Different sources of autologous mononuclear cells and stem cells for critical lower limb ischaemia (Protocol)

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Different sources of autologous mononuclear cells and stem cells for critical lower limb ischaemia

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ABSTRACT

This is the protocol for a review and there is no abstract. The objectives are as follows:

To assess the efficacy and safety of autologous mononuclear cells (MNCs) and stem cells derived from different sources in patients with critical lower limb ischaemia (CLI).

BACKGROUND

Description of the condition

Peripheral arterial disease (PAD) affects 3% to 10% of the population (Norgren 2010). It is a prevalent health problem all over the world and is associated with a significant burden in terms of morbidity and mortality, due to intermittent claudication and critical limb ischaemia (CLI). Intermittent claudication is the most common form of PAD and is generally managed conservatively. CLI is a more severe form of PAD and is characterised by rest pain, ulcerations, and gangrene. Revascularisation, either surgical or endovascular, aiming to improve blood flow to the affected extremity is the gold standard therapy for patients with severe PAD. However, this treatment modality cannot be applied to over 30% of patients because of excessive operative risk and unfavourable vascular involvement (Sasajima 1997). Moreover, revascularisation may not be successful owing to the presence of extensive atherosclerotic plaque and the low rates of long-term patency in severe PAD (Conrad 2011). Hence, many patients are reliant on medical therapy that may only slow disease progression temporarily and the only remaining option for relief of pain or treatment of gangrene is amputation of the affected limb (Botti 2012). Of note, after one year, 30% of patients with CLI undergo amputation (Norgren 2007). An estimated 120 to 150 amputations are performed per million people per year, and one-quarter of these
patients require long-term institutional care or professional assistance at home (Norgren 2007). There is a critical need to develop novel strategies to promote vascular regeneration (neovascularisation) in patients with CLI who are not suitable for conventional treatments. The current literature suggests that stem cell therapy is a relatively safe, feasible, and possibly effective therapy for patients with CLI, and stem cells may be considered as an alternative treatment for patients who are not suitable for revascularisation and best medical therapy.

**Description of the intervention**

Although initial clinical studies on stem cell therapy have been encouraging, current evidence from large scale randomised controlled clinical trials (RCTs) comparing active treatment with placebo is limited, leading to a previous Cochrane review concluding that there was “insufficient evidence to support cell therapy in clinical practice” (Moazzami 2011). The procedures are generally safe and well tolerated and there have been extensive clinical studies involving patients with PAD utilising stem cells derived from various sources. The types of cells used for implantation to date have been bone marrow (BM) mononuclear cells (BM-MNCs) (Durdu 2006; Miyamoto 2006; Tateishi-Yuyama 2002), peripheral blood mononuclear cells (PB-MNCs) (Huang 2004; Kawamura 2006; Lenk 2005; Matsui 2003), granulocyte colony-stimulating factor (G-CSF)-mobilised PB-MNCs (M-PBMNC) (Huang 2005; Lenk 2005; Ishida 2005), CD34 antigen-positive MNCs (Inaba 2002; Kawamoto 2009), CD133 antigen-positive MNCs (Burt 2010), BM-mesenchymal stem cells (BM-MSCs) (Dash 2009; Lu 2011), and recently in small series of patients adipose tissue-MSCs (AT-MSCs) (Lee 2012). Implantation of cells into patients via several routes including intramuscular, intra-arterial, or a combination of both, have yielded promising results in patients with PAD. Intramuscular injection is usually performed through multiple injections at the level of the gastrocnemius muscles, while intra-arterial infusion is usually performed via the femoral artery. MNCs and stem cells derived from different sources may have different clinical outcomes in patients with PAD. Stem cells obtained from different sources may vary in biological (plasticity, self renewal, differentiation, homing, migration, secretion of trophic factors) and immunological (modulating immune response) properties. This may be attributed to the inherent biological properties of the stem cells or changes to the cells that may occur during cell enrichment and culture. For example, injection of G-CSF that is used to mobilise BM-derived progenitor cells can significantly enhance the formation of several growth factors involved in vascular repair (Huang 2007). In addition, the apheresis procedure results in the transient cleavage of the chemokine receptor (directly involved in stem cell homing) from M-PBMNCs (Honold 2006).

**How the intervention might work**

To date, the mechanisms by which the transplanted cells improve clinical outcomes in patients with PAD are still unclear. Experimental animal studies indicate that BM-derived cells contribute to vascular and muscle regeneration by physically integrating into the tissue or by secreting growth factors, or both (Fadini 2007; Honold 2006). BM adult stem cells with angiogenic potential such as endothelial progenitor cells (EPCs) and MSCs have the capability to stimulate the formation of new blood vessels (Schatteman 2004). MSCs are reported to promote angiogenesis because of their capacity to differentiate into endothelial cells (ECs) and vascular smooth muscle (Pittenger 1999; Reyes 2002) and to stimulate EC proliferation and migration. EPCs have direct angiogenic action, supporting angiogenesis through their ability to secrete paracrine mediators (Jarajapu 2010). Furthermore, MSCs support neo-angiogenesis by releasing soluble factors that stimulate EPCs sprouting from pre-existing blood vessels (Cobellis 2010; Jarajapu 2010). Therefore, cell transplantation into ischaemic limbs may promote neo-angiogenesis by providing precursor cells capable of vascular transdifferentiation, and by supplying multiple angiogenic cytokines, growth factors and homing signals for mural cells or pericytes for microvascular stabilisation (Benoit 2013; Kaelin 2008). The combination of these mechanisms is responsible for augmenting vascular repair and ameliorating tissue perfusion, which leads to reversal of ischaemia of the affected limb.

**Why it is important to do this review**

It is important to do this review to determine whether different sources or methods of MNC and stem cell preparation have different effects on clinical outcomes following transfer into CLI patients; and whether a combination versus a single type of MNC or stem cell treatment improves ischaemic symptoms and survival in patients with CLI. Currently the data comparing the angiogenic potency of cells derived from different sources are limited. Questions regarding the optimal cell dose, cell phenotype, cell processing, route of delivery, and frequency of application remain open. The current proposed meta-analysis attempts to address some of these challenging issues based on the published data on cell therapy trials in patients with PAD. Obtaining a comprehensive insight to these key points is critical in designing the optimal cell-based therapy program for patients with CLI as well as in identifying critical areas for improvement and in making recommendations for future clinical trials. Additionally, other sources of stem cells might become available such as placenta and stored autologous cord blood. It is not yet known whether cells from these sources would be as effective as cells derived from BM or PB in treating CLI and studies involving cells from new sources will be included in future updates.
OBJECTIVES

To assess the efficacy and safety of autologous mononuclear cells (MNCs) and stem cells derived from different sources in patients with critical lower limb ischaemia (CLI).

METHODS

Criteria for considering studies for this review

Types of studies
Randomised controlled trials (RCTs). We will exclude cluster-randomised trials (due to the difficulties in adjusting for the unit of analysis) and cross-over studies (due to the possible ‘contaminating’ effect of one intervention on another).

Types of participants
Study participants will be adult patients diagnosed with CLI who are not candidates for revascularisation and did not show any improvement in response to best standard medical therapy. There will be no age restriction.

Types of interventions
Intervention: administration of autologous MNCs or stem cells from a particular source of MNCs and stem cells into patients with CLI.
Comparison: administration of autologous MNCs or stem cells from any other source of MNCs and stem cells into patients with CLI.
We will also include studies that compare the same source of MNCs or stem cells but under different therapeutic regimes, for example, different dosages or routes of injection.

Types of outcome measures

Primary outcomes
1. All-cause mortality
2. Amputation-free survival
3. Reduction in pain as assessed by a validated visual analogue scale (VAS)

Secondary outcomes
1. The number of ulcers healed and the change in ulcer size as measured by clinical assessment and grid maps
2. Improvement in angiogenesis as measured by a change in transcutaneous oxygen tension (TcPO2)
3. Improvement in vascularity and blood supply as measured by the ankle brachial index (ABI)
4. Change in pain-free walking distance (PFWD) as measured by a change in walking distance (metres), walking duration (minutes), and walking speed (mph)
5. Adverse effects and safety. Adverse effects include an inflammatory reaction at the stem cell implantation site (grade I to IV), cardiovascular abnormalities, and thromboembolic complications. Safety is measured as the rate of adverse events and the rate of withdrawal

Search methods for identification of studies

Electronic searches
The Cochrane Peripheral Vascular Diseases Group Trials Search Co-ordinator (TSC) will search the Specialised Register and the Cochrane Central Register of Controlled Trials (CENTRAL), part of The Cochrane Library at www.thecochranelibrary.com. See Appendix 1 for details of the search strategy which will be used to search CENTRAL. The Specialised Register is maintained by the TSC and is constructed from weekly electronic searches of MEDLINE, EMBASE, CINAHL, AMED, and through handsearching relevant journals. The full list of the databases, journals, and conference proceedings which have been searched, as well as the search strategies used, are described in the Specialised Register section of the Cochrane Peripheral Vascular Diseases Group module in The Cochrane Library (www.thecochranelibrary.com).
The following trial databases will be searched by the TSC for details of ongoing and unpublished studies:
- World Health Organization International Clinical Trials Registry Platform (http://apps.who.int/trialsearch/);
- ClinicalTrials.gov (http://clinicaltrials.gov/);
- Current Controlled Trials (http://www.controlled-trials.com/);

Authors’ searches
The authors will search PubMed using the strategy shown in Appendix 2.

