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Local intramuscular transplantation of autologous mononuclear cells for critical lower limb ischaemia (Review)

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[Intervention Review]

Local intramuscular transplantation of autologous mononuclear cells for critical lower limb ischaemia

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ABSTRACT

Background

Peripheral arterial disease is a major health problem, and in about 1% to 2% of patients the disease progresses to critical limb ischaemia (CLI). In a substantial number of patients with CLI, no effective treatment option other than amputation is available and around a quarter of these patients will require a major amputation during the following year. This is an update of the review first published in 2011.

Objectives

To determine the effectiveness and safety of local intramuscular transplantation of autologous adult bone marrow mononuclear cells (BMMNCs) as a treatment for critical limb ischaemia (CLI).

Search methods

For this update the Cochrane Peripheral Vascular Diseases Group Trials Search Co-ordinator searched the Specialised Register (last searched February 2014) and the Cochrane Central Register of Controlled Trials (CENTRAL; 2014, Issue 1).

Selection criteria

We included all randomised controlled trials of CLI in which participants were randomly allocated to intramuscular administration of autologous adult BMMNCs or control (either no intervention or conventional conservative therapy). We excluded studies on patients with intermittent claudication.

Data collection and analysis

Two authors independently selected trials, assessed trials for eligibility and methodological quality, and extracted data. Disagreements were resolved by consensus or by the third author.

Main results

Only two small studies, with a combined total of 57 participants, met our inclusion criteria and were finally included. They were classified as having a moderate risk of bias with unclear issues regarding their methods, and according to the GRADE approach, the overall quality of the evidence would be considered as moderate. In one study the effects of intramuscular injections of BMMNCs in the ischaemic lower limbs of patients with CLI were compared with control (standard conservative treatment). No deaths were reported and no significant difference

was observed between the two groups for either pain (P = 0.37) or the ankle brachial index (ABI) parameter. However, the treatment group showed a significantly smaller proportion of participants undergoing amputation compared with the control group (P = 0.026).

In the other study, following subcutaneous injections of granulocyte colony-stimulating factor (G-CSF) for five days, peripheral blood derived mononuclear cells were collected and then transplanted by intramuscular injections into ischaemic lower limbs. The effects were compared with daily intravenous prostaglandin E1 injections (control group). No deaths were reported. Pain reduction was greater in the treatment group than in the control group (P < 0.001) as was increase in ABI (mean increase 0.13 versus 0.02, P < 0.01). The treatment group experienced a statistically significant increase in pain-free walking distance (PFWD) compared with the control group (mean increase 306.4 m versus 78.6 m, P = 0.007). A smaller proportion of participants underwent amputation in the treatment group compared with the control group (0% versus 36%, P = 0.007).

Authors' conclusions

The data from the published trials suggest that there is insufficient evidence to support this treatment. These results were based on only two trials which had a very small number of participants. Therefore evidence from larger randomised controlled trials is needed in order to provide adequate statistