STEM CELLS FOR TREATMENT OF CARDIOVASCULAR DISEASES: AN UMBRELLA REVIEW OF RANDOMIZED CONTROLLED TRIALS

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ABSTRACT

AIMS: Stem cells are a promising therapy for various medical conditions. The literature regarding their adoption for the clinical care of cardiovascular diseases (CVD) is still conflicting. Therefore, our aim is to assess the strength and credibility of the evidence on clinical outcomes and application of stem cells derived from systematic reviews and meta-analyses of intervention studies in CVD.

METHODS and RESULTS: Umbrella review of systematic reviews with meta-analyses of randomized controlled trials (RCTs) using placebo/no intervention as control group. For meta-analyses of RCTs, outcomes with a random-effect p-value< 0.05, the GRADE (Grading of Recommendations Assessment, Development and Evaluation) assessment was used, classifying the evidence from very low to high. From 184 abstracts initially identified, 11 meta-analyses (for a total of 34 outcomes) were included. Half of the outcomes were statistically significant ($p<0.05$), indicating that stem cells are more useful than placebo. High certainty of evidence supports the associations of the use of stem cells with a better left ventricular end systolic volume and left ventricular ejection fraction (LVEF) in acute myocardial infarction; improved exercise time in refractory angina; a significant lower risk of amputation rate in critical limb ischemia; a higher successful rate in complete healing in case of lower extremities ulcer; and better values of LVEF in systolic heart failure, as compared to placebo.

CONCLUSION and RELEVANCE: The adoption of stem cells in clinical practice is supported by a high certainty of strength in different CVD, with the highest strength in acute myocardial infarction and refractory angina.

Key words: cardiovascular disease; stem cells; umbrella review; meta-analysis; randomized controlled trials.

INTRODUCTION

Stem cells are characterized by: (i) self-renewal – the ability to go through numerous symmetric or asymmetric cell division cycles maintaining the undifferentiated state; this features distinguishes them from progenitor cells; (ii) potency – the potential to differentiate into several specialized cell types (Fortier, 2005). According to the latter feature they can be classified as: totipotent cells with the ability to differentiate into embryonic and extraembryonic cell types (i.e. zygote); pluripotent cells that differentiate into cell linages from all three germ layers with the exception of extrafetal tissues; whereas multipotent cells have ability to differentiate into a limited number of types from one germ layer; oligopotent cells that can differentiate into a only few type of cells; and unipotent cells that can produce cells of their own type. Furthermore, stem cells can be differentiate in: embryonic (ESC) (pluripotent cells, obtained from preimplantation-stage embryos), the use of which is still controversial; adult/somatic (ASC), found in many tissues of adult organisms (i.e. mesenchymal, adipose, neural, cardiac etc.); and induced pluripotent stem cells (iPSC), created through the induction of embryonic genes' expression into somatic cells (Fortier, 2005).

Notably, in many tissues ASCs serve as a sort of internal repair system, dividing to replenish other cells (National Institutes of Health, 2006). This finding gave rise to the "regenerative medicine" defined as the "process of replacing, engineering or regenerating human or animal cells, tissues or organs to restore or establish normal function" (Mason and Dunnill, 2008).

This promising approach has been applied to treat several diseases including cardiovascular disease (CVDs). A primary goal of cardiac cell-based therapy is to repopulate areas of damaged myocardium with three types of cells capable of engraftment: cardiomyocytes, vascular smooth muscle and endothelial cells (Zipes et al., 2018). Even if a large variety of cellular substrates with different

potency have been proposed for cardiac regenerative therapy (namely bone marrow mononuclear cells, skeletal myoblasts, mesenchymal stem cells, mesenchymal progenitor cells, endothelial precursor cells, and cardiac-derived stem cells), it has to be established whether these cells are able to productively supply one or more of the three key cardiac cell types within damaged myocardium, and initial clinical trials have generated mixed results (Zipes et al., 2018).

A 2018 review on legislature and restrictions in application of stem cells in clinical practice reported that a number of studies embellish their results, choosing to mostly represent findings on secondary outcomes without the inclusion of data on adverse effects (Poulos, 2018). One group of researchers also reported that the greatest number of discrepancies came from studies reporting greatest potential benefit for patients (Nowbar et al., 2014). Other studies have also reported on "stimulus triggered acquisition of pluripotency" only to later, following inability of independent confirmation of the results, admit that the whole research data was fabricated (Obokata et al., 2015). Such misreporting leads to widen the gap between expectations and reality.

Recently, in order to address the breadth of the literature of complex health behaviors and outcomes, an increasing emphasis has been placed on "umbrella reviews" that offer the possibility to obtain a wide picture of the topic of interest highlighting if the evidences are consistent or if contradictory findings exist, and allow to explore and detail the reasons why.

To the best of our knowledge, no attempt has been performed so far to capture the breadth of outcomes associated with clinical use of stem cells in cardiovascular diseases and to systematically assess the quality and the strength of the evidence of systematic reviews with meta-analyses of its clinical application. Therefore, our aim is to assess the strength and credibility of the evidence on clinical

outcomes and application of stem cells derived from systematic reviews and meta-analyses of randomized controlled studies.

METHODS

This work followed a pre-planned, but unpublished protocol, available on request to the corresponding author. For this work we followed the PRISMA guidelines (Liberati et al., 2009; Moher et al., 2009).

Data sources and searches

We conducted an umbrella review, searching the MEDLINE, Scopus, Embase databases from inception until 23th March 2020 with the following search: "(Meta-Analysis[ptyp] OR metaanaly*[tiab] OR meta-analy*[tiab]) AND (stem cell* [tiab] OR precursor cell* [tiab] OR progenitor cell* [tiab]) AND (cardiovascular OR stroke OR cerebrovascular OR transient ischemic attack OR transient ischaemic attack OR peripheral vascular OR myocardial infarction OR coronary heart disease OR ischemic heart disease OR ischaemic heart disease OR hypertensive heart disease OR angina OR cardiac failure OR heart failure OR congestive heart failure OR cardiovascular mortality)." We then hand-searched the reference lists of eligible articles and reviews in this field.

Study selection

We considered eligible the following categories of studies: 1. Meta-analyses of RCTs including at least one arm being administrated stem cells and one placebo; 2. Meta-analyses including people affected by any CVD. Meta-analyses were included only if they reported study-specific information

(i.e. effect size, 95% confidence intervals, sample size) or if those metrics could be inferred from the data presented.

The study selection was performed by two authors independently (NV, JD). Disagreements were resolved through consensus with another independent author (LS). Full texts of all potentially eligible articles were consequently evaluated by the same two authors and any disagreement was resolved with another independent author (LS).

Data extraction

For each eligible MA, two investigators (GNF, SLR) independently extracted the following data: name of the first author, year of publication, study population, study design, outcome, number of studies, intervention, comparison, effect size reported with its 95% CI.

On a second phase the same two authors extracted the following information for each original article: (I) PMID/doi; (II) meta-analysis author; (III) year of meta-analysis; (IV) first author name of individual studies included in the meta-analysis; (V) year of publication; (VI) main CVD condition; (VII) cell type; (VIII) type of intervention; (IX) way of administration; (X) effect size metrics used in the meta-analysis; (XI) number of people treated with stem cells and treated with placebo; (XII) follow-up duration; (XIII) outcomes of interest.

Next, the study-specific estimated relative risk for any side effects or negative outcome (risk ratio [Sloan et al., 2009], odds ratio [OR], hazard ratio [Sleeman et al., 2012], incident risk ratio, standardized mean differences [SMDs], mean differences [MD]), along with their 95% CIs, were extracted.

If two meta-analyses were available for the same outcome, the one included the largest in terms of studies considered and, if equal in terms of numerosity of studies, the most recent one was used.

Outcomes

Any health outcome, adverse events and side effects potentially associated to CVD and related to the use of stem cells was included.

Risk of bias assessment

The methodological quality of each included meta-analysis was assessed with the Assessment of multiple systematic reviews (AMSTAR) 2 tool (available at https://amstar.ca/Amstar-2.php), which is a recent update of AMSTAR, by two independent investigators (GNF, JD). The AMSTAR2 ranks the quality of a meta-analysis from critically low to high according to 16 predefined items (Shea et al., 2017).

Data synthesis and analysis

For each meta-analysis, we estimated the summary effect size and its 95% CIs through a random-effects model. We also estimated the prediction interval (PI) and its 95% CI, which further accounts for between-study effects and estimates the certainty of the association if a new study addresses that same association (IntHout et al., 2016; Higgins et al., 2009; Serghiou and Goodman, 2018). Between-study inconsistency was estimated with the I^2 metric, with values $\geq$ 50% indicative of high heterogeneity and $\geq$ 75% very large heterogeneity (Higgins and Thompson, 2002). We calculated the evidence of small-study effects (i.e. whether small studies inflated effect sizes) using the regression asymmetry test (Egger et al., 1997) with a p-value < 0.10. We considered the effect size of the largest study included for each outcome, determining if it was statistically significant (p-value <0.05) or not. All statistical analyses were conducted in Stata, version 14.0 (StataCorp), and R, version 3.3.0 (R Foundation for Statistical Computing).

Grading the evidence

Evidence from meta-analyses of RCTs was assessed in terms of the significance of the summary effect, using a p-value <0.05 as the threshold for statistical significance. When the p-value for the random effect was <0.05, we evaluated the evidence using the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) assessment. We also reported 95% PIs (excluding the null or not), the presence of large heterogeneity (I^2 >50%), small study effects (P>0.10), if the largest study in terms of participants, and excess significance (P>0.10) as possible indicators of quality of the available evidence.

RESULTS

Literature review

The initial search yielded 184 articles. After removing the duplicates, we started our selection and evaluated 184 papers, with 52 assessed as full text. As reported in the PRISMA flow-chart (**Figure 1**), we identified 11 meta-analyses as eligible, with 34 outcomes (Fan et al., 2019; Fernandes et al., 2019; Jayaraj et al., 2019; Jeong et al., 2018; Jiang et al., 2016; Jones et al., 2019; Kuswardhani and Soejitno, 2011; Liu et al., 2015; Marquis-Gravel et al., 2014; Sun et al., 2015; Velagapudi et al., 2019).

Meta-analyses of RCTs (vs. placebo)

Supplementary Table 1 shows the descriptive findings and the ancillary analyses of the 34 outcomes included in this umbrella review. The way of administration of the stem cells was heterogeneous as well as the type of intervention and the stem cells used. The patients were mainly affected by coronary heart disease (19/34 outcomes; 6 by acute myocardial infarction, 7 by refractory angina, 6 by ischemic heart disease), followed by peripheral artery disease (6/34) and finally by other CVD (6/34), in particular systolic heart failure (n=5). Among the outcomes included, the most frequent were echocardiographic parameters (n=8) and mortality (n=4). In total, the RCTs included 410 participants with 223 randomized to stem cell intervention and 187 to placebo.

Among the 34 outcomes included, 17 were statistically significant ($p<0.05$). High heterogeneity ($I^2 \geq 50\%$) was present in 5/34 outcomes, small-study effect was present in only one outcome, the largest study in terms of participants was statistically significant in 5/34, as reported in **Supplementary Table 1**.

Using the GRADE approach (Guyatt et al., 2008), we categorized the significant outcomes by the type of CVD included. In **Table 1**, we reported the data regarding outcomes related to coronary heart disease. Overall, the use of stem cells was associated, with a high certainty of evidence, with a better left ventricular end systolic volume (LVESV) (404 randomized to stem cells vs. 387 placebo; MD=-5.52; 95%CI: -7.68 to -3.36) and Left Ventricular Ejection Fraction (LVEF) (344 stem cells vs. 345 placebo; MD=2.60; 95%CI: 1.11-4.09) in people affected by acute myocardial infarction. Moreover, a high certainty of evidence supported the use of stem cells in improving exercise time in refractory angina (162 stem cells vs. 140 placebo; MD= 58.62; 95%CI: 21.19-96.06) (**Table 1**). The other outcomes were supported by different degrees of certainty from moderate to very low.

Similarly, as reported in **Table 2**, the use of stem cells was associated, supported by a high level of certainty, with a significant lower risk of amputation rate in critical limb ischemia (163 stem cells and 143 placebo; OR=0.30; 95% CI: 0.16-0.57) and a higher successful rate in complete healing in case of lower extremities ulcers (124 stem cells and 106 placebo RR= 2.16; 95%CI: 1.47-3.16).