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**Searching other resources**

To identify further eligible studies, we will inspect the reference lists of articles that we have retrieved via the strategies as outlined above and also from the relevant Cochrane reviews that assess MNCs as interventions.

**Data collection and analysis**

**Selection of studies**

Two review authors (NAI, MKAAH) will independently screen the titles and abstracts of the articles that are retrieved in the first round of the search, aiming to exclude articles that are clearly irrelevant. After the initial step of screening, we will have a shortlist of articles that appear to be relevant to our review. Three review authors (SFAW, NAM, and MAMI) will then assess the shortlisted articles in greater detail by using the abstract and full text to identify articles that will be included in this meta-analysis. In instances where there are disagreements between the authors on article selection, a fourth author (NML) will act as an arbiter.

We will accept published and unpublished studies, both in full article and abstract forms, as long as assessment of risk of bias is possible and relevant data are available. When required, we will contact authors of unpublished studies and studies available only as abstracts with a request for further information.

We will screen for duplicate publications of the same trial, and will contact the trial authors for clarification when necessary.

**Data extraction and management**

Two review authors (NAI, MKAAH) will independently extract and code all data for each included study using a pro forma designed specifically for this review. We will extract the following information on each study: study design, participants, setting, sample size, nature of intervention, comparison, outcomes, methods (unit of allocation and analysis), and results. We will screen for duplicate entry of patients, where possible, by matching the initial number of patients recruited against the total number along each step in the conduct of the study. If we discover a discrepancy (for example if the total number in the later stage of the study exceeds the initial number), we will attempt to look for an explanation in the article (for example multiple enrolment of the same patient in different hospital admissions). We will contact the authors of the study for clarification if necessary.

If we find duplicate publications, we will assess data from all versions to avoid duplicate extraction.

We will resolve any disagreement among the review authors by discussion leading to a consensus.

**Assessment of risk of bias in included studies**

Two review authors (NAI and NML) will independently assess each included study using the Cochrane Collaboration tool for assessing risk of bias (Higgins 2011). This tool addresses six specific domains:

1. sequence generation;
2. allocation concealment;
3. blinding;
4. incomplete outcome data;
5. selective outcome reporting;
6. other issues (e.g. extreme baseline imbalance).

We will make a judgment on each of the criteria above as to whether the study is of high, low, or unclear risk of bias. We will assess blinding for each category of outcome (objective and subjective) separately where possible. We will complete a 'Risk of bias' table for each eligible study. We will resolve any disagreement among the review authors by discussion leading to a consensus. We will present an overall assessment of the risk of bias using the 'Risk of bias' graph and 'Risk of bias' summary.

**Measures of treatment effect**

For dichotomous data, we will use risk ratio (RR) to measure outcome estimates of the same scale. We will estimate the number needed to treat for an additional beneficial outcome (NNTB) from the pooled risk difference (RD). For continuous data, we will pool measures at a similar time point using mean difference (MD). If pooled analyses are not possible, we will report results of the studies individually.

**Unit of analysis issues**

We will use each individual patient as our unit of analysis. As we will not include cluster-RCTs and cross-over studies, we do not expect any of the unit of analysis issues that may arise from analysing such studies.

**Dealing with missing data**

We will assess the dropout rate of each study and whether an intention-to-treat analysis was performed. To assess whether the dropout rate is worrying, we will inspect the event rates for the intervention and the comparison groups. We will then use a 'worst-case scenario' method for the primary outcomes (Guyatt 1993).

For instance, in negatively-worded outcomes (such as mortality), for a trial that favours the intervention group we assume that all dropouts from the intervention group have developed the outcome, and all dropouts from the comparison group have not developed the outcome. We will then analyse the results to see if such an assumption changes the direction of the results (for example from favouring the intervention group to favouring the comparison group). If so, we will consider the dropout rate to be worrying and make a corresponding note in the table that corresponds
to the characteristics of the study and its accompanying risk of bias assessment table under the heading of 'incomplete outcome data'. We will make the reverse assumption when a trial favours the comparison group.

Assessment of heterogeneity

We will assess all the included studies in terms of their clinical and methodological characteristics, including the following.

1. Aetiology of the disease.
2. Baseline characteristics of the participants (age, gender, race and ethnicity, co-morbidity group).
3. Nature of intervention (different regimens of implantation, different types of cells, different preparations and dosages of cells injected).
4. Types of co-interventions.
5. Duration of follow-up period.
6. Methodological quality (as detailed in the assessment of risk of bias section, e.g. studies at high risk of bias, which are defined as studies with unclear or no allocation concealment, and studies where participants, care givers or investigators are not blinded, or where blinding is unclear).

We will visually inspect the forest plots for any evidence of heterogeneity of treatment effects. We will use the $I^2$ statistic (Deeks 2011) to measure inconsistency in the results, with a value of greater than 50% indicating substantial statistical heterogeneity. If we find significant statistical heterogeneity but consider the studies suitable for a meta-analysis based on the clinical and methodological characteristics, then we will use the random-effects model to provide the pooled effect estimates.

Assessment of reporting biases

We will specifically assess publication bias in our review using a funnel plot if there are 10 or more studies included in the analysis. If publication bias is implied by a significant asymmetry of the funnel plot, we will include a statement in our results with a corresponding note of caution in our discussion.

Data synthesis

We will use Review Manager to perform meta-analysis of the included studies (RevMan 5.2). We will use a fixed-effect model unless we find significant heterogeneity, in which case we will employ the strategies as outlined in the previous section on assessment of heterogeneity. We will follow the strategies detailed in the Cochrane Handbook for Systematic Reviews of Interventions (Higgins 2011) in our data management. Our primary data analyses will follow the intention-to-treat principle; namely, we will use the original number of participants allocated to each study arm as the denominator in the subsequent analyses.

Subgroup analysis and investigation of heterogeneity

We plan to carry out subgroup analyses (where data are available) for studies describing patients:

1. with different severity of CLI (e.g. rest pain versus tissue loss),
2. with different aetiology of CLI (e.g. atherosclerosis obliterans (ASO) versus thromboangiitis obliterans (TAO)),
3. with and without significant co-morbidity (e.g. smokers, diabetes, hypercholesterolaemia, and hypertension),
4. with different age groups,
5. with different gender,
6. with different race or ethnicity,
7. injected with cells obtained using different preparation techniques (e.g. fresh versus cultured, non-selected versus selected),
8. injected with different doses of cells,
9. injected with single versus a combination of cell products,
10. injected via different routes (intra-arterial versus intramuscular);

or when:

1. the intervention is administered with and without co-intervention,
2. studies are undertaken in patients with different follow-up periods.

Sensitivity analysis

We will perform sensitivity analysis to assess the impact of excluding studies based on the following criteria.

1. Significant or worrying dropout rates, as defined under the heading Dealing with missing data.
2. Significant methodological issues identified in the assessment of risk of bias. For the purpose of this systematic review, we will take the following criteria to indicate a significant risk of bias: studies with unclear or no allocation concealment; and studies where participants, care givers, or investigators are not blinded, or where blinding is unclear. We will perform sensitivity analysis separately for allocation concealment and blinding.
References

Additional references

Benoit 2013

Botti 2012

Burt 2010

Cobelliis 2010

Conrad 2011

Dash 2009

Deeks 2011

Durdu 2006

Fadini 2007

Guyatt 1993
Guyatt GH, Sackett DL, Cook DJ. Users’ guides to the medical literature. II. How to use an article about therapy or prevention. A. Are the results of the study valid? Evidence-Based Medicine Working Group. JAMA 1993;270(21):2598–601.

Higgins 2011

Honold 2006

Huang 2004

Huang 2005

Huang 2007

Inaba 2002

Ishida 2005

Jarajapu 2010

Kaelin 2008
Kawamoto 2009

Kawamura 2006

Lee 2012

Lenk 2005

Lu 2011

Matsui 2003

Miyamoto 2006

Moazzami 2011

Norgren 2007

Norgren 2010

Pittenger 1999

RevMan 5.2 [Computer program]

Reyes 2002

Sasajima 1997

Schatteman 2004

Tateishi-Yuyama 2002

* Indicates the major publication for the study.
Appendix 1. CENTRAL search strategy

