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[Intervention Review]

Stem cell therapy for treatment of thromboangiitis obliterans (Buerger's disease)

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ABSTRACT

Background

Thromboangiitis obliterans, also known as Buerger's disease, is a non-atherosclerotic, segmental inflammatory pathology that most commonly affects the small- and medium-sized arteries, veins, and nerves in the upper and lower extremities. The etiology is unknown, but involves hereditary susceptibility, tobacco exposure, immune and coagulation responses. In many cases, there is no possibility of revascularization to improve the condition. Stem cell therapy is an option for patients with severe complications, such as ischemic ulcers or rest pain.

Objectives

To assess the effectiveness and safety of stem cell therapy in individuals with thromboangiitis obliterans (Buerger's disease).

Search methods

The Cochrane Vascular Information Specialist searched the Cochrane Vascular Specialised Register, CENTRAL, MEDLINE, Embase, CINAHL and AMED databases and World Health Organization International Clinical Trials Registry Platform and ClinicalTrials.gov trials registers to 17 October 2017. The review authors searched the European grey literature OpenGrey Database, screened reference lists of relevant studies and contacted study authors.

Selection criteria

Randomized controlled trials (RCTs) or quasi-RCTs of stem cell therapy in thromboangiitis obliterans (Buerger's disease).

Data collection and analysis

The review authors (DC, DM, FN) independently assessed the studies, extracted data and performed data analysis.

Main results

We only included one RCT (18 participants with thromboangiitis obliterans) comparing the implantation of stem cell derived from bone marrow with placebo and standard wound dressing care in this review. We identified no studies that compared stem cell therapy (bone marrow source) versus stem cell therapy (umbilical cord source), stem cell therapy (any source) versus pharmacological treatment and stem cell therapy (any source) versus sympathectomy. Ulcer healing was assessed in the form of ulcer size. The mean ulcer area decreased more in the stem cell implantation group: from 5.04 cm² (standard deviation (SD) 0.70) to 1.48 cm² (SD 0.56) compared with the control group: mean ulcer size area decreased from 4.68 cm² (SD 0.62) to 3.59 cm² (SD 0.14); mean difference (MD) -2.11 cm², 95% confidence interval (CI) -2.49 to -1.73; 1 study, 18 participants; very low-quality evidence. Pain-free walking distance showed more of an improvement

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in the stem cell implantation group: from mean of 38.33 meters (SD 17.68) to 284.44 meters (SD 212.12) compared with the control group: mean walking distance increased from 35.66 meters (SD 19.79) to 78.22 meters (SD 35.35); MD 206.22 meters, 95% CI 65.73 to 346.71; 1 study; 18 participants; very low-quality evidence.

Outcomes such as rate of amputation, pain, amputation-free survival and adverse effects were not assessed.

The quality of evidence was classified as very low, with only one study, small numbers of participants, high risk of bias in many domains and missing information regarding tobacco exposure status.

Authors' conclusions

Very low-quality evidence suggests there may be an effect of the use of bone marrow-derived stem cells in the healing of ulcers and improvement in the pain-free walking distance in patients with Buerger's disease. High-quality trials assessing the effectiveness of stem cell therapy for treatment of patients with thromboangiitis obliterans (Buerger's disease) are needed.

PLAIN LANGUAGE SUMMARY

Stem cell therapy for treatment of thromboangiitis obliterans (Buerger's disease)

Background

Thromboangiitis obliterans, also known as Buerger's disease, is a condition characterized by recurring progressive inflammation and clotting in small- and medium-sized arteries and veins of the hands and feet. Its cause is unknown, but it is most common in men with a history of tobacco use. It is responsible for ulcers and extreme pain in the limbs of young smokers. In many cases, mainly in patients with the most severe form, there is no possibility of improving the condition with surgery, and therefore, alternative treatments are used. Stem cell therapy is an experimental treatment performed through the implantation of cells (from bone marrow, umbilical cord, peripheral blood etc.) which are capable of becoming new blood vessels, improving local circulation and contributing to the healing of ulcers and relieving rest pain. This review assessed the effectiveness of stem cell therapy in the treatment of patients with thromboangiitis obliterans (Buerger's disease).

Key results

Only one randomized controlled study (18 participants with thromboangiitis obliterans) comparing the implantation of stem cells derived from bone marrow with placebo and standard wound dressing care was included in this review (most recent search was 17 October 2017). We identified no studies that compared stem cell therapy from different sources, stem cell therapy versus drug treatment and stem cell therapy versus sympathectomy (surgical cutting of a sympathetic nerve). The results showed a decrease in ulcer size and improvement in pain-free walking distance in the group receiving the stem cell implantation compared with the group receiving placebo and standard wound dressing care.

Outcomes such as rate of amputation, pain, amputation-free survival and adverse effects were not assessed.

Quality of the evidence

We classified the quality of evidence as very low, because there was only one study, small numbers of participants, and high risk of bias in many domains and missing information regarding tobacco exposure status.

Conclusions

Very low-quality evidence suggests there may be an effect of the use of bone marrow-derived stem cells in the healing of ulcers and improving the pain-free walking distance in patients with Buerger's disease. High-quality trials assessing the effectiveness of stem cell therapy for treatment of patients with thromboangiitis obliterans (Buerger's disease) are needed.

SUMMARY OF FINDINGS

Summary of findings for the main comparison. Stem cell therapy (any source) compared with any other modality of treatment for thromboangiitis obliterans (Buerger's disease)

Stem cell therapy (any source) compared with any other modality of treatment for thromboangiitis obliterans (Buerger's disease)

Patient or population: individuals with thromboangiitis obliterans (Buerger's disease)

Settings: not stated

Intervention: stem cell therapy (bone marrow source)

Comparison: placebo

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No. of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Placebo	Stem cell therapy (bone marrow source)				
Rate of amputation	-	-	-	-	-	The single included study in this review did not report on this outcome
Ulcer healing (size area) Follow-up: 12 weeks	The mean ulcer size in control group was 3.59 cm²	The mean ulcer size in the intervention group was 2.11 cm² lower (2.49 lower to 1.73 lower)		18 (1 RCT)	⊕⊕⊕⊕a, b, c very low	
Pain Measured using a validated pain score or scale, or quality of life questionnaire	-	-	-	-	-	The single included study in this review did not report on this outcome
Adverse effects Local or systemic inflammatory response, cardiovascular abnor-	-	-	-	-	-	The single included study in this review did not report on this outcome

malities, and thromboembolic complications					
Amputation-free survival	-	-	-	-	The single included study in this review did not report on this outcome
Pain-free walking distance Follow-up: 12 weeks	The mean pain-free walking distance in control group was 78.22 m	The mean pain-free walking distance in the intervention group was 206.22 m higher (65.73 higher to 346.71 higher)	18 (1 RCT)	⊕⊕⊕⊕ ^{a, b, c} very low	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: confidence interval; **cm:** centimeter; **m:** meter; **RCT:** randomized controlled trial

GRADE Working Group grades of evidence

High quality: further research is very unlikely to change our confidence in the estimate of effect.

Moderate quality: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low quality: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low quality: we are very uncertain about the estimate.

^aHigh risk of bias in allocation concealment and selective reporting, downgraded by one level.

^bAbsence of patient's smoking history, downgraded by one level.

^cSmall number of participants and one single study (doubt about reproducibility of data), downgraded by one level.

BACKGROUND

Description of the condition

Thromboangiitis obliterans, also known as Buerger's disease, is a non-atherosclerotic, occlusive, thrombotic, segmental, inflammatory pathology that most commonly affects small- and medium-sized arteries, veins, and nerves in the upper and lower extremities (Olin 2000). Von Winiwarter first described a person with the disease in 1879 (von Winiwarter 1879), but it was Leo Buerger, in 1908, who published a detailed description of the pathological findings on 11 amputated limbs and named the disease (Buerger 1908).

The prevalence of the disease among individuals with peripheral arterial disease (PAD) varies from as low as 0.5% to 5.6% in Western Europe to as high as 45% to 63% in India, and from 16% to 66% in Japan and Korea (Cachovan 1988; Malecki 2009; Olin 2000).

Despite knowledge of the fundamental role that smoking plays in the development and progression of this ischemic condition, the etiology of Buerger's disease is still unknown (Matsushita 1991; Olin 1990; Silbert 1945). The role of tobacco in the etiopathogenesis of Buerger's disease is unquestionable, but the specific mechanism is not yet fully understood. It is not known which exact component of tobacco is involved in the pathogenesis (Olin 1990). The work of Rahman 2000 showed that a type of cigarette (called a "bidi") made from small and unprocessed tobacco leaves appears to have a greater potential for triggering Buerger's disease than regular cigarettes.

The most commonly accepted theory is a possible connection between hereditary factors (related to specific human leukocyte antigen haplotypes, such as A9, B5, and DR4), immune factors (hypersensitivity to collagen type I, III, and IV; antibodies directed against G-protein receptors; increased production of interleukin-6, -10, and -12; increased expression of the adhesion molecules intercellular adhesion molecule 1, vascular adhesion molecule 1, and E-selectin; increased expression of plasminogen activator inhibitor 1 along the internal elastic lamina); and coagulation disorders in people exposed to tobacco without the development and perpetuation of the disease (Malecki 2009). Interest is also increasing in a possible infectious etiology following the discovery of oral flora micro-organism DNA in occlusive thrombi in individuals with Buerger's disease and moderate-to-severe periodontitis (Iwai 2005; Li 2008). Another hypothesis is a possible pathogenic role of rickettsial infection in Buerger's disease among persons in low-socioeconomic conditions (Bartolo 1987; Fazeli 2013).

Features distinguishing Buerger's disease from atherosclerosis include the peculiar distribution of pathology (with involvement of both the upper and lower extremities), associated superficial venous thrombosis, a paucity of atherosclerotic risk factors, and normal proximal large arteries (Weinberg 2012).

Diagnosis and complications

The typical individual with thromboangiitis obliterans is a young man (aged < 45 years), with a current or previous history of tobacco use, presenting with progressive claudication, ischemic ulcers, or pain at rest (Olin 2000). Approximately 76% of affected individuals have ischemic ulcerations at the time of presentation (Olin 2006). To date, there are no unanimous diagnostic criteria for Buerger's disease in the medical literature. The most commonly

used are Shionoya's criteria, which comprise: (1) smoking history; (2) onset of symptoms before the age of 50 years; (3) infrapopliteal arterial occlusions; (4) involvement of either arm or phlebitis migrans; and (5) absence of atherosclerotic risk factors other than smoking (Shionoya 1983). All criteria should be present. Other important and more complete diagnostic criteria were formulated by Olin 2000: (1) age younger than 45 years; (2) current or recent history of tobacco use; (3) presence of distal extremity ischemia indicated by claudication, pain at rest, ischemic ulcers, or gangrene, and documented by noninvasive vascular testing; (4) exclusion of autoimmune disease, hypercoagulable state, and diabetes mellitus; (5) exclusion of a proximal source of embolization by echocardiography and arteriography; and (6) consistent arteriographic findings in the clinically involved and noninvolved limbs.

The disease is usually confined to the distal circulation and is almost always infrapopliteal in the legs and distal to the brachial artery in the arms. The distal and diffuse nature of the disease culminates in critical limb ischemia (typical chronic ischemic rest pain or ischemic skin lesions (either ulcers or gangrene), or both for more than two weeks) in approximately 76% to 81% of affected individuals, with little to no chance of revascularization (Olin 2006).

In individuals diagnosed with limb ischemia, as in Buerger's disease, clinical evaluation is made according to the Fontaine classification or the Rutherford classification for PAD. The Fontaine classification has four stages: (I) asymptomatic; (II) intermittent claudication; (III) rest pain; and (IV) ulceration or gangrene, or both (Fontaine 1954). Novo 2004 described a modified Fontaine classification, the Leriche-Fontaine classification: (I) asymptomatic or effort pain; (IIa) effort pain or pain-free walking distance further than 200 meters; (IIb) pain-free walking distance less than 200 meters; (IIIa) rest pain and ankle arterial pressure higher than 50 mm Hg; (IIIb) rest pain and ankle arterial pressure lower than 50 mm Hg; (IV) and trophic lesions, necrosis, or gangrene. The Rutherford classification for PAD has seven categories: (0) asymptomatic; (1) mild claudication; (2) moderate claudication; (3) severe claudication; (4) rest pain; (5) minor tissue loss, non-healing ulcer, or focal gangrene with diffuse pedal ischemia; (6) major tissue loss extending above the transmetatarsal level; and (7) a functional foot that is no longer salvageable (Rutherford 2005).

According to Cooper 2004, the risk of any extremity amputation during 15.6 years of follow-up in a person with Buerger's disease is 25% at five years, 38% at 10 years, and 46% at 20 years.

Description of the intervention

There is no standard treatment for Buerger's disease. Treatment of an individual with Buerger's disease is based primarily on the complete abolition of smoking. Concomitantly, depending on the degree of ischemia, the measures are similar to those adopted in individuals with PAD of atherosclerotic etiology: in those with intermittent claudication, the performance of supervised exercise; for those with critical limb ischemia, increased limb perfusion via surgical revascularization, sympathectomy, use of pharmacological agents, etc. as well as analgesia, and wound and extremity care (Rutherford 2005).

The surgical revascularization of limbs in individuals with Buerger's disease is thought to reduce pain and promote the healing of trophic changes but is controversial due to the high index of

graft occlusion. The large distal involvement seen in Buerger's disease greatly impairs surgery and long-term patency. An article by [Sasajima 1997](#), which presented the results of 18 years' experience in revascularization in the infrainguinal territory, reported on the performance of 71 grafts with an autologous vein in 61 individuals with Buerger's disease and the occurrence of 38 (62%) graft occlusions. Among the possible causes for the high rate of graft failure are: the fact that the distal anastomosis is usually performed in a diseased artery and is subject to frequent vasospasms; the progression of inflammatory disease itself; the use of 'poor quality' veins for the graft (because they are also affected by inflammation); and vein stenosis due to myointimal hyperplasia.