Finally, as shown in **Table 3**, the use of stem cells was associated with better values of LVEF in systolic heart failure (MD= 6.24; 95%CI: 4.64-7.84), with a high certainty of evidence.

Risk of bias

The assessment of the risk of bias in the meta-analyses included is reported in **Supplementary Table 2**. Nine meta-analyses were rated as critically low, whilst two low. The main reasons of this downgrading were poor explanation of the inclusion criteria (item 3) and the absence of a list of excluded studies (item 7) as well as poor information regarding the source of funding in the studies included (item 10).

Discussion

Despite the improvement in diagnostic and treatments, CVDs and especially myocardial infarction (MI) are still the leading cause of mortality and morbidity in industrialized countries (Itoh et al., 2016; Members et al., 2010; Miquerol and Kelly, 2013). Drugs and interventional therapies cannot save or restore dead cardiomyocytes and heart transplantation remains the only effective therapy in patients with severe heart failure; however, is associated with high cost, shortage of donors' organ and post-operative issues that limit its use (Guo et al., 2020). Therefore, new and innovative therapeutic approaches are needed. In this sense, the clinical application of stem cells could be important since, contrary to heart transplantation, can be derived from the same patients or from alive donors, increasing the availability of this treatment in daily clinical practice (Williams and Hare, 2011).

The discovery of resident cardiac stem cells led to a fervid research aiming to assess the efficacy and feasibility of stem cells transplantation therapy in CVDs trying to overcome the actual limitations. Indeed, in recent decades, many studies demonstrated that stem cells therapy could be used as an attractive therapeutic approach to prevent and treat several CVDs (Williams and Hare, 2011). Many sources of stem cells could be potentially used as demonstrated with several *in-vitro* and *in-vivo* models; among those, skeletal myoblasts, bone marrow mononuclear cells, resident cardiac stem cells and iPSC have been used in clinical trials (Williams and Hare, 2011).

Mesenchymal stem cells (MSCs) represent the most frequent administered type of stem cells for therapeutic purposes. Firstly identified in bone marrow, they have been isolated in different tissues such as adipose, endometrial and peripheral or cord blood which nowadays represent the easiest sources of MSCs (Yamada et al., 2007). After transplantation MSCs could differentiate in cardiomyocytes, vascular smooth muscle and endothelial cells. Moreover, MSCs reduce the

inflammatory response and fibrosis (Guo et al., 2020). Although many clinical trials reported encouraging results, the administration of stem cell therapy in the clinical routine is hampered by several limitations: (i) differentiation abilities and immunoregulatory properties of MSCs are diverse and depending on the source of collection and culture conditions; (ii) tumorigenicity: the risk of stem cells neoplastic transformation should never be neglected, even if it is really low for ASCs; (iii) immunogenicity: autologous cells can avoid rejection, but their identification and isolation represent an expensive and time-consuming process; (iii) limited amount: to implant a sufficient number of MSCs in-vitro expansion is needed but spontaneous senescence limit cell death; (iv) tissue targeting: open-surgery and intravascular routes are two potential approaches but the first cannot be used for advanced-stage heart disease or complicated patients, whereas with the latter the challenge is to target and delivery stem cells in the exact injured area; (v) storage/shipping issues have to be overcome too; (vi) finally an important limitation to be considered regards the inflammatory microenvironment which have an significant impact on stem cell survival and engraftment rate (Guo et al., 2020; Stubbendorff et al., 2013; Tang et al., 2018; Van der Spoel et al., 2011). For example, several cytokines and other inflammatory factors can greatly contribute in finalizing or blocking the positive effects of stem cells (Guo et al., 2020). Along this line, further studies are needed to better understand the correct timing and the correct use of stem cells also in relation of the inflammatory state. MI is a classic example of an evolving inflammatory condition, in which the necrotic area is populated by neutrophils in the early phase, then by macrophages/lymphocytes and at last by fibroblast. Each of these types of cells is recruited based on specific chemotactic programs, and the use of stem cells should be adopted only in the first phases, where there are the possibilities but also the conditions to permit their action. All these technical and conceptual limits have undoubtedly prevented the potential long-term efficacy of stem cells transplantation.

Our results, coming from an umbrella review approach, showed with a high certainty of evidence that the use of stem cells improves different clinical and echocardiographic outcomes. Patients with coronary artery disease treated with stem cells therapies had reduced left ventricular and ischemic scar volumes and a better LVEF; these instrumental evidences are accompanied by an increased exercise time, a longer exercise distance on 6-minute walking test and exercise tolerance in refractory angina. Moreover, a reduced mortality was shown. Altogether, our findings suggest a promising role of stem cells in coronary heart disease, the most important cause of mortality in Western countries. However, even if it seems that stem cells are useful in refractory angina, this will probably remain a relatively narrow ‘‘niche’’ for stem cells.

The findings of our work should be interpreted considering shortcomings and potential limitations. The use of pre-established tools for quality assessment of evidence in RCTs, which relies on the data reported in the included meta-analysis, even if individually does not produce a lack of credibility, can cumulatively bring some biases. We used I^2 metric, with values $\geq$ 50% as one of the criteria for class I evidence (convincing) in order to assign the best-evidence grade only to robust associations and without hints of bias. However, I^2 estimates can also carry uncertainty, and clinical heterogeneity may be substantial even in the absence of statistical heterogeneity.

It is known that meta-analyses have considerable limitations (Ioannidis, 2016) and their results depend on the choice of the estimate from each primary study and its representation in the meta-analysis. Moreover, applying the criteria suggested by the AMSTAR 2 for evaluating the quality of meta-analyses, we unfortunately observed the presence of low/critically low rating, highlighting several potential biases. This evidence is mainly driven by missing information in item 2 (protocol published before the meta-analysis), 7 (list of excluded studies), or 13 (risk of bias that was not accurately accounted in the interpretation/discussion of the review). It is important that future meta-

analyses in this area utilize AMSTAR 2 as a checklist to ensure that the meta-analyses are of a high or very high quality.

In conclusion, stem cells therapy is a safe and promising approach to repair damaged myocardial tissues. However, barriers for routine implementations are present and further experimental studies are needed to acquire knowledge in order to overcome immunological and theratomic issues.

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TABLES AND FIGURES

Figure 1. PRISMA flow-chart.

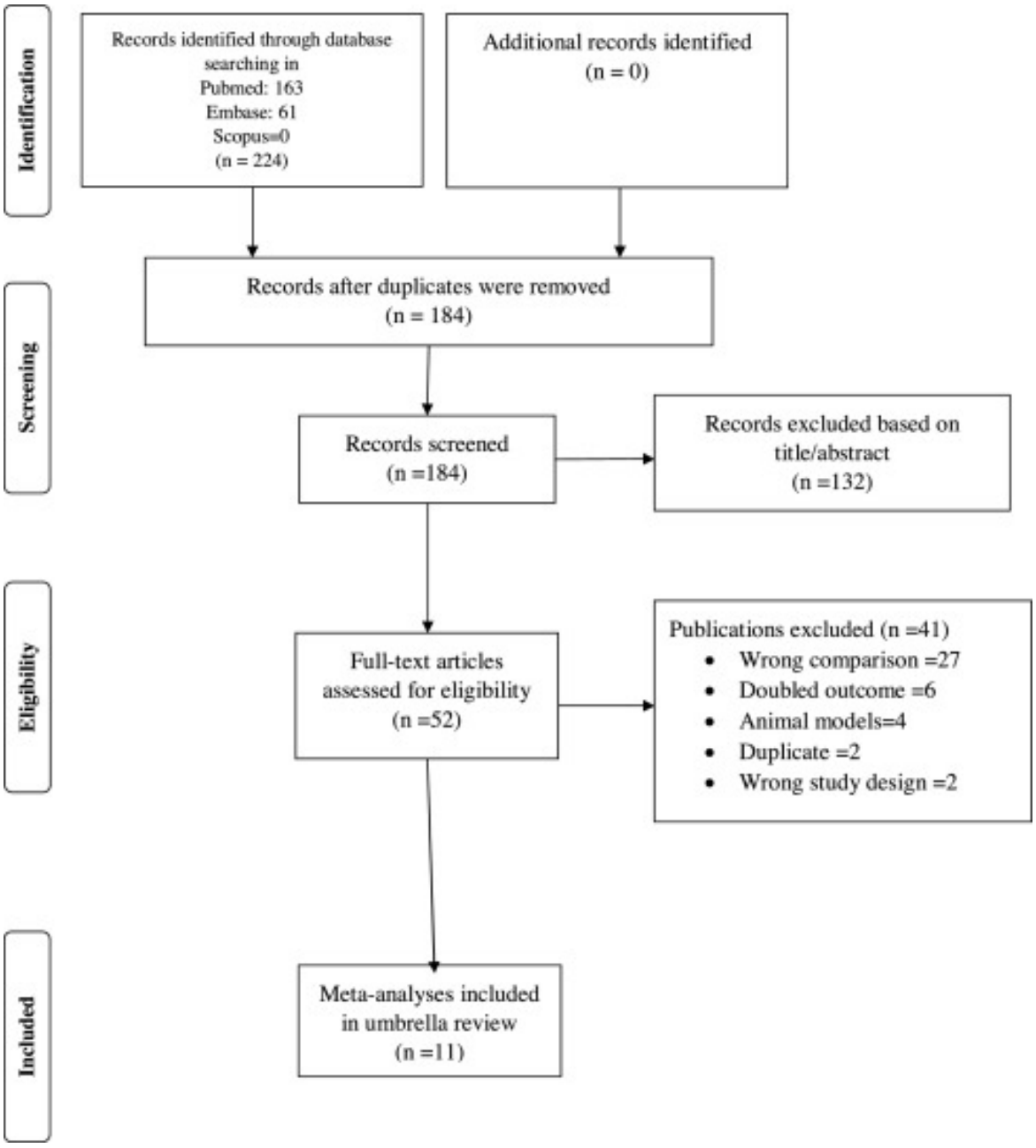

Table 1. GRADE evidence for randomized controlled trials investigating outcomes related to coronary heart disease.

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**stem cells**	**placebo/ no intervention**	**Relative (95 % CI)**	**Absolute (95 % CI)**		
LVESV (in AMI)												
13	randomised trials	not serious	not serious	not serious	not serious	none	404	387	–	MD **5.52 lower** (7.68 lower to 3.36 lower)	⊕⊕⊕⊕ HIGH	8
LVEF (in AMI)												
10	randomised trials	not serious	not serious	not serious	not serious	none	344	345	–	MD **2.6 higher** (1.11 higher to 4.09 higher)	⊕⊕⊕⊕ HIGH	8
6MWD (in IHD)												

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**stem cells**	**placebo/ no intervention**	**Relative (95 % CI)**	**Absolute (95 % CI)**		
5	randomised trials	serious [a]	not serious	not serious	serious [b]	none	163	80	–	MD **27.68 SD higher** (16.18 higher to 39.07 higher)	⊕⊕◯◯ LOW	7
Scar mass (IHD)												
3	randomised trials	serious [c]	very serious [d]	not serious	serious [b]	none	94	65	–	MD **0.96 lower** (1.86 lower to 0.07 lower)	⊕◯◯◯ VERY LOW	7
Mortality (refractory angina)												
8	randomised trials	serious [a]	not serious	not serious	not serious	none	314	212	**OR 0.24** (0.10 to 0.60)	–	⊕⊕⊕◯ MODERATE	9

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**stem cells**	**placebo/ no intervention**	**Relative (95 % CI)**	**Absolute (95 % CI)**		
Exercise time (refractory angina)												
3	randomised trials	not serious	not serious	not serious	not serious	none	162	140	–	**58.62 higher** (21.19 higher to 96.06 higher)	⊕⊕⊕⊕ HIGH	7
Angina frequency (refractory angina)												
4	randomised trials	not serious	very serious [d]	not serious	not serious	none	180	146	–	MD **2.79 lower** (4.8 lower to 0.77 lower)	⊕⊕◯◯ LOW	8
Exercise tolerance (refractory angina)												
7	randomised trials	serious [a]	not serious	not serious	not serious	none	261	150	–	SMD **0.26 SD higher** (0.06	⊕⊕⊕◯ MODERATE	8

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**stem cells**	**placebo/ no intervention**	**Relative (95 % CI)**	**Absolute (95 % CI)**		
										higher to 0.47 higher)		
MACE (refractory angina)												
8	randomised trials	serious [a]	not serious	not serious	not serious	none	314	212	**OR 0.42** (0.19 to 0.94)	–	⨁⨁⨁◯ MODERATE	9

6MWD: 6-Minute Walking Distance; AMI: Acute Myocardial Infarction; CI: Confidence interval; IHD: Ischemic Heart Disease; LVEF: Left Ventricular Ejection Fraction; LVESV: Left Ventricular End Systolic Volume; MACE: Major Adverse Cardiovascular Events; MD: Mean difference; OR: Odds ratio; SMD: Standardized mean difference.

