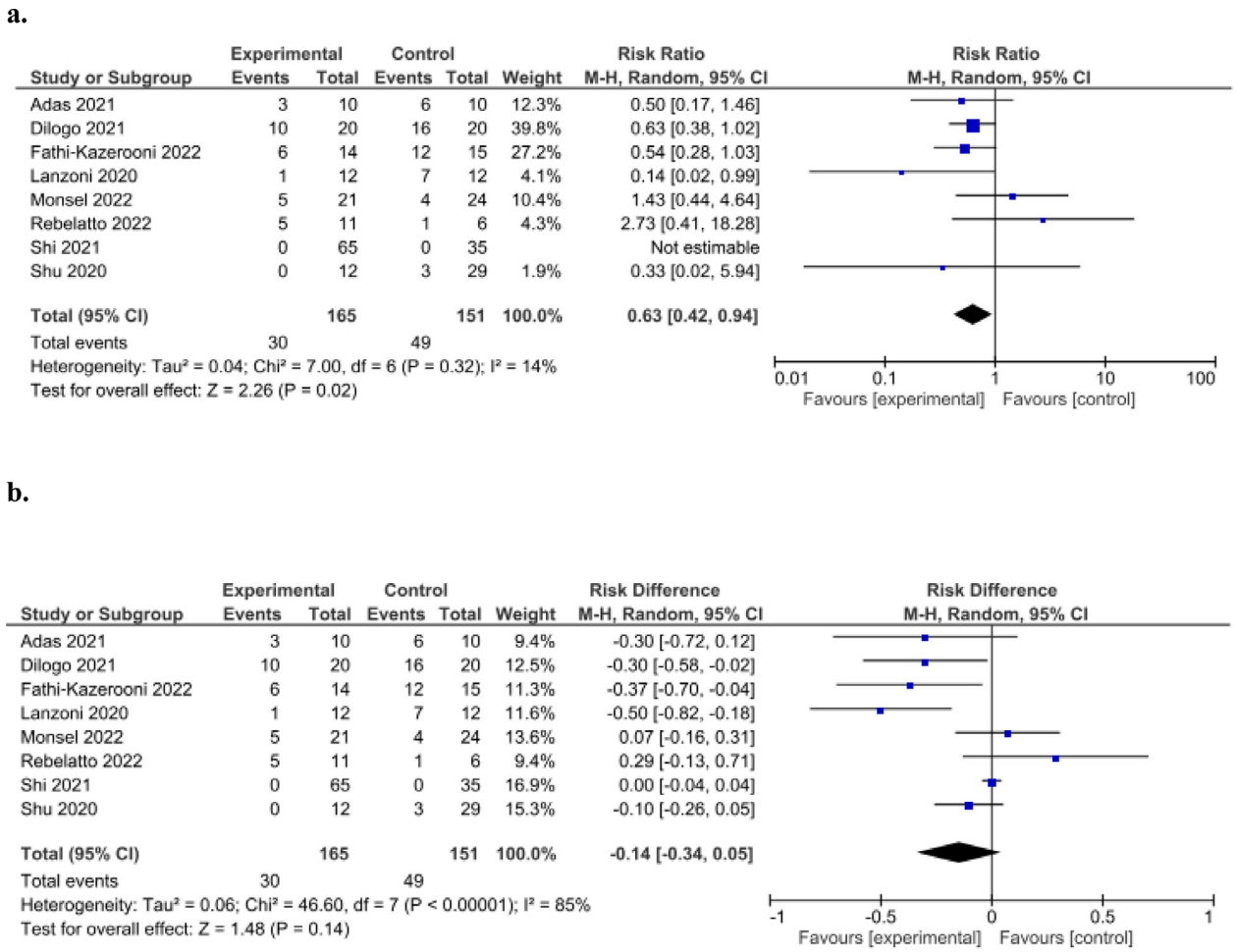


Fig. 2. (A) Forest plot demonstrating significantly decreased relative risk of death at study endpoint in patients administered MSCs (experimental) compared with patients not administered MSCs (control). (B) Forest plot demonstrating that absolute risk of death at study endpoint was not significantly reduced in patients administered MSCs compared with controls. Control groups received standard of care for COVID-19 at time of hospital admission, which varied depending on the institution. *df*, degrees of freedom.