Surgical treatment through lumbar sympathectomy is a surgical modality used to prevent amputations and provide pain relief at rest through the vasodilatory effects resulting from a decreased sympathetic response in the affected limb. [Nakajima 1998](#) reported an improvement in symptoms of up to 60% following lumbar sympathectomy in individuals with Buerger's disease. However, the importance of this procedure is reduced by the fact that the effects of amputation prevention and the ability of the procedure to provide pain relief remain unproven.

A recent Cochrane Review ([Cacione 2016](#)) assessed the effectiveness of pharmacological agents in individuals with Buerger's disease and critical limb ischemia. According to this review, there is moderate-quality evidence to suggest that intravenous iloprost (a prostacyclin analog) is more effective than aspirin for eradicating rest pain and healing ischemic ulcers in individuals with thromboangiitis obliterans, but that oral iloprost is not more effective than placebo. There is very low- and low-quality evidence to suggest that there is no difference between prostacyclin (iloprost and clinprost) and the prostaglandin analog alprostadil for ulcer healing and relief of pain, respectively, in individuals with severe thromboangiitis obliterans.

Other therapeutic modalities include the microsurgical flap and omental transfer, arterialization of the venous arch of the foot, and spinal cord stimulation in cases of severe pain ([Rutherford 2005](#)).

Another alternative treatment for individuals with Buerger's disease and critical limb ischemia is stem cell therapy, in which stem cells are injected into the affected area ([Boda 2009](#)).

Stem cells are undifferentiated and proliferative cells (in vitro and in vivo) that have the ability to differentiate into a specialized adult cell type, with the potential to become any tissue in the human body ([Herberts 2011](#)). Theoretically, they may promote ulcer healing, neovascularization, and the regeneration of nerve cells. Stem cells can be pluripotent or multipotent. Pluripotent cells have the capacity to become any type of cell in the human body, being categorized as embryonal stem cells and induced pluripotent stem cells. Embryonal stem cells are derived from totipotent cells of the inner cell mass of the blastocyst (an early stage of the mammalian embryo), and are capable of unlimited and undifferentiated proliferation in vitro. Induced pluripotent stem cells are a type of pluripotent stem cell artificially derived from an adult differentiated somatic cell that is nonpluripotent and induced through a 'forced' expression of specific genes, related to the expression of transcription factors. Multipotent adult or somatic stem cells are found in differentiated tissues, generally with maintenance, regeneration, and tissue-replacing functions. Multipotent somatic stem cells can differentiate into limited cell

types whereas pluripotent cells have the capacity to differentiate into any type of cell. Sources of somatic stem cells include bone marrow, cord blood, and even adipose tissue ([Pessina 2006](#)). Endothelial progenitor cells are somatic stem cells derived from the bone marrow endothelial lineage ([Asahara 1997](#)).

Prior to the injection of concentrated stem cells into the tissue of the affected limb (e.g. calf muscles), stem cells are collected from source (e.g. bone marrow cells from the ilium bone), and isolated by blood cell separation and cytofluorometric analysis. Potential adverse effects of stem cells administered for vascular purposes include the induction of angiogenic diseases, such as proliferative retinopathy, accelerated arteriosclerosis due to proinflammatory growth factors, enhanced restenosis, arrhythmias, teratoma formation, and ectopic calcification ([Durdu 2006](#)).

How the intervention might work

Buerger's disease is characterized by a distal vaso-occlusive limb pathology. Neovascularization and arteriogenesis may have an important role in stabilizing ischemia. Experimental studies have demonstrated that many lineages of cells within the bone marrow may maintain the ability to differentiate into one or more cellular components of the vascular bed and so may incorporate directly into the vessel wall of the newly formed or remodeled vessels, thus promoting neovascularization ([Kinnaird 2004](#)). Endothelial progenitor cells may improve neovascularization not only by direct incorporation but also through a paracrine action (production of multiple angiogenic cytokines, growth factors, and chemokines) that could facilitate arteriogenesis, and inhibit endothelial and smooth muscle cell apoptosis ([Kinnaird 2004](#)). An experimental study by [Kalka 2000](#), reported that the transplantation of endothelial progenitor cells (culture-expanded) into an animal model of limb ischemia improved neovascularization and blood flow recovery, and reduced limb necrosis and auto-amputation by 50% compared with controls.

Why it is important to do this review

Buerger's disease is a debilitating condition that can affect productive, young people. In many cases, limb revascularization to improve the condition is not possible. Stem cell therapy may offer a possible alternative treatment option. Therefore, a systematic review assessing the effectiveness and safety of stem cell therapy in individuals with Buerger's disease is opportune and relevant.

OBJECTIVES

To assess the effectiveness and safety of stem cell therapy in individuals with thromboangiitis obliterans (Buerger's disease).

METHODS

Criteria for considering studies for this review

Types of studies

We searched for randomized controlled trials (RCTs) and quasi-RCTs.

Types of participants

We included individuals clinically diagnosed with Buerger's disease.

We evaluated trials that included a mix of participants diagnosed with Buerger's disease and non-Buerger's disease and incorporated these into the review if the inclusion criteria were satisfied. In this case, we extracted and assessed results and outcomes only in participants with Buerger's disease in both groups (intervention and comparison). We intended to contact the study authors of such trials if data for participants with Buerger's disease were not readily available. If we were unable to obtain data stratified by Buerger's disease we classified these studies as 'awaiting classification'.

Types of interventions

We planned to assess stem cell therapies for treating individuals with Buerger's disease through the following comparisons.

- Stem cell therapy (bone marrow source) versus stem cell therapy (umbilical cord source)
- Stem cell therapy (any source) versus pharmacological treatment
- Stem cell therapy (any source) versus sympathectomy
- Stem cell therapy (any source) versus any other modality of treatment

Types of outcome measures

Primary outcomes

- Rate of amputation: major amputation (defined as amputation of the lower or upper limb above the ankle or the wrist, respectively); and minor amputation (defined as amputation of a hand or foot or any part of)
- Ulcer healing
- Pain: assessed using a validated pain score or scale, or quality of life questionnaire
- Adverse effects such as local or systemic inflammatory responses evaluated using clinical (erythema, local pain, wound infection) and biological (C-reactive protein, fibrinogen, white blood cell count) assessments; cardiovascular abnormalities (e.g. arrhythmias); immunological manifestations (e.g. anaphylactic reaction, graft-versus-host disease); thromboembolic complications (e.g. disseminated intravascular coagulation); donor-to-recipient transmission of bacterial, viral, fungal, or prion pathogens (microbiological contamination); and the development of benign or malignant neoplasms

Secondary outcomes

- Amputation-free survival
- Walking distance or pain-free walking, assessed using a treadmill test or validated questionnaire (e.g. Walking Impairment Questionnaire)

Search methods for identification of studies

Electronic searches

The Cochrane Vascular Information Specialist conducted systematic searches of the following databases for RCTs and quasi-RCTs without language, publication year or publication status restrictions.

- The Cochrane Vascular Specialised Register via the Cochrane Register of Studies (CRS-Web searched from inception to 18 October 2017).
- The Cochrane Central Register of Controlled Trials (CENTRAL) Cochrane Register of Studies Online (CRSO 2017, issue 9).
- MEDLINE (Ovid MEDLINE Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE Daily and Ovid MEDLINE) (searched from 1 January 2017 to 17 October 2017).
- Embase Ovid (searched from 1 January 2017 to 18 October 2017).
- CINAHL Ebsco (searched from 1 January 2017 to 18 October 2017).
- AMED Ovid (searched from 1 January 2017 to 18 October 2017).