Explanations.

[a] Poor information regarding randomization (unbalance between active and control group in sample sizes).

[b] Small sample size (one arm with less than 100 participants).

[c] 30−50 % of the RCTs included at high risk of bias.

[d] I2 >.75 %.

Table 2. GRADE evidence for randomized controlled trials investigating outcomes related to peripheral artery disease.

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**stem cells**	**placebo/ no intervention**	**Relative (95 % CI)**	**Absolute (95 % CI)**		
Amputation rate (Critical Limb Ischaemia)												
7	randomised trials	not serious	not serious	not serious	not serious	none	163	133	OR 0.30 (0.16 to 0.57)	–	⊕⊕⊕⊕ HIGH	9
Complete healing (Lower extremity ulcers)												
9	randomised trials	not serious	not serious	not serious	not serious	none	124	106	RR 2.16 (1.47–3.16)	–	⊕⊕⊕⊕ HIGH	9
Ulcer size (Lower extremity ulcers)												
4	randomised trials	not serious	not serious	not serious	very serious [a]	none	54	48	–	MD 0.62 lower (1.17 lower to 0.06 lower)	⊕⊕◯◯ LOW	7

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**stem cells**	**placebo/ no intervention**	**Relative (95 % CI)**	**Absolute (95 % CI)**		
Partial healing (Lower extremity ulcers)												
3	randomised trials	not serious	not serious	not serious	very serious [a]	none	31	29	RR 3.07 (1.14–8.27)	–	⨁⨁◯◯ LOW	6
ABI (PAD)												
4	randomised trials	not serious	not serious	not serious	serious [a]	none	60	69	–	MD 0.55 higher (0.18 higher to 0.88 higher)	⨁⨁⨁◯ MODERATE	7

ABI: Ankle Brachial Index; CI: Confidence interval; OR: Odds ratio; PAD: Peripheral Artery Disease; RR: Risk ratio; MD: Mean difference.

Explanations.

[a] Small sample size (<100 in both arms).

Table 3. GRADE evidence for randomized controlled trials investigating outcomes related to other cardiovascular diseases.

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**stem cells**	**placebo/ no intervention**	**Relative (95 % CI)**	**Absolute (95 % CI)**		
LVEF (systolic heart failure)												
6	randomised trials	not serious	not serious	not serious	not serious	none	159	111	–	MD **6.24 higher** (4.64 higher to 7.84 higher)	⨁⨁⨁⨁ HIGH	8
NYHA (systolic heart failure)												
3	randomised trials	not serious	not serious	not serious	very serious [a]	none	72	55	–	SMD **0.38 SD lower** (0.68 lower to 0.07 lower)	⨁⨁◯◯ LOW	8
LVEF (Cardiomyopathy)												

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**stem cells**	**placebo/ no intervention**	**Relative (95 % CI)**	**Absolute (95 % CI)**		
4	randomised trials	very serious [b]	serious [c]	not serious	not serious	publication bias strongly suspected [d]	124	120	–	MD **4.87 higher** (1.32 higher to 8.43 higher)	⨁◯◯◯ VERY LOW	8

CI: Confidence interval; LVEF: Left Ventricular Ejection Fraction; MD: Mean difference; NYHA: New York Heart Association Functional Class; SMD: Standardised mean difference.

Explanations.

[a] Sample size < 100 in both arms.

[b] >30 % RCTs included at high risk of bias.

[c] I2 between 50 and.75 %.

[d] Egger's test (p-value) <0.05.

APPENDIX A

Supplementary Table 1. Descriptive characteristics of the studies included

Intervention	Way of administration	Population	Outcome	N of studies	SC	Placebo	Type of ES	Mean ES (RE) LL, UL	P	I^2	Small study effects	Largest study significant	95% PI LL, UP
MSCT	IntraCoronary. TransEndocardial. IntraVenous	HF (sys)	LVEF	6	159	111	WMD	6.24 (4.64, 7.84)	2.22E-14	23.8	no	no	2.73, 9.75
BMSCT	IntraCoronary. IntraVenous	Human AMI	LVESV	13	404	387	MD	-5.52 (-7.68, -3.36)	5.41E-07	15.8	no	yes	-9.71, -1.33
MSCT	IntraMyocardial. IntraVenous	Human IHD	6MWD	5	163	80	MD	27.62 (16.18 39.07)	2.22E-06	0	no	no	9.05, 46.2
ASCT	IntraMuscular. IntraArterial	Human Lower Extremity Ulcers	Complete healing	9	124	106	RR	2.16 (1.47, 3.16)	0.00008	0	no	no	1.36, 3.42
ASCT	IntraMuscular	Human Crtitical Limb Ischaemia	Amputation rate	7	163	133	OR	0.3 (0.16, 0.57)	0.0003	0	no	no	0.13, 0.69
BMSCT	IntraCoronary. IntraVenous	Human AMI	LVEF	10	344	345	MD	2.6 (1.11, 4.09)	0.001	32.8	no	yes	-0.94, 6.14
MSCT	IntraMyocardial	Refractory angina	Mortality	8	314	212	OR	0.24 (0.1, 0.6)	0.002	0	no	yes	0.05, 1.06
MSCT	IntraMyocardial	Refractory angina	Exercise time	3	162	140	MD	58.62 (21.19, 96.06)	0.002	0	no	no	-184, 301
SCT	IntraMuscular. IntraArterial	Human PAD	ABI	4			MD	0.55 (0.18, 0.88)	0.003	0	no		-0.23, 1.3
BMSCT	IntraMyocardial. IntraCoronary	Human CMP	LVEF	4	124	120	MD	4.87 (1.32, 8.43)	0.007	70.3	no	yes	-10.11, 19.85
MSCT	IntraMyocardial	Refractory angina	Angina frequency	4	180	146	MD	-2.79 (-4.8, -0.77)	0.007	96.2	no	no	-11.51, 5.98
MSCT	IntraMyocardial	Refractory angina	Exercise tolerance	7	261	150	SMD	0.26 (0.06, 0.47)	0.01	0	no	no	0, 0.53
MSCT	TransEndocardial. IntraCoronary. IntraVenous	HF (sys)	NYHA	3	72	55	WMD	-0.38 (-0.68, -0.07)	0.02	47.6	no	no	-3.51, 2.76