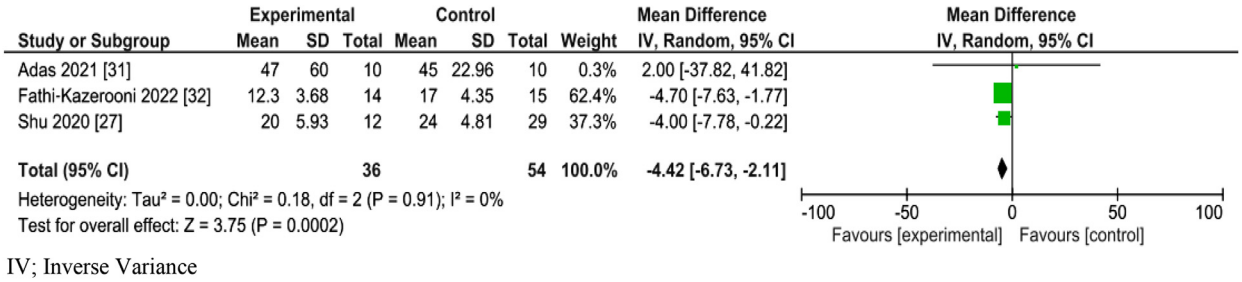
P = 0.06, I^2 = 56%, MSC mean of the median, 34.2, IQR, 43.1, control mean of the median, 72.8, IQR, 105.6) (see supplementary Figure 10). Interferon gamma (IFN-γ) and IL-2 levels at the study endpoint were also reported in three studies each. Meta-analysis demonstrated no significant difference in levels of IFN-γ (SMD, −0.72, 95% CI, −1.77 to −0.33, P = 0.18, I^2 = 83%, MSC mean of the median, 88.8, IQR, 101.2, control mean of the median, 268.0, IQR, 363.5) (see supplementary Figure 11) or IL-2 (SMD, −0.41, 95% CI, −1.52 to 0.71, P = 0.48, I^2 = 76%, MSC mean of the median, 130.7, IQR, 134.0, control mean of the median, 328.6, IQR, 425.4) (see supplementary Figure 12) at the study endpoint in patients administered MSCs compared with controls. All other biomarkers were reported infrequently and could not be pooled through meta-analysis.

Other outcomes

Other outcomes measured across studies, including oxygenation levels, immune cell levels, length of time in the ICU, number of patients requiring supplemental oxygen, length of time on mechanical ventilation, radiological parameters, virological and/or antibody responses and clinical scale scores, are summarized in the supplementary materials.

Adverse events

Adverse event reporting for all studies is summarized in Table 4. Treatment-related adverse events associated with MSC infusion occurred in five of the eight studies. However, rates of non-treatment-related adverse events (i.e., adverse events occurring in control



IV; Inverse Variance

Fig. 3. 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 time of hospital admission, which varied depending on the institution. *df*, degrees of freedom; SD, standard deviation.

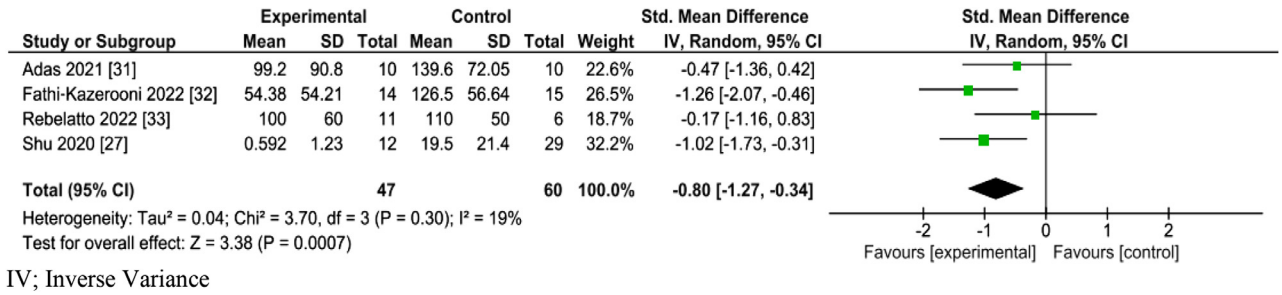


Fig. 4. Forest plot demonstrating significantly lower CRP levels at study endpoint in patients administered MSCs (experimental) compared with controls. Control groups received standard of care for COVID-19 at time of hospital admission, which varied depending on the institution. *df*, degrees of freedom; SD, standard deviation; Std., Standard.

groups) were similar to those of treatment-related adverse events, occurring in five of the eight studies. Furthermore, adverse events associated with MSC infusion were mild and resolved spontaneously or with minimal supportive treatment in all patients. In addition, meta-analysis demonstrated no significant difference in relative (RR: 0.85 [0.65-1.11, 95% CI, p=0.23, I^2=0%]) or absolute (RD: -0.02 [-0.07-0.03, 95% CI, p=0.43, I^2=0%]) risk of adverse events between the MSC and control groups (Figure 5). Five of the studies reported the occurrence of severe adverse events in patients who were administered MSCs. However, most severe adverse events occurring in the MSC arm were deemed by the site investigators to be unrelated to MSC infusion. Furthermore, non-treatment-related severe adverse events (i.e., severe adverse events occurring in control groups) were more common than treatment-related severe adverse events, occurring in six of the eight included studies.

Risk of bias

RoB was assessed for each outcome reported in RCTs using the Cochrane Risk of Bias 2 tool [20]. RoB analysis for the authors' primary outcome of risk of death at study endpoint can be seen in supplementary Table 1. Seven studies [28–34] were found to have low RoB, and one study [27] had RoB of "some concerns," as the method of randomization was unclear, and it was unclear whether there were deviations from intended interventions and whether there was selection of reported results. With regard 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 the hospital, risk of requiring mechanical ventilation at study endpoint), each study had the same RoB classification for both individual RoB domains and overall RoB (low RoB, n = 7, "some concerns," n = 1) (see supplementary Table 1).

FASTER Approval criteria evaluation

With regard to the extent to which evidence presented in this third edition of the living systematic review met the authors' proposed FASTER Approval criteria, which were described in the first two iterations of the living systematic review [8,9], three of the domains were considered satisfactory (number of studies, sample size, study populations), two of the domains were considered unclear (study characteristics, RoB) and two of the domains were considered unsatisfactory (outcome measurement, product characterization) (Table 6).

Evolution of trial characteristics, included studies and primary effect estimate

A full breakdown of changes in terms of studies included, number and types of trials (uncontrolled, non-randomized controlled, randomized controlled) and primary effect estimate (mortality at study endpoint) over the three iterations of the authors' living systematic review can be seen in supplementary Table 2.

Current status of trials examining the use of MSC products for COVID-19

To summarize the details of all currently registered trials examining the use of MSCs as a therapeutic intervention for COVID-19, the authors included a comprehensive table in the supplementary materials (see supplementary Table 2). This table was adapted from Liao *et al.* [7] to reflect the current landscape surrounding clinical trials for MSC-based products as a therapeutic intervention for COVID-19. In terms of current trial status, 11 trials are still recruiting patients, 11 trials are not yet recruiting patients and one trial has been withdrawn because of lack of ethics committee approval. In terms of the number of additional patients who can be expected from registered trials that have yet to be published, another 840 patients are expected, with 599 of these patients being administered MSCs. In terms of RCTs, an additional 568 patients are expected, with 324 of these patients being administered MSCs. Other characteristics of registered trials, including MSC tissue sources, clinical trial databases used and countries of origin, can be seen in supplementary Table 3.