#1 MeSH descriptor: [Arteriosclerosis] this term only
#2 MeSH descriptor: [Arteriolosclerosis] this term only
#3 MeSH descriptor: [Arteriosclerosis Obliterans] this term only
#4 MeSH descriptor: [Atherosclerosis] this term only
#5 MeSH descriptor: [Arterial Occlusive Diseases] this term only
#6 MeSH descriptor: [Intermittent Claudication] this term only
#7 MeSH descriptor: [Ischemia] this term only
#8 MeSH descriptor: [Peripheral Vascular Diseases] explode all trees
#9 MeSH descriptor: [Vascular Diseases] this term only
#10 MeSH descriptor: [Leg] explode all trees and with qualifiers: [Blood supply - BS]
#11 MeSH descriptor: [Femoral Artery] explode all trees
#12 MeSH descriptor: [Popliteal Artery] explode all trees
#13 MeSH descriptor: [Iliac Artery] explode all trees
#14 MeSH descriptor: [Tibial Arteries] explode all trees
#15 (atherosclero* or arteriosclero* or PVD or PAOD or PAD)
#16 (arter*) near (*occlus* or steno* or obstruct* or lesio* or block* or obliter*)
#17 (vascular) near (*occlus* or steno* or obstruct* or lesio* or block* or obliter*)
#18 (vein*) near (*occlus* or steno* or obstruct* or lesio* or block* or obliter*)
#19 (veno*) near (*occlus* or steno* or obstruct* or lesio* or block* or obliter*)
#20 (peripher*) near (*occlus* or steno* or obstruct* or lesio* or block* or obliter*)
#21 peripheral near/3 dis*
#22 arteriopathic
#23 (claudic* or hinken*)
#24 (isch* or CLI)
#25 dysvascular*
#26 leg near/4 (obstruct* or occlus* or steno* or block* or obliter*)
#27 limb near/4 (obstruct* or occlus* or steno* or block* or obliter*)
#28 (lower near/3 extrem*) near/4 (obstruct* or occlus* or steno* or block* or obliter*)
#29 (aort* or iliac or femoral or popliteal or femoro* or fempop* or crural) near/3 (obstruct* or occlus*)
#30 #1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 or #24 or #25 or #26 or #27 or #28 or #29
#31 MeSH descriptor: [Stem Cell Transplantation] explode all trees
#32 MeSH descriptor: [Hematopoietic Stem Cell Mobilization] explode all trees
#33 MeSH descriptor: [Hematopoietic Stem Cells] explode all trees
#34 MeSH descriptor: [Stem Cells] this term only
#35 MeSH descriptor: [Bone Marrow Cells] explode all trees
#36 (mononuclear or endothelial or mesenchymal) near/3 cell*:ti,ab,kw (Word variations have been searched)
#37 (stem or progenitor or precursor or therap*) near/3 cell*:ti,ab,kw (Word variations have been searched)
#38 ((embryo* or fetal or foetal or umbilical or marrow or cord) near/5 cell*):ti,ab,kw (Word variations have been searched)
#39 (BM-MNC* or PB-MNC* or M-PBNCC* or AT-MSC*):ti,ab,kw (Word variations have been searched)
#40 #31 or #32 or #33 or #34 or #35 or #36 or #37 or #38 or #39
#41 #30 and #40 in Trials

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## Appendix 2. Authors’ PubMed search strategy

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<td></td>
</tr>
<tr>
<td>#2</td>
<td>(limb arteriosclerosis obliterans [Title/Abstract] OR foot ulcer [Title/Abstract] OR diabetic foot [Title/Abstract] OR arteriosclerosis obliterans [Title/Abstract] OR thromboangiitis obliterans [Title/Abstract] OR buerger's disease [Title/Abstract])</td>
<td></td>
</tr>
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<td>#4</td>
<td>Search Peripheral Arterial Diseases [MeSH Terms]</td>
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<td>#5</td>
<td>#1 OR #2 OR #3 OR #4</td>
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</tr>
<tr>
<td>#6</td>
<td>(mononuclear cells[Title/Abstract] OR mesenchymal stem cells[Title/Abstract] OR therapeutic angiogenesis[Title/Abstract] OR bone marrow transplantation[Title/Abstract] OR adult stem cells[Title/Abstract])</td>
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<td>#8</td>
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<td>#10</td>
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<td>#13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19</td>
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<tr>
<td>#21</td>
<td>Search (animals [mh] NOT humans [mh])</td>
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</table>
CONTRIBUTIONS OF AUTHORS

SFAW: conceived the title of this protocol.
All review authors wrote the protocol.

DECLARATIONS OF INTEREST

None known

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Internal sources

• No sources of support supplied

External sources

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