Intervention	Way of administration	Population	Outcome	N of studies	SC	Placebo	Type of ES	Mean ES (RE) LL, UL	P	I^2	Small study effects	Largest study significant	95% PI LL, UP
ASCT	IntraMuscular	Human Lower extremity ulcers	Partial healing	3	31	29	RR	3.07 (1.14, 8.27)	0.03	0	no	no	1.06, 4.45
ASCT	IntraMuscular. IntraArterial	Human Lower extremity ulcers	Ulcer size	4	54	48	MD	-0.62 (-1.17, -0.06)	0.03	32.6	no	no	-2.46, 1.22
MSCT	IntraMyocardial. IntraCoronary	Human IHD	Scar mass	3	94	65	MD	-0.96 (-1.86, -0.07)	0.04	84.3	no	yes	-11.85, 9.92
MSCT	IntraMyocardial	Refractory angina	MACE	8	314	212	OR	0.42 (0.19, 0.94)	0.04	37	no	yes	0.06, 2.96
MSCT	IntraMyocardial. IntraVenous. IntraCoronary	Human IHD	Rehospitalization	8	255	180	OR	0.57 (0.31, 1.03)	0.06	0	no	no	0.27, 1.2
MSCT	IntraCoronary. TransEndocardial. IntraVenous	HF (sys)	Readmission	5	222	222	RR	0.68 (0.45, 1.02)	0.06	37.2	no	no	0.22, 2.07
MSCT	IntraMyocardial. IntraVenous. IntraCoronary	Human IHD	Death	13	389	300	OR	0.6 (0.3, 1.21)	0.15	0	no	no	0.26, 1.39
MSCT	IntraCoronary. TransEndocardial	HF (sys)	6MWD	6	168	100	WMD	29.42 (-12.64, 71.42)	0.17	74.2	no	no	-105.94, 164.77
BMSCT	IntraMyocardial. IntraCoronary	Human CMP	LVEDD	3	83	80	MD	-2.19 (-5.59, 1.31)	0.22	25.7	no	yes	-33.48, 29.1
BMSCT	IntraCoronary	Human AMI	Rehospitalization	3	161	163	OR	0.39 (0.08, 1.84)	0.24	0	no	no	0, 8586
BMSCT	IntraCoronary	Human AMI	Recurrent MI	4	201	203	OR	0.41 (0.09, 1.93)	0.26	14.1	yes	no	0.01, 28.99
MSCT	IntraCoronary. TransEndocardial. IntraVenous	HF (sys)	Death	9	320	286	RR	0.72 (0.4, 1.3)	0.27	0	no	no	0.25, 1.85
SCT	IntraMuscular	Human PAD	Ulcer healing rate	3			OR	2.07 (0.44, 9.87)	0.36	0	no		0, 51546
ASCT	IntraMuscular	Human Crtitical Limb Ischaemia	ABI	4	84	85	WMD	0.02 (-0.02, 0.06)	0.37	0	no	no	-0.08, 0.12

Intervention	Way of administration	Population	Outcome	N of studies	SC	Placebo	Type of ES	Mean ES (RE) LL, UL	P	I^2	Small study effects	Largest study significant	95% PI LL, UP
MSCT	IntraMyocardial. IntraVenous. IntraCoronary	Human IHD	LVESV	10	418	371	MD	-4.562 (-15.91, 6.79)	0.43	94	no	no	-43.12, 34
MSCT	IntraMyocardial	Refractory angina	Stroke	3	179	90	OR	0.5 (0.08, 3.2)	0.47	0		no	
MSCT	IntraMyocardial	Refractory angina	MI	3	179	90	OR	0.77 (0.36, 1.64)	0.49	0		no	
BMSCT	IntraCoronary. IntraVenous	Human AMI	Death	13	423	483	OR	1.03 (0.35, 3.03)	0.95	0	no	no	0.23, 4.74
BMSCT	IntraCoronary. IntraVenous	Human AMI	LVEDV	13	404	387	MD	-2.91 (0.11, 0.06)	23.5	23.5	no	no	-9.55, 3.74
MSCT	IntraMyocardial. IntraVenous. IntraCoronary	Human IHD	LVEF	13	469	415	MD	3.84 (2.33, 5.34)	<0.0001	42.5	no	no	-0.27, 7.95

Abbreviations:

6MWD: 6-Minute Walking Distance; **ABI**: Ankle Brachial Index; **AMI**: Acute Myocardial Infarction; **ASCT**: Autologous Stem Cell Transplant; **BMC**: Bone Marrow Concentrate; **BMNC**: Bone Marrow Mononuclear Cells; **BMSCT**: Bone Marrow-Derived Mesenchymal Stem Cells Transplant; **CMP**: Nonischemic Cardiomyopathy; **ES**: Effect Size; **HF**: Heart Failure; **IHD**: Ischemic Heart Disease; **LL**: Confidence Interval Lower Limit; **LVEDD:** Left Ventricular End-Diastolic Diameter; **LVEDV:** Left Ventricular End-Diastolic Volume; **LVEF:** Left Ventricular Ejection Fraction; **LVSV**: Left Ventricular Stroke Volume; **MACE**: Major Adverse Cardiovascular Events; **MD**: Mean Difference; **MI**: Myocardial Infarction; **MSCT**: Mesenchymal Stem Cells Transplant; **NYHA**: New York Heart Association Functional Class; **OR**: Odds Ratio; **P**: p-value; **PAD**: Peripheral Artery Disease; **PI**: Prediction Interval; **RE**: Random Effect; **RR**: Risk Rati; **SC**: Steam cells; **SCT**: Steam cell Transplant; **SMD**: Standardized Main Difference; **UCB**: Umbilical Cord Blood Derived Stem Cells; **UL**: Confidence interval upper limit; **WMD**: Weighted Mean Difference.