Table 4
Adverse events and severe adverse events 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
Shu [27]	•	•	•	•	•
Lanzoni [28]	•	–	–	•	–
Dilogo [29]	•	•	•	•	•
Shi [30]	–	•	–	•	•
Adas [31]	•	•	•	•	•
Fathi-Kazerooni [32]	•	–	•	–	•
Rebelatto [33]	•	–	–	–	–
Monsel [34]	•	•	•	–	•
Total	7	5	5	5	6

AEs, adverse events; SAEs, severe adverse events.

Table 5
Concomitant therapies reported in studies.

Study	Antiviral agents	Antibiotic agents	Glucocorticoids	Transfusion-based interventions	Other interventions
Shu [27]	Abidor, oseltamivir	Moxifloxacin	Yes	None	None
Lanzoni [28]	BAT	BAT	BAT	BAT	BAT
Dilogo [29]	Oseltamivir	Azithromycin	None	None	None
Shi [30]	Antiviral drugs	Antibiotics	Yes	None	None
Adas [31]	Favipiravir, HCQ	Piperacillin-tazobactam	Yes	None	Enoxaparin
Fathi-Kazerooni [32]	BSC	BSC	BSC	BSC	BSC
Rebelatto [33]	Antiviral drugs	Antibiotics	Yes	None	Anticoagulants
Monsel [34]	None	None	Yes	None	None

BAT, best available therapy; BSC, best standard of care; HCQ, hydroxychloroquine.

Discussion

This latest analysis and update to the authors' living systematic review and meta-analysis suggests that MSCs are safe and may be effective for treating COVID-19. With repeated waves of the pandemic, viral mutations, evolving immunity patterns and myriad alternative treatment options, it is not surprising that published studies have embraced a broad range of comparative treatments in control groups, which clouds the ability to analyze results regarding effectiveness. With the publication of additional future studies, network meta-analysis may be a way to account for differences between control groups [35]. Additionally, the authors observed marked inter-study heterogeneity and low rates of reporting details concerning MSC product characterization with regard to ISCT criteria [36]. Despite the fact that the strength of the conclusions in this iteration of the authors' living systematic review was augmented by restricting included studies to RCTs, inadequate MSC product characterization and inter-study heterogeneity continue to be persistent barriers to performing a more informative meta-analysis. Although regulatory approval and widespread clinical adoption of MSCs for COVID-19 appear unlikely at this point in the pandemic, addressing these limitations in future clinical trials may increase the likelihood of more accelerated adoption of MSC-based therapies for other diseases and conditions. Use of a master protocol may address these limitations if timely assessments of efficacy are a priority.

Although MSCs decreased relative risk of death at the study endpoint in this iteration of the authors' living systematic review, no difference in absolute risk of death at the study endpoint was observed between patients administered MSCs and controls. Moreover, MSCs failed to reduce the absolute and relative risk of death at 28 days in a subset of studies reporting at this specific time point. Given the current trajectory of the pandemic [37] and the increasing number of