The Information Specialist modeled search strategies for other databases on the search strategy designed for CENTRAL. Where appropriate, they were combined with adaptations of the highly sensitive search strategy designed by Cochrane for identifying RCTs and controlled clinical trials (as described in Chapter 6 of the *Cochrane Handbook for Systematic Reviews of Interventions*; Higgins 2011). Search strategies for major databases are provided in [Appendix 1](#).

The Information Specialist also searched the following trials registries on 17 October 2017.

- World Health Organization International Clinical Trials Registry Platform (www.who.int/trialsearch).
- ClinicalTrials.gov (www.clinicaltrials.gov).

Searching other resources

We searched the grey literature produced in Europe by consulting the OpenGrey Database (www.opengrey.eu). We used the terms 'Buerger's disease', 'thromboangiitis obliterans', 'von Winiwarter disease', and word variations to perform our search ([Appendix 2](#)).

We searched the reference lists of relevant articles retrieved by the electronic searches for additional citations. We contacted study authors to inquire about ongoing or unpublished studies.

Data collection and analysis

Selection of studies

The three review authors (DC, DM, FN) independently assessed all studies that were identified by the search strategy for inclusion. We resolved any disagreements through discussion.

Data extraction and management

The three review authors (DC, DM, FN) extracted data from all eligible studies using Cochrane Vascular's data extraction template. Where there were discrepancies, we resolved them through discussion. We entered the data into Review Manager 5 for further analyses ([Review Manager 2014](#)).

Assessment of risk of bias in included studies

The three review authors (DC, DM, FN) independently assessed the included studies for risk of bias using Cochrane's 'Risk of bias' tool as described in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). The information about the risk of bias of the included studies is presented in the form of a table and graph.

Measures of treatment effect

Dichotomous (categorical) data

We planned to present the results as summary risk ratios (RRs) with 95% confidence intervals (CIs).

Continuous data

We used the mean difference (MD) with 95% CI where there was consistency in the outcome measure, or planned to use the standardized mean difference (SMD) to combine trials that measure the same outcome but use different methods.

Time-to-event data

We planned to use hazard ratios (HRs) with 95% CIs to measure the treatment effect for any time-to-event outcomes.

Unit of analysis issues

We considered the individual participant as the unit of randomization.

Dealing with missing data

We contacted contact authors by email about methodological issues and unpublished results, but none of them answered the solicitation. Where possible, we had planned to analyze all outcome measures on an intention-to-treat basis by including data from all randomized participants.

Assessment of heterogeneity

We planned to quantify the heterogeneity among the eligible studies using the Chi² test and I² statistic, specifically using the formula $I^2 = (Q - df/Q) \times 100\%$ where Q is the Chi² statistic and df represents the degree of freedom. We planned to interpret the I² statistic values as:

- 0% to 25% = low heterogeneity;
- 25% to 75% = moderate heterogeneity;
- more than 75% = substantial heterogeneity (Higgins 2011).

We planned to investigate the possible causes of heterogeneity, where substantial heterogeneity is detected according to the criteria above.

Assessment of reporting biases

We planned to explore publication bias through the use of funnel plots and explore the presence of time-lag bias in both published and unpublished trials if sufficient eligible trials were available.

Data synthesis

We used Review Manager 5 to perform data synthesis (Review Manager 2014). We planned to use a fixed-effect model meta-analysis if the studies were estimating the same intervention effect and had low or moderate heterogeneity (Higgins 2011).

We planned to use a random-effects model meta-analysis if we detected substantial heterogeneity between studies (Higgins 2011).

Subgroup analysis and investigation of heterogeneity

If sufficient information had been available, we had intended to perform subgroup analyses according to the following parameters.

- Tobacco exposure (cigarette, cannabis, or any other form of smoking, either measured in a laboratory or declared) after the intervention.
- Severity of the ischemia, according to the Fontaine (Fontaine 1954), or Rutherford classification (Rutherford 2005).
- Source of stem cells (bone marrow cells or umbilical cord blood, etc.).

Sensitivity analysis

We had intended to perform sensitivity analyses by looking separately at sponsored and publication bias, and by excluding studies at high risk of bias according to our 'Risk of bias' judgments. However, we did not perform sensitivity analyses as we included only one study.

Summary of findings

Based on the methods described in Chapter 11 of the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011), we presented the findings of this review in a 'Summary of findings' table. We presented results for the main comparison of the review (see Types of interventions) and for outcomes:

- rate of amputation;
- ulcer healing;
- pain;
- adverse effects;
- amputation-free survival; and
- walking distance or pain-free walking.

For each assumed risk cited in the table(s), we provided a source and rationale. We prepared the table using the GRADE profiler (GRADEpro software). We used the GRADE approach to assess the quality of the evidence as high, moderate, low, or very low based on the risk of bias, inconsistency, indirectness, imprecision, and publication bias (Atkins 2004; Higgins 2011). If meta-analysis was not possible, we presented the results in a narrative 'Summary of findings' table format.

RESULTS

Description of studies

See Characteristics of included studies, Characteristics of excluded studies, Characteristics of studies awaiting classification and Characteristics of ongoing studies tables.

Results of the search

A flow diagram of the search results is shown in Figure 1.

Figure 1. Study flow diagram.

Included studies

We only included one randomized controlled trial (RCT) in this review ([Dash 2009](#)).

[Dash 2009](#) reported on a study of 24 participants with chronic non-healing ulcers among people with diabetic foot and

Buerger's disease. Eighteen participants with Buerger's disease were randomized for implantation of stem cells derived from bone marrow or placebo and standard wound dressing care. Outcomes assessed were ulcer size, pain-free walking distance and biochemical parameters (fasting blood glucose, hepatic enzymes and renal function tests) in both groups at every two-week interval

following intervention, until 12 weeks after the start of the study. See [Characteristics of included studies](#).

We identified no studies that compared stem cell therapy (bone marrow source) versus stem cell therapy (umbilical cord source), stem cell therapy (any source) versus pharmacological treatment and stem cell therapy (any source) versus sympathectomy.

Excluded studies

We excluded five studies because they were not RCTs ([Dong 2013](#); [Kawamoto 2009](#); [Lee 2012](#); [Matoba 2008](#); [Ra 2016](#)). See also the [Characteristics of excluded studies](#) table.

Risk of bias in included studies

See [Figure 2](#) and [Figure 3](#) for a graphical summary of the methodological quality of the included study, based on the 'Risk of bias' domains.

Figure 2. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

Figure 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

Allocation

We classified [Dash 2009](#) at low risk for random sequence generation and high risk for allocation concealment. Random sequence was generated by numbers in sealed envelopes. However, the envelopes were not described as opaque, which is considered a possible source of allocation concealment bias.

Blinding

We classified [Dash 2009](#) at unclear risk of performance and detection bias, because no information about blinding of participants and personnel and outcome assessments was available.

Incomplete outcome data

We classified [Dash 2009](#) at unclear risk of bias for attrition because no information about losses of follow-up in the trial were reported in the study paper.