Supplementary Table 2. AMSTAR 2 quality assessment of meta-analyses.

	AMSTAR 2 items [a, c]																
Author, Year [Reference]	**1**	**2** [b]	**3**	**4** [b]	**5**	**6**	**7** [b]	**8**	**9** [b]	**10**	**11** [b]	**12**	**13** [b]	**14**	**15** [b]	**16**	**Overall rating** (based on critical domains)[d]
Fan, 2019	Y	Y	N	PY	Y	Y	N	Y	Y	N	Y	N	N	Y	Y	Y	CRITICALLY LOW
Jones, 2019	Y	Y	N	PY	Y	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y	LOW
Velagapudi, 2018	Y	N	N	PY	Y	Y	N	Y	N	N	Y	N	N	Y	Y	N	CRITICALLY LOW
Jeong, 2018	Y	PY	N	PY	Y	Y	N	Y	Y	N	Y	N	Y	Y	Y	Y	LOW
Kuswardhani, 2011	Y	PY	N	PY	N	Y	N	Y	PY	N	Y	Y	N	Y	Y	N	CRITICALLY LOW
Marquis-Gravel, 2014	Y	N	N	PY	Y	Y	N	Y	Y	N	Y	N	N	Y	Y	N	CRITICALLY LOW
Sun, 2015	Y	N	N	PY	Y	Y	N	Y	PY	N	Y	Y	N	Y	Y	Y	CRITICALLY LOW
Liu, 2015	Y	N	N	PY	Y	Y	Y	Y	Y	N	Y	N	N	Y	Y	Y	CRITICALLY LOW
Jiang, 2016	Y	N	N	PY	Y	Y	N	Y	Y	N	Y	N	N	Y	Y	Y	CRITICALLY LOW
Jaravi, 2019	Y	N	N	N	Y	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y	CRITICALLY LOW
Fernandes,2019	Y	N	N	PY	Y	Y	N	Y	Y	N	Y	Y	N	Y	Y	Y	CRITICALLY LOW

[a] Yes, No, Other
[b] Critical Domains
[c] AMSTAR 2 items:

1. **Did the research questions and inclusion criteria for the review include the components of PICO (Population, Intervention, Comparator group, Outcome)?** YES/NO. For yes, must have all four.
2. **Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?** YES, PARTIAL YES, NO. For Partial YES: the authors state that they had a written protocol or guide that included ALL the following (review question(s), a search strategy, inclusion/exclusion criteria, a risk of bias assessment). For YES: as for partial yes, plus the protocol should be registered and should also have specified: a meta-analysis/synthesis plan, if appropriate, and a plan for investigating causes of heterogeneity, justification for any deviations from the protocol.
3. **Did the review authors explain their selection of the study designs for inclusion in the review?** YES/NO. For YES, the review should satisfy one of the following: explanation for including only RCTs, or explanation for including only NRSI, or explanation for including both RCTs and NRSI.

4. **Did the review authors use a comprehensive literature search strategy?** YES, PARTIAL YES, NO. for PARTIAL YES must have all of the following: searched at least 2 databases (relevant to research question), provided key word and/or search strategy, justified publication restrictions (eg. Language). For YES should also have all of the following: searched the reference lists/biographies of included studies, searched trial/study registries, included/consulted content experts in the field, searched for grey literature where relevant, conducted search within 24 months of completion of the review.
5. **Did the review authors perform study selection in duplicate?** YES/NO. for YES, either ONE of the following: at least two reviewers independently agreed on selection of eligible studies and achieved consensus on which studies to include OR two reviewers selected a sample of eligible studies and achieved good agreement (at least 80 per cent) with the remainder selected by one reviewer.
6. **Did the review authors perform data extraction in duplicate?** YES/NO. For YES, either one of the following: at least two reviewers achieved consensus on which data to extract from included studies OR two reviewers extracted data from a sample of eligible studies and achieved good agreement (at least 80 per cent) with the remainder extracted by one reviewer.
7. **Did the review authors provide a list of excluded studies to justify the exclusions?** YES, PARTIAL YES, NO. FOR partial yes must provide a list of all potentially relevant studies that were read in full text form but excluded from the review. For YES must also have justified the exclusion from the review of each potentially relevant study.
8. **Did the review authors describe the included studies in adequate detail?** YES, PARTIAL YES, NO. For PARTIAL YES, must describe all of the following: populations, interventions, comparators, outcomes, research designs. For YES should also have all of the following: described populations in detail, described intervention and comparator in detail (including doses where relevant), described study setting, timeframe or follow-up.
9. **Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review? For RCTs**: YES, PARTIAL YES, NO, INCLUDES ONLY NRSI. For PARTIAL YES must have assessed RoB from unconcealed allocation and lack of blinding of patients and assessors when assessing outcomes (unnecessary for objective outcomes such as all cause mortality); for YES must also have assessed RoB from allocation sequence that was not truly random and selection of the reported result from among multiple measurements or analyses of a specified outcome. **For NRSI** (Non Randomized Studies of Intervention)**:** YES, PARTIAL YES, NO, INCLUDES ONLY RCTs. For PARTIAL YES must have assessed RoB from confounding and from selection bias. For YES, must also have assessed methods used to ascertain exposures and outcomes, and selection of the reported results from among multiple measurements or analyses of a specified outcome.
10. **Did the review authors report on the sources of funding for the studies included in the review?** YES/NO. For YES: must have reported on the sources of funding for individual studies included in the review. Note: reporting that the reviewers looked for this information but it was not reported by study authors also qualifies
11. **If meta-analysis was performed, did the review authors use appropriate methods for statistical combination of results? For RCTs:** YES, NO, NO META-ANALYSIS. For YES: the authors justified combining the data in a meta-analysis and they used an appropriate weighted technique to combine study results and adjusted for heterogeneity if present and investigated the causes of heterogeneity. **For NRSI:** YES, NO, NO META-ANALYSIS CONDUCTED. For YES: the authors justified combining the data in a meta-analysis and they used an appropriate weighted technique to combine study results, adjusting for heterogeneity if present, and they statistically combined effects estimates from NRSI that were adjusted for confounding, rather than combining raw data, or justified combining raw data when adjusted effect estimates were not available, and they reported separate summary estimates for RCTs and NRSI separately when both were included in the review.