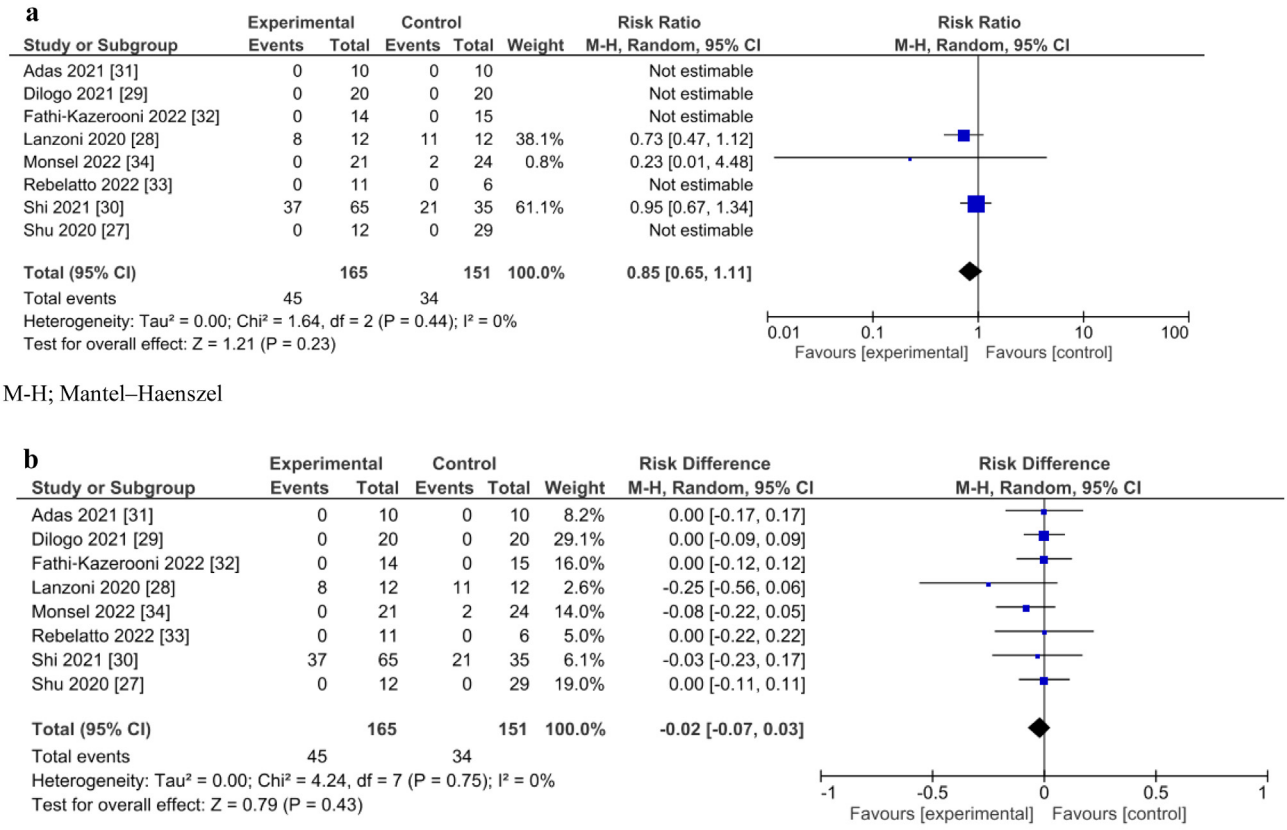


Fig. 5. Forest plot demonstrating no significant difference in (A) relative and (B) absolute risk of experiencing adverse events in patients administered MSCs (experimental) compared with controls. Control groups received standard of care for COVID-19 at time of hospital admission, which varied depending on the institution. *df*, degrees of freedom.

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.