Selective reporting

In this bias domain, we classified [Dash 2009](#) at high risk of bias. The outcome 'pain' was not adequately assessed because the reported information was limited to information about a "striking improvement in pain relief", without any objective measure of pain

or a proportion of participants who experienced pain improvement. Missing data about the number of amputations among the participants also contributed to rating the study as high risk of reporting bias.

Other potential sources of bias

[Dash 2009](#) did not describe tobacco exposure before and after the treatment and we judged this to be at high risk of other bias.

Effects of interventions

See: [Summary of findings for the main comparison Stem cell therapy \(any source\) compared with any other modality of treatment for thromboangiitis obliterans \(Buerger's disease\)](#)

Stem cell therapy (any source) versus any other modality of treatment: stem cell therapy (bone marrow source) versus placebo

One study assessed this comparison ([Dash 2009](#)).

Primary outcomes

Rate of amputation

This outcome was not assessed by [Dash 2009](#).

Ulcer healing

[Dash 2009](#) assessed ulcer size at the beginning of the study and 12 weeks after treatment. The study authors reported an improvement in ulcer size area with a larger decrease in ulcer area in the stem cells implantation group: mean ulcer area decreased from 5.04 cm² (standard deviation (SD) 0.70) to 1.48 cm² (SD 0.56), compared with the control group: mean decrease from 4.68 cm² (SD 0.62) to 3.59 cm² (SD 0.14); mean difference (MD) -2.11, 95% confidence interval (CI) -2.49 to -1.73; 1 study, 18 participants; very low-quality evidence ([Analysis 1.1](#)).

Pain

This outcome was not assessed by [Dash 2009](#).

Adverse effects

This outcome was not assessed by [Dash 2009](#).

Secondary outcomes

Amputation-free survival

Amputation-free survival was not assessed by [Dash 2009](#).

Pain-free walking distance

[Dash 2009](#) assessed pain-free walking distance at the beginning of the study and 12 weeks after treatment. The study authors reported more of an improvement in pain-free walking distance in the stem cell implantation group: the mean pain-free walking distance increased from 38.33 meters (SD 17.68) to 284.44 meters (SD 212.12), compared with the control group: mean pain-free walking distance increased from 35.66 meters (SD 19.79) to 78.22 meters (SD 35.35); MD 206.22 meters, 95% CI 65.73 to 346.71; 1 study, 18 participants, very low-quality evidence ([Analysis 1.2](#)).

DISCUSSION

Summary of main results

Only one study was included in this review, reporting the efficacy of stem cells derived from bone marrow in 18 participants with thromboangiitis obliterans (Buerger's disease) and chronic non-healing ulcers.

The findings of this review suggest there may be an effect of the use of bone marrow-derived stem cells in the healing of ulcers and improvement in the pain-free walking distance in patients with thromboangiitis obliterans (see [Summary of findings for the main comparison](#)). The safety of the therapy is unclear because the study authors only assessed selected biochemical parameters (fasting blood glucose, hepatic enzymes and renal function tests) in the evaluation of adverse effects, without mention of possible clinical effects of the therapy.

Overall completeness and applicability of evidence

The objectives of this review were to assess the effectiveness of stem cell therapy of any source (bone marrow, umbilical cord, peripheral blood etc.), in people with thromboangiitis obliterans presenting different levels of limb ischemia (intermittent claudication, rest pain, ischemic ulcers and gangrene) compared with other therapies currently performed in the treatment of thromboangiitis obliterans. However, given the limited evidence available, we were unable to meet the objectives.

Only one study fulfilled the eligibility criteria to be included in this review, reporting 18 patients with thromboangiitis obliterans of a severe level of disease (critical limb ischemia). The intervention was performed with stem cells from bone marrow and, consequently, there is uncertainty about the effectiveness of stem cells from other sources (peripheral blood and umbilical cord, for example). Questions about the performance of other therapies (pharmacological agents, sympathectomy etc.) compared with stem cell treatment were also not answered because placebo was the comparator of the single included study.

Besides, only two outcomes were evaluated (ulcer healing and pain-free walking distance), without stating information about other relevant outcomes, such as rate of amputation and pain improvement. In fact, pain assessment characteristics, such as the scale of evaluation and proportion of patients who experienced improvement, were not described in the article. Another critical and not adequately assessed feature was the smoking status before and after intervention in the thromboangiitis obliterans participants, which may substantially change any of the outcomes studied. An additional important point to highlight is the complexity in the preparation of the stem cells administered in patients, which makes the process expensive and can make it difficult to apply in the lowest resource regions around the world. Thus, the external validity of the available evidence generated is extremely limited, implying impracticability of routine stem cell therapy in patients with Buerger's disease.

Quality of the evidence

We summarized the quality of the evidence for the main comparisons in [Summary of findings table 1](#).

We classified the quality of the evidence as very low. We downgraded the quality of evidence by two levels due to serious limitations in the design (high risk of bias in critical domains) and one further level because of imprecision (small number of participants and only one study included).

Potential biases in the review process

We could not evaluate the significant number of studies awaiting classification (7 trials) because the results were not available. We attempted to communicate with those responsible for the studies to try to incorporate the results and therefore to reduce this bias. Unfortunately, there was no response from the trialists.

Agreements and disagreements with other studies or reviews

We identified one systematic review which incorporates evaluation of treatment of patients with Buerger's disease and atherosclerosis with stem cell therapies ([Fadini 2010](#)). [Fadini 2010](#) incorporated controlled and non-controlled studies, randomized and non-randomized trials using autologous bone marrow or granulocyte colony-stimulating factor mobilized peripheral blood cells to treat peripheral artery disease (atherosclerosis and thromboangiitis obliterans). The non-controlled trials suggest a significantly larger improvement relating to change in ankle-brachial index, oxygen transcutaneous pressure, pain scale and pain-free walking distance in patients with thromboangiitis obliterans than in patients with atherosclerosis after treatment with stem cell therapy.

AUTHORS' CONCLUSIONS

Implications for practice

Very low-quality evidence suggests there may be an effect of the use of bone marrow-derived stem cells in the healing of ulcers and improvement in the pain-free walking distance in patients with thromboangiitis obliterans.

High-quality trials assessing the effectiveness of stem cell therapy for treatment of people with thromboangiitis obliterans are needed.

Implications for research

We suggest future trials investigating stem cell therapy for treatment of thromboangiitis obliterans should incorporate the following characteristics.

- Clearly describe the methods of randomization and concealment of allocation.
- Include participants with mild/moderate ischemia, such as intermittent claudication.

- Describe the proportion of active tobacco users before and after the treatment.
- Evaluate essential outcomes, such as pain (utilizing validated scores/scales) and rate of amputations.
- Evaluate time without amputation (amputation-free survival).
- Assess adverse effects with clinical and laboratory parameters.
- Investigate the effectiveness of other sources of stem cells and their performance against other therapies for Buerger's disease, such as pharmacological agents and sympathectomy.

