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

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HIGLIGHTS

- Umbrella review of systematic reviews with meta-analyses.
- Randomized controlled trials using placebo/no intervention as control group.
- 11 meta-analyses (for a total of 34 outcomes) were included.
- Half of the outcomes were statistically significant ($p<0.05$).
- Stem cells are more useful than placebo for treatment of cardiovascular diseases.

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.

Keywords: 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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Figure 1.

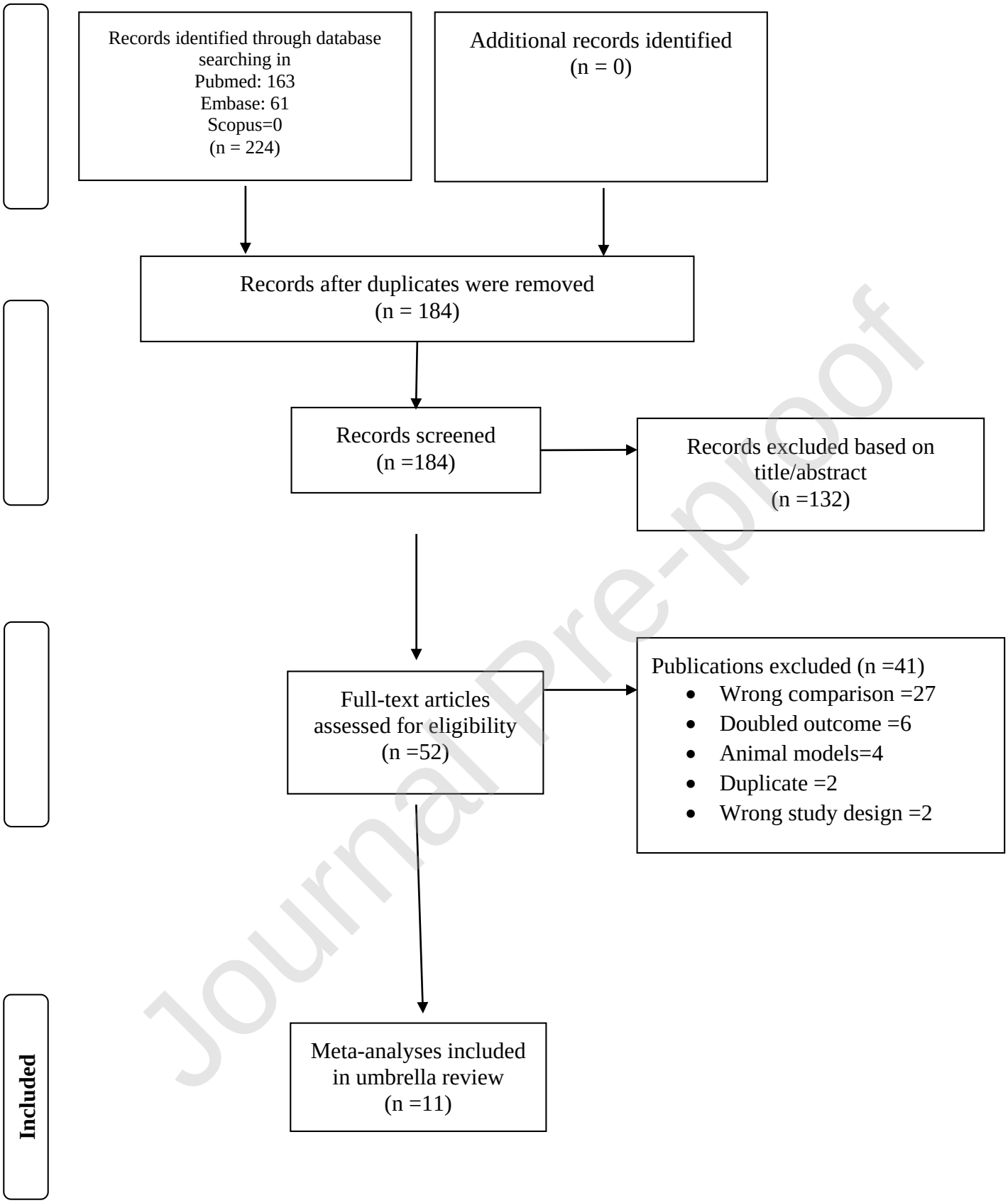

Table 1. GRADE evidence for randomized controlled trials investigating outcomes related to coronary heart disease.

Certainty assessment							№ of patients	
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	stem cells	placebo/ no intervention
LVESV (in AMI)								
13	randomised trials	not serious	not serious	not serious	not serious	none	404	387
LVEF (in AMI)								
10	randomised trials	not serious	not serious	not serious	not serious	none	344	345
6MWD (in IHD)								
5	randomised trials	serious [a]	not serious	not serious	serious [b]	none	163	80
Scar mass (IHD)								
3	randomised trials	serious [c]	very serious [d]	not serious	serious [b]	none	94	65
Mortality (refractory angina)								
8	randomised trials	serious [a]	not serious	not serious	not serious	none	314	212
Exercise time (refractory angina)								

Certainty assessment							№ of patients	
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	stem cells	placebo/ no intervention
3	randomised trials	not serious	not serious	not serious	not serious	none	162	140
Angina frequency (refractory angina)								
4	randomised trials	not serious	very serious [d]	not serious	not serious	none	180	146
Exercise tolerance (refractory angina)								
7	randomised trials	serious [a]	not serious	not serious	not serious	none	261	150
MACE (refractory angina)								
8	randomised trials	serious [a]	not serious	not serious	not serious	none	314	212

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. I^2 >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	stemcells	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 to 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
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 to 8.27)	-	⨁⨁◯◯ LOW	6
ABI (PAD)												

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	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			
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	stemcells	placebo/ no intervention	Relative (95 % CI)	Absolute (95 % CI)	Certainty	Importance
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)												
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. I^2 between 50 and 75%

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