Number of studies	• Sufficient number and similar enough to perform meta-analysis that achieves the required power for determining efficacy. See sample size.	✓ Eight controlled studies identified
Study characteristics	• Controlled with contemporary and similar control groups. Randomized is preferable. Concomitant therapies should be controlled.	✓ RCTs: n = 8 ? Concomitant therapies not always controlled
Sample size	• To reduce mortality from 20% to 10%, 199 subjects needed in intervention group(s) [24].	✓ Sample size = 316 total, with 165 patients in treatment (MSC) arm
Study population	• Severe or critical COVID-19 in hospitalized patients (most common).	✓ Most patients presented with severe (62.3%) or critical (24.4%) COVID-19
Outcome measurement	• Mortality at day 28. • WHO response criteria. • Secondary: IL-6 levels; hospitalization; ICU admission; pulmonary function at 1 month, 6 months and 12 months. • Safety and AE reporting.	? Mortality at 28 days (n = 5) ✘ WHO response criteria (n = 0) ? IL-6 levels (n = 6); hospitalization (n = 4); ICU admission (n = 1); pulmonary function at 1 month (n = 3), 6 months (n = 0) and 12 months (n = 0) ✓ Safety laboratory values (n = 3) and AE reporting (n = 8)
Product characterization	• MSCs produced and characterized according to ISCT criteria.	✘ Full criteria (n = 2) ✓ Positive/negative surface markers (n = 7) ? MSC viability (n = 6) ? Trilineage differentiation potential (n = 2), ? Plastic adherence (n = 3)
RoB	• Studies with high risk of potential bias should not be included in meta-analysis.	? "Some concerns" RoB: n = 1

Checkmark indicates criteria satisfied, question mark indicates uncertainty as to whether criteria satisfied and "x" indicates criteria not met.
AE, adverse event; WHO, World Health Organization.

approved therapeutics [38,39], MSCs likely need to demonstrate a significant mortality benefit to achieve regulatory approval and mainstream clinical translation in light of the resource intensity associated with production and distribution of cell-based therapies [40].

It was interesting to observe that when the study examining MSC-conditioned medium [32] was removed from the authors' analysis regarding mortality at the study endpoint, the observed beneficial effects were lost. Although more studies are needed, it may be worth examining the possibility that infused MSCs may be eliminated by the host immune response more rapidly than factors in conditioned medium [41,42]. It was originally thought that MSCs were completely immune-privileged, without concern of immune rejection [43,44]; however, recent studies have suggested that rapid clearance by the host immune response can occur [45,46]. This may be particularly relevant when MSCs are infused into a highly pro-inflammatory environment [41], such as with COVID-19 [47]. Strategies to prolong MSC persistence have been explored [41,48,49], and the use of MSC-secreted factors may achieve the therapeutic effects of MSCs with less concern regarding host immune rejection. Additional studies of MSC-conditioned medium and extracellular vesicles are ongoing.

An improvement in hospital stay was observed and consistent with observations in earlier iterations of the authors' systematic review [8,9]. Shortened hospital stays suggest attenuation of disease severity and have been correlated with less severe disease course and lower mortality rates [50,51]. Moreover, shortened hospital stays alleviate pressure on the health care system, which is important during pandemics with widespread societal implications [52,53]. Reduced hospital stays have been observed with other therapies, including corticosteroids [54,55], remdesivir [56,57] and SARS-CoV-2-specific vaccines [58,59]. Thus, given the complexities and challenges associated with widespread distribution of cellular therapies [60,61], reducing the duration of hospitalization alone may be insufficient to buoy widespread support for MSC therapy.

Although MSCs significantly lowered CRP levels at the study endpoint, patients who were administered MSCs failed to show appreciable decreases in other pro-inflammatory markers such as IL-6 and IFN-γ. In the context of viral infection, pro-inflammatory factors help to resolve infection through several mechanisms, including promotion and optimization of T-cell response, enhancement of migration and phagocytic activity of macrophages, prevention of viral-induced cell apoptosis and regulation of IgG isotype switching [62,63]. However, infection with SARS-CoV-2 can result in excess production of pro-inflammatory factors, leading in some cases to the development of cytokine storm and associated organ dysfunction or multiorgan failure, which can be fatal [64,65]. Although the anti-inflammatory actions of MSCs may result from a decrease in infiltration of infected tissues by pro-inflammatory immune cells and through polarization of pro-inflammatory immune cells to anti-inflammatory phenotypes [66,67], the authors' analysis suggests that MSCs fail to exert significant anti-inflammatory effects in the context of COVID-19. This is further corroborated by the lack of significant differences the authors observed regarding CD4+ and CD8+ T-cell levels at the study endpoint in patients administered MSCs compared with controls. It has been suggested that pre-conditioning of MSCs may be necessary to generate potent anti-inflammatory properties for therapeutic use [68,69]. Techniques to augment the anti-inflammatory properties of MSCs include pre-culturing with pro-inflammatory cytokines in three-dimensional culture conditions and pre-conditioning with chemical agents and/or through gene transfer approaches [70,71]. As none of the studies in the authors' review reported augmenting the therapeutic properties of MSCs using any of these techniques, this may explain the observed inability of MSCs to reduce pro-inflammatory cytokine levels and decrease mortality