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CHARACTERISTICS OF STUDIES

Characteristics of included studies [ordered by study ID]

Dash 2009

Methods	Study design: RCT parallel group
Participants	<p>Country: India N° patients: 24 (18 with Buerger's disease) Setting: not stated Mean age: not specified for Buerger's disease Gender: not specified</p> <p>Inclusion criteria:</p> <ul style="list-style-type: none"> age above 21 and below 65 years non-healing ulcers that do not show reduction in size during 1-month treatment period with good pressure reduction and ulcer therapy participant willing and able to read, understand, and sign the study-specific informed consent <p>Exclusion criteria:</p> <ul style="list-style-type: none"> presence of septicemia presence of any hematological disorder presence of concurrent infection at wound site severe malnutrition pregnancy presence of lower-extremity ulcers due to other specific causes, e.g. syphilitic foot ulcer, fungal ulcer, and tubercular ulcers current participation in another clinical trial <p>Tobacco status: not specified</p>
Interventions	Treatment: bone marrow-derived MSCs with standard wound dressing

Dash 2009 (Continued)

Control: standard wound dressing

Therapeutic procedure

Bone marrow (30 mL to 50 mL) was aspirated aseptically from the iliac crest of patients under sedation in a syringe primed with heparin (1000U/mL). Mononuclear cells were isolated by layering onto a Ficoll-Hypaque density gradient (1:2; Stem Cell Technologies). The mononuclear fraction was plated onto T75-cm³ flasks (BD Biosciences, San Jose, CA) in a culture medium containing Dulbecco's Modified Eagle Medium (DMEM), low-glucose medium, 15% knock-out serum (Invitrogen), 200 mM Glutamax (Invitrogen), penicillin (100 units/mL), and streptomycin (100 mg/mL) at a temperature of 37°C in a humidified atmosphere that contained 5% CO₂. The non-adherent cells were removed after 48 h of culture and fresh medium was added. Subsequently, the medium was replenished every 48 h. Once the cells became confluent, they were dissociated with 0.25% trypsin/0.53 mM EDTA and reseeded at the rate of 1 x 10⁵ cells/cm² plated on a T75-cm² flask. After 3–5 days of culture, the cells reached 90% confluence and were subcultured for subsequent propagation. The short-term culture plates at days 5 and 7 were tested with the Trypan Blue dye exclusion test for viability and then used in cell therapy after screening for mycoplasma and endotoxin.

The cell-surface antigen expression of cultured cells was used for the characterization of the MSC phenotype. Immunocytochemistry was performed for characterization of cultured cells. Cells from day 5 were trypsinized with 0.05% trypsin-EDTA, diluted in MSC medium, and allowed to adhere to four-well chamber sterile culture slides for 15 min. These slides were then placed in 100 mm x 15 mm dishes, covered with 2 mL of MSC medium, and allowed to grow at 37°C, 5% CO₂ for 24 h. Cells were analyzed by different cell-surface markers such as CD34, CD90-Alexa Fluor 594 (BD Pharmingen, CA, USA), and CD105-FITC (Chemicon International, Inc., USA) by the immunofluorescent method. The cultured cells were fixed in 4% paraformaldehyde for 15–20 min at 48°C, washed with 1 rinse buffer (20 mM Tris HCl, pH 7.4, 0.15 g NaCl, 0.05% Tween-20), permeabilized with Triton X-100 for 10 min, and again washed with rinse buffer. Normal 4% goat serum was applied for 30 min at room temperature as a blocking solution. The primary antibodies were diluted at a working concentration, incubated for 2 h at room temperature, and, after incubation, washed three to four times with rinse buffer. FITC-labeled goat anti-mouse immunoglobulin G (IgG) FITC and Alexa Fluor 594 were used as a secondary antibody, appropriate to the isotype of primary antibody.

Treatment with 4,6-diamidino-2-phenylindole (DAPI; 5 mg/mL, Fluka Chemie GMBH) was done for 5 min for nuclear staining. The mouse isotype was served as negative control.

Administration of cultured cells.

The cultured cells were harvested by a standard trypsinization method at days 5–7 according to their viability and confluence. The cell suspension was centrifuged at 1500 rpm for 5 min, and the supernatant was discarded. The harvested cells were then washed several times with Dulbecco phosphate-buffered saline to remove any culture contaminants. The cell pellet was then washed several times in preservative-free saline solution and finally suspended in 5–6 mL of normal saline to be applied to the ulcer. A single application of MSCs was performed. Cell count was done before implantation. The cell count at the time of harvest was around 45–60 x 10⁶/mL of suspension. Prior to the application of cultured cells, the ulcer was properly debrided to allow cultured cells to come into contact with viable wound tissue. The MSC suspension was administered via a syringe with a 25-gauge needle intramuscularly into the ischemic limb and along the edges of non-healing ulcers on the average of > 1 x 10⁶ cells were applied per cm² of ulcer area. Cultured cells were also placed topically on the ulcer. In the cases of ulcers due to Buerger disease, the injection sites were selected according to the angiographic findings and included calf muscles (soleus and gastrocnemius), the popliteal fossa, as well as the ulcer area. The aspirate was held in place with a dressing, and a bolster of rolled gauze pads was placed over the dressing and around the wound to prevent leakage of the cells. The dressing was replaced every day in the same manner during the follow-up period.

Follow-up: 12 weeks

Outcomes

Wound size, pain-free walking distance, and biochemical parameters (fasting blood glucose, hepatic enzymes and renal function tests)

Dash 2009 (Continued)

Notes Quote : " ...S.C.B Medical College Cuttack, Orissa, India, for supporting internal funding from 2006 to 2007 for this research work"

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomization with numbers in sealed envelopes
Allocation concealment (selection bias)	High risk	Numbers in sealed envelopes, but no details about opacity of the envelopes
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Quote: "...comparing one new treatment with placebo..". However, there is no description in the study report about how placebo is administered
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Study report does not describe whether the outcome assessments were done by the same person responsible for recruitment
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Study report does not describe if there were losses to follow-up
Selective reporting (reporting bias)	High risk	Quote: "In the implant group, striking improvement in pain relief was observed within 12 weeks as compared to the control group." Objective evaluation of pain (relevant outcome) was not properly performed. Information about number of amputations among participants is missing.
Other bias	High risk	Proportion of active tobacco users before and after intervention was not described

EDTA: ethylenediamine tetraacetic acid

FITC: fluorescein isothiocyanate

MSC: mesenchymal stem cells

RCT: randomized controlled trial

Characteristics of excluded studies [ordered by study ID]

Study	Reason for exclusion
Dong 2013	Not a RCT
Kawamoto 2009	Not a RCT
Lee 2012	Not a RCT
Matoba 2008	Not a RCT
Ra 2016	Not a RCT

RCT: randomized controlled trial

Stem cell therapy for treatment of thromboangiitis obliterans (Buerger's disease) (Review)

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Characteristics of studies awaiting assessment [ordered by study ID]

Gu 2007

Methods	Controlled trial (unclear if randomized)
Participants	Patients with Buerger's disease and arteriosclerosis in critical limb ischemia
Interventions	Autologous bone marrow stem cell implantation versus autologous peripheral blood stem cell implantation
Outcomes	Improvement of pain, cold sensation and numbness; increase of ankle-brachial index, transcutaneous oxygen pressure (TcPO ₂), angiography, amputation rate and improvement of foot wound healing
Notes	Results for the group of participants with Buerger's disease were not available