12. **If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?** YES, NO, NO META-ANALYSIS INCLUDED. For YES: included only low risk of bias RCTs or, if the pooled estimate was based on RCTs and/or NRSI at variable RoB, the authors performed analysis ton investigate possible impact of RoB on summary estimates of effect.
13. **Did the review authors account for RoB in individual studies when interpreting/discussing the results of the review?** YES/NO. for YES: included only low risk of bias RCTs or, if RCTs with moderate or high RoB, or NRSI were included, the review provided a discussion of the key impact of RoB on the results
14. **Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?** YES/NO. For Yes: there was no significant heterogeneity in the results OR if heterogeneity was present the authors performed an investigation of sources of any heterogeneity in the results and discussed the impact of this on the results of the review
15. **If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?** YES, NO, NO META-ANALYSIS CONDUCTED. For YES: performed graphical statistical tests for publication bias and discussed the likelihood and magnitude of impact of publication bias
16. **Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?** YES/NO. For Yes: the authors reported no competing interests OR the authors described their funding sources and how they managed potential conflicts of interest.

[d] Rating overall confidence in the results of the review:
HIGH: *no on one non-critical weakness*: the systematic review provides an accurate and comprehensive summary of the results of the available studies that address the question of interest
MODERATE: *more than one non critical weakness* (multiple non-critical weaknesses may diminish confidence in the review and it may be appropriate to move the overall appraisal down from moderate to low confidence): the systematic review has more than one weakness but no critical flaws. It may provide an accurate summary of the results of the available studies that were included in the review
LOW: *one critical flaw with or without non-critical weaknesses*: the review has a critical flaw and may not provide an accurate and comprehensive summary of the available studies that address the question of interest
CRITICALLY LOW: *more than one critical flaw with or without non-critical weaknesses*: the review has more than one critical flaw and should not be relied on to provide an accurate and comprehensive summary of the available studies

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[c] AMSTAR items:
1. Was an 'a priori' design provided? The research question and inclusion criteria should be established before the conduct of the review. *Note: Need to refer to a protocol, ethics approval, or pre-determined/a priori published research objectives to score a "yes."*
2. Was there duplicate study selection and data extraction? There should be at least two independent data extractors and a consensus procedure for disagreements should be in place. *Note: 2 people do study selection, 2 people do data extraction, consensus process or one person checks the other's work.*

3. Was a comprehensive literature search performed? At least two electronic sources should be searched. The report must include years and databases used (e.g., Central, EMBASE, and MEDLINE). Key words and/or MESH terms must be stated and where feasible the search strategy should be provided. All searches should be supplemented by consulting current contents, reviews, textbooks, specialized registers, or experts in the particular field of study, and by reviewing the references in the studies found. *Note: If at least 2 sources + one **Appendix** strategy used, select "yes" (Cochrane register/Central counts as 2 sources; a grey literature search counts as **Appendix**).*

4. Was the status of publication (i.e., grey literature) used as an inclusion criterion? The authors should state that they searched for reports regardless of their publication type. The authors should state whether or not they excluded any reports (from the systematic review), based on their publication status, language etc. *Note: If review indicates that there was a search for "grey literature" or "unpublished literature," indicate "yes." SIGLE database, dissertations, conference proceedings, and trial registries are all considered grey for this purpose. If searching a source that contains both grey and non-grey, must specify that they were searching for grey/unpublished lit.*

5. Was a list of studies (included and excluded) provided? A list of included and excluded studies should be provided. *Note: Acceptable if the excluded studies are referenced. If there is an electronic link to the list but the link is dead, select "no."*

6. Were the characteristics of the included studies provided? In an aggregated form such as a table, data from the original studies should be provided on the participants, interventions and outcomes. The ranges of characteristics in all the studies analyzed e.g., age, race, sex, relevant socioeconomic data, disease status, duration, severity, or other diseases should be reported. *Note: Acceptable if not in table format as long as they are described as above.*

7. Was the scientific quality of the included studies assessed and documented? 'A priori' methods of assessment should be provided (e.g., for effectiveness studies if the author(s) chose to include only randomized, double-blind, placebo controlled studies, or allocation concealment as inclusion criteria); for other types of studies alternative items will be relevant. *Note: Can include use of a quality scoring tool or checklist, e.g., Jadad scale, risk of bias, sensitivity analysis, etc., or a description of quality items, with some kind of result for EACH study ("low" or "high" is fine, as long as it is clear which studies scored "low" and which scored "high"; a summary score/range for all studies is not acceptable).*

8. Was the scientific quality of the included studies used appropriately in formulating conclusions? The results of the methodological rigor and scientific quality should be considered in the analysis and the conclusions of the review, and explicitly stated in formulating recommendations. *Note: Might say something such as "the results should be interpreted with caution due to poor quality of included studies." Cannot score "yes" for this question if scored "no" for question 7.*

9. Were the methods used to combine the findings of studies appropriate? For the pooled results, a test should be done to ensure the studies were combinable, to assess their homogeneity (i.e., , Chi-squared test for homogeneity, I2). If heterogeneity exists a random effects model should be used and/or the clinical appropriateness of combining should be taken into consideration (i.e., , is it sensible to combine?). *Note: Indicate "yes" if they mention or describe heterogeneity, i.e., , if they explain that they cannot pool because of heterogeneity/variability between interventions.*

10. Was the likelihood of publication bias assessed? An assessment of publication bias should include a combination of graphical aids (e.g., funnel plot, other available tests) and/or statistical tests (e.g., Egger regression test, Hedges-Olken). *Note: If no test values or funnel plot included, score "no". Score "yes" if mentions that publication bias could not be assessed because there were fewer than 10 included studies.*

11. Was the conflict of interest included? Potential sources of support should be clearly acknowledged in both the systematic review and the included studies. *Note: To get a "yes," must indicate source of funding or support for the systematic review AND for each of the included studies.*