The lack of reporting specifics regarding MSC characterization in accordance with minimal criteria established by the ISCT [36] remains an issue in studies addressing the treatment of COVID-19 that the authors identified in previous iterations of this systematic review [8,9]. The issue has been previously identified more broadly in MSC studies [17,72], despite the publication of two editions of ISCT guidelines [36,73], and was identified as a barrier in a position paper released by the US Food and Drug Administration in 2014 [74]. This position paper highlighted that despite a growing number of MSC-based trial submissions to the Food and Drug Administration, MSC products are becoming increasingly diverse and trials rarely report product characterization in sufficient detail to satisfy published ISCT criteria [36,73]. Rigorous MSC characterization can provide assurance that patients consistently receive a safe and effective therapeutic product [74]. Issues related to cell product characterization are not unique to MSC-based studies. A systematic review examining the use of endothelial colony–forming cells as a therapeutic intervention in pre-clinical animal models found that very few studies characterized these cells according to established minimal standards [75]. Strengthening the reporting and adherence to standardized product

Table 7
Hypothetical master protocol for use of MSCs as a therapeutic intervention for COVID-19.

Diagnosis of COVID-19	COVID-19 diagnosis using RT-qPCR
Classification of COVID-19 severity	Classification of COVID-19 severity using NIH guidelines[a]
Patient characteristics	Should be balanced between control and MSC groups
	Ensure adequate distinction between adult and pediatric trials
	Patients with conditions that may complicate interpretation of study results (e.g., hematological malignancies, autoimmune conditions) should not be included
Study procedures	RCT
	Minimum number of patients: N = 100 (n = 50 for MSCs, n = 50 for control)
	Investigators, outcome assessors and patients should be blinded to the intervention that is given
MSC intervention characteristics	UC-MSCs should be used
	3×10^6 cells/kg administered as three doses of 1×10^6 cells/kg 2 days apart or according to patient symptoms (improvement versus deterioration); additional doses as per criteria
	Use of WHO OSCI for clinical improvement
	MSCs should be administered via intravenous infusion
	UC-MSC product should satisfy all ISCT criteria
Concomitant therapies	Should be balanced between MSC and control arms
Outcome measures	Outcomes should be standardized across studies and measured at common time points
	Endpoints should include the following:
	Mortality at 28 days (n = 5)
	WHO OSCI criteria at 28 days
	Levels of pro-inflammatory and anti-inflammatory biomarkers (e.g., IL-6, CRP, IL-2) and immune cell levels (e.g., neutrophils, CD4+ T cells, CD8+ T cells) at 7 days, 14 days and 28 days
	Number of patients in hospital and ICU at 28 days
	Number of patients on supplemental oxygen or mechanical ventilation at 28 days
	Changes in pulmonary imaging (e.g., ground-glass opacities, pleural effusion) at 7 days, 14 days and 28 days
	Occurrence of treatment-related and non-treatment-related AEs and SAEs during the study period, including frequency, time points and severity
	Levels of safety laboratory biomarkers (e.g., troponin, blood urea nitrogen, lactate dehydrogenase) at 7 days, 14 days and 28 days
Follow-up	Minimum inpatient follow-up of 5 days after last MSC infusion and minimum outpatient follow-up of 28 days

AEs, adverse events; NIH, National Institutes of Health; RT-qPCR, reverse transcription quantitative polymerase chain reaction; SAEs, severe adverse events; UC-MSC, umbilical cord-derived MSC; WHO OSCI, World Health Organization Ordinal Scale for Clinical Improvement.
[a] Clinical spectrum of SARS-CoV-2 infection section of COVID-19 treatment guidelines document provided by NIH.

characterization should reduce heterogeneity and improve confidence in study results from cell therapy studies.