JPRN-C000000330

Methods	Randomized trial
Participants	Peripheral arterial disease patients (atherosclerosis and Buerger disease) with intermittent claudication
Interventions	Peripheral blood mononuclear cell therapy versus placebo
Outcomes	Maximum walking distance, pain-free walking distance, recovery time of lower leg blood pressure after exercise, ankle-brachial index at 6 months after treatment as compared with those before treatment
Notes	No results available

JPRN-UMIN000002280

Methods	Randomized trial
Participants	Peripheral arterial disease patients (arteriosclerosis obliterans, Buerger's disease)
Interventions	G-CSF-mobilized peripheral blood mononuclear cells transplantation versus recommended treatment
Outcomes	<p>Primary outcome</p> <p>Progression-free survival</p> <p>Secondary outcomes</p> <ul style="list-style-type: none"> • Changes of Fontaine classification or Rutherford classification • Overall survival • Time-to-amputation • Amputation-free survival • Ulcer/gangrene size • Severity of ischemic pain in lower limbs

JPRN-UMIN000002280 (Continued)

- Ankle-brachial index (toe-brachial index in measurable patients)
- ICD and ACD

Notes	No results available
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Majumdar 2015

Methods	Controlled trial (unclear if randomized)
Participants	Patients with Buerger's disease in critical limb ischemia
Interventions	Bone marrow mesenchymal stromal cells (BMMSC) manufactured from pooled obtained from healthy volunteer donors
Outcomes	Improvement in rest pain and ulcer healing, ankle-brachial index and quality of life
Notes	Abstract only, insufficient results available for inclusion in review

NCT01446055

Methods	Randomized controlled trial
Participants	Patients with chronic limb ischemia
Interventions	Autologous BM-MNC processed by ResQ Separator versus conventional manual method
Outcomes	<p>Primary outcomes</p> <p>Cell treatment-related adverse event (time frame: 2 weeks after bone marrow transplantation)</p> <ul style="list-style-type: none"> • Temperature, pulse, respiration, blood pressure • Routine analysis of blood and urine • Liver function (ALT, AST), renal function (blood urea nitrogen, creatinine), function of coagulation (APTT, PT, Fib, TT) • ECG • Local inflammatory response • Cell treatment-related death • Cell treatment-related unexpected amputation <p>Secondary outcomes</p> <ul style="list-style-type: none"> • Ulcer size - measuring ulcer area (cm²) and depth (mm) of limb: for each ulcer, photographically record the area and depth with a ruler in order to calculate the ulcer area in square mm • Rest pain score. (Time frame: post bone marrow transplantation: 1, 3, 6, 12 months). Scoring the rest pain based on the degree of pain as following five scales: level-0 point: no pain; level-1 point: occasional pain which can be recalled; level-2 points: pain often but can be tolerated, without or with a little analgesics; level-3 points: often with need of general analgesics; level-4 points: affects sleeping due to the pain, pain difficult to alleviate with general pain medication. Points before and after bone marrow transplantation • Cold sensation score (time frame: post bone marrow transplantation: 1, 3, 6, 12 months) based on a sense of cold as following five scales: level-0 point: no cold sensation; level-1 point: occasionally cold feeling; level-2 points: often with cold feeling; level-3 points: significantly cold feeling, and can be significantly improved when using a local insulation; level-4 points: significantly cold feeling, and cannot be significantly improved when using a local insulation

NCT01446055 (Continued)

- Claudication distance (m) (time frame: post bone marrow transplantation: 1, 3, 6, 12 months). Measurement of claudication distance (m): for patients with intermittent claudication, treadmill exercise test (no tilt, speed 3 km/h) is employed to measure claudication distance
- Resting ABI (time frame: post bone marrow transplantation: 1, 3, 6, 12 months). Measurement of ABI: measure arterial pressure with a laser Doppler, and then calculate the ABI, that is a ratio of ankle arterial blood pressure to brachial arterial blood pressure at rest
- Resting TcPO₂ (mmHg) (time frame: post bone marrow transplantation: 1, 3, 6, 12 months). Transcutaneous oxygen pressure (TcPO₂) should be measured at the same site in the ischemic limb at rest
- Collateral vessel score (time frame: post bone marrow transplantation: 1, 3, 6, 12 months). Collateral vessel score: using digital subtraction angiography or computed tomographic angiography to score the collateral vessel formation. A mean score is obtained for each ischemic limb by 3 independent interventionists based on the following 4-level score: 0 (no new collateral vessels), 1 (a little new collateral vessels), 2 (moderate new collateral blood vessels), 3 (rich new collateral)
- Amputation rate (time frame: post bone marrow transplantation: 1, 3, 6, 12 months). Amputation rate and level is recorded
- Skin microcirculation measurement (time frame: 1, 3, 6, 12 months post transplantation) using PeriMed 'laser-Doppler flowmetry' measure the skin microcirculation on the same site in the ischemic limb at rest
- Resting TBI (time frame: post bone marrow transplantation: 1, 3, 6, 12 months). Measurement of TBI: measure arterial pressure with a laser Doppler, and then calculate TBI, that is a ratio of toe arterial blood pressure to brachial arterial blood pressure

Notes	According to Clinicaltrial.gov the recruitment status of trial is unknown
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Wen 2010

Methods	Randomized trial
Participants	Patients with critical limb ischemia (diabetic foot, atherosclerosis obliterans and thromboangiitis obliterans)
Interventions	Autologous peripheral blood mononuclear cell transplantation versus control group
Outcomes	Improvement in limb pain, ulcer healing, laser Doppler flowmetry, ankle-brachial pressure index, angiographic score, lower limb amputation
Notes	Results for the group of patients with Buerger's disease were not available

ABI: ankle-brachial index

ALT: alanine aminotransferase

APTT: activated partial thromboplastin time

AST: aspartate transferase

ACD: acute claudication distance

BM-MNC: bone marrow mononuclear cells

ECG: electrocardiography

Fib: fibrinogen

G-CSF: granulocyte-colony stimulating factor

ICD: intermittent claudication distance

PT: prothrombin time

TBI: toe-brachial index

TT: thrombin time

Characteristics of ongoing studies [ordered by study ID]

Stem cell therapy for treatment of thromboangiitis obliterans (Buerger's disease) (Review)

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NCT02501018

Trial name or title	Study to assess the efficacy and safety of CLBS12 in patients with critical limb ischemia (CLI)
Methods	Randomized controlled trial
Participants	Patients with Buerger's disease and atherosclerosis obliterans and critical limb ischemia
Interventions	<ul style="list-style-type: none"> Biological: CLBS12 - intramuscular transfusion of CLBS12. Other Name: CD34+ cells Drug: standard of care is defined as pharmacotherapy with approved drugs (e.g. antiplatelets, anticoagulants, and vasodilators including prostanooids)
Outcomes	CLI-free is determined by assessing the Rutherford score (≤ 3) by the investigator and a central adjudication committee
Starting date	1 November, 2017
Contact information	Scott Volk - 9493468784- svolk@caladrius.com
Notes	Estimated study completion date: July 2020

CLI: critical limb ischemia

DATA AND ANALYSES

Comparison 1. Stem cell therapy (any source) versus any other modality of treatment: stem cell therapy (bone marrow source) versus placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Ulcer size at 12 weeks	1	18	Mean Difference (IV, Fixed, 95% CI)	-2.11 [-2.49, -1.73]
2 Pain-free walking distance at 12 weeks	1	18	Mean Difference (IV, Fixed, 95% CI)	206.22 [65.73, 346.71]

Analysis 1.1. Comparison 1 Stem cell therapy (any source) versus any other modality of treatment: stem cell therapy (bone marrow source) versus placebo, Outcome 1 Ulcer size at 12 weeks.