Trials included in the authors' analysis were modest in size and heterogeneous with regard to study design, outcome reporting and product characterization. This heterogeneity and lack of sufficient sample size meant that the authors' meta-analyses lacked adequate statistical power to provide definitive conclusions regarding the efficacy of MSCs as a therapeutic intervention for COVID-19 [76,77]. By contrast, many well-designed and sufficiently powered clinical trials have been completed for other COVID-19 therapeutics, including vaccines, antivirals and antibody-based therapies [78–80]. These interventions have been widely adopted across the globe to curtail the impacts of COVID-19 [81–83]. One strategy to accelerate the completion of studies that share greater homogeneity is the use of a master protocol [84,85]. Master protocols would provide guidance regarding recommended sample sizes, patient populations, MSC product characteristics, administration strategies, outcome measures and blinding procedures to be followed. This would allow for standardization of these aspects across individual clinical studies, which would facilitate the pooling of results from more studies in a meaningful meta-analysis. The authors provide a proposed outline of a master protocol in Table 7. Although the authors' proposal provides only an initial template and framework, a more refined master protocol could evolve through expert consensus and collaboration. Umbrella trials using master protocols have previously been launched for the treatment of patients with cancer who have different subtypes, mutations or disease manifestations and where treatment interventions and outcome reporting are aligned [86,87].

The authors' study has limitations that are worth mentioning. First, the number of studies and patients included in the authors' analysis remains modest. Although the authors could have achieved a larger sample size had the inclusion criteria to all controlled studies been kept regardless of randomization status, the authors believe that the benefits of restricting studies to high-quality RCTs outweigh the downsides of a reduced number of studies and patients. Variable outcome reporting across the studies included in the authors' review is also a persistent issue and a major hurdle to performing a robust meta-analysis. The only outcome reported across all studies was mortality. However, mortality was not measured at a uniform time point across all studies, further limiting the informative nature of this analysis. Moreover, only two studies reported sufficient MSC product characterization details to satisfy all ISCT criteria [36]. This inadequate product characterization further limits the confidence in the authors' pooled results.

Conclusions

The authors' living systematic review and meta-analysis of RCTs demonstrates continued uncertainty regarding the use of MSCs as a therapeutic intervention for COVID-19. Although MSCs reduced relative risk of death at the study endpoint, absolute risk of death at the study endpoint and several important secondary clinical outcomes were not significantly different in patients administered MSCs compared with controls. These mixed benefits contrast with the more promising results the authors initially observed when all study types were considered. A more refined estimate of the treatment effect with higher-quality evidence coupled with evolving comparative treatments in control arms continues to influence the evidence supporting the use of MSCs in the treatment of COVID-19. Although MSCs may never achieve mainstream clinical use in the treatment of COVID-19 given the current trajectory of the pandemic, the potential role of master protocols in accelerating the acquisition of high-quality data from studies that share more similarities should be investigated and may be applicable to future clinical trials of MSCs for other diseases and conditions.

Declaration of Competing Interest

DSA is a paid medical consultant with Canadian Blood Services.

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Author Contributions

Conception and design of the study: AMK and DSA. Acquisition of data: AMK, AJMB, RS, DSA, MML and DAF. Analysis and interpretation of data: AMK, AJMB, DSA, MML and DAF. Drafting or revising the manuscript: AMK and DSA. All authors have approved the final article.

Availability of Data and Materials

Datasets are available from the corresponding author upon request.

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