Study or subgroup	Stem cell (bone marrow)		Placebo		Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
	N	Mean(SD)	N	Mean(SD)			
Dash 2009	9	1.5 (0.6)	9	3.6 (0.1)		100%	-2.11[-2.49,-1.73]
Total ***	9		9			100%	-2.11[-2.49,-1.73]
Heterogeneity: Not applicable							
Test for overall effect: Z=10.97(P<0.0001)							
<div style="display: flex; justify-content: space-between; width: 100%;"> Stem cell (bone marrow) -5 -2.5 0 2.5 5 Placebo </div>							

Analysis 1.2. Comparison 1 Stem cell therapy (any source) versus any other modality of treatment: stem cell therapy (bone marrow source) versus placebo, Outcome 2 Pain-free walking distance at 12 weeks.

APPENDICES

Appendix 1. Database searches October 2017

Source	Search strategy	Hits retrieved
CENTRAL (via CRSW)	1 Buerger AND CENTRAL:TARGET 66 2 Buerger* AND CENTRAL:TARGET 86 3 MESH DESCRIPTOR Thromboangiitis Obliterans EXPLODE ALL AND CENTRAL:TARGET 14 4 thromboang* near/2 oblit* AND CENTRAL:TARGET 0 5 thromboang* near oblit* AND CENTRAL:TARGET 35 6 endangitis obliterans AND CENTRAL:TARGET 0 7 Winiwarter AND CENTRAL:TARGET 2 8 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 102	102
Clinicaltrials.gov	Buerger Disease	12
ICTRP Search Portal	Buerger* OR Thromboangiitis	35
MEDLINE	1 exp Thromboangiitis Obliterans/ 3258 2 Buerger*.ti,ab. 1245 3 "Thromboangiitis Obliterans".ti,ab. 863 4 Winiwarter.ti,ab. 78 5 endangitis obliterans.ti,ab. 39 6 (thromboang* adj2 oblit*).ti,ab. 1009 7 or/1-6 3627 8 randomized controlled trial.pt. 496115 9 controlled clinical trial.pt. 99212	6

(Continued)

10 randomized.ab. 382886
11 placebo.ab. 186525
12 drug therapy.fs. 2112658
13 randomly.ab. 260070
14 trial.ab. 402522
15 or/8-14 2872866
16 exp animals/ not humans.sh. 4674886
17 15 not 16 2563723
18 7 and 17 363
19 2017*.ed. 806881
20 18 and 19 6

EMBASE	1 exp Thromboangiitis Obliterans/ 3136	11
	2 Buerger*.ti,ab. 1354	
	3 "Thromboangiitis Obliterans".ti,ab. 821	
	4 Winiwarter.ti,ab. 52	
	5 endangitis obliterans.ti,ab. 19	
	6 (thromboang* adj2 oblit*).ti,ab. 946	
	7 1 or 2 or 3 or 4 or 5 or 6 3507	
	8 randomized controlled trial/ 477673	
	9 controlled clinical trial/ 451560	
	10 random\$.ti,ab. 1251095	
	11 randomization/ 75988	
	12 intermethod comparison/ 230680	
	13 placebo.ti,ab. 262028	
	14 (compare or compared or comparison).ti. 455807	
	15 ((evaluated or evaluate or evaluating or assessed or assess) and (compare or compared or comparing or comparison)).ab. 1659012	
	16 (open adj label).ti,ab. 60438	
	17 ((double or single or doubly or singly) adj (blind or blinded or blindly)).ti,ab. 201937	
	18 double blind procedure/ 144132	
	19 parallel group\$1.ti,ab. 20882	
	20 (crossover or cross over).ti,ab. 89851	
	21 ((assign\$ or match or matched or allocation) adj5 (alternate or group\$1 or intervention\$1 or patient\$1 or subject\$1 or participant\$1)).ti,ab. 269943	

(Continued)

22 (assigned or allocated).ti,ab. 318650
23 (controlled adj7 (study or design or trial)).ti,ab. 281099
24 (volunteer or volunteers).ti,ab. 218066
25 trial.ti. 238175
26 or/8-25 3866518
27 7 and 26 241
28 2017*.dc. 1499596
29 27 and 28 11

CINAHL	1 (MM "Thromboangiitis Obliterans") 45	3
	2 TX Buerger* 79	
	3 TX "Thromboangiitis Obliterans" 68	
	4 TX Winiwarter 0	
	5 TX endangitis obliterans 0	
	6 TX endangitis obliterans 0	
	7 S1 OR S2 OR S3 OR S4 OR S5 OR S6 117	
	8 MH "Clinical Trials" 90,486	
	9 TX trial 234,301	
	10 TX "single-blind*" 8,610	
	11 TX "double-blind*" 751,004	
	12 TX "treatment as usual" 700	
	13 TX randomly 41,076	
	14 S8 OR S9 OR S10 OR S11 OR S12 OR S13 945,550	
	15 S7 AND S14 39	
	16 EM 2017 155,198	
	17 S15 AND S16 3	
AMED	1 exp Thromboangiitis Obliterans/ 3	0
	2 Buerger*.ti,ab. 15	
	3 "Thromboangiitis Obliterans".ti,ab. 6	
	4 Winiwarter.ti,ab. 0	
	5 endangitis obliterans.ti,ab. 1	
	6 (thromboang* adj2 oblit*).ti,ab. 6	
	7 1 or 2 or 3 or 4 or 5 or 6 21	
	8 2017*.yr. 47	

(Continued)

9 7 and 8 0

Appendix 2. OpenGrey Database search strategy

buerger's disease	3
thromboangiitis obliterans	2
von Winiwarter disease	0
buerger's disease OR thromboangiitis obliterans OR von Winiwarter disease	3

CONTRIBUTIONS OF AUTHORS

- DC: contact person; protocol drafting, addressing clinical comments from the referees, acquiring trial reports, trial selection, data extraction, data analysis, data interpretation, review drafting, and future review updates
- FN: data extraction, data analysis, data interpretation, and review drafting
- DM: protocol drafting, acquiring trial reports, trial selection, data extraction, data analysis, data interpretation, and review drafting

DECLARATIONS OF INTEREST

- DC: none known
- FN: none known
- DM: none known

SOURCES OF SUPPORT

Internal sources

- No sources of support supplied

External sources

- Chief Scientist Office, Scottish Government Health Directorates, The Scottish Government, UK.

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NOTES

Parts of the methods section of this protocol are based on a standard template established by Cochrane Vascular.

INDEX TERMS

Medical Subject Headings (MeSH)

*Bandages; *Bone Marrow Transplantation; Thromboangiitis Obliterans [*therapy]; Walk Test; Wound Healing

MeSH check words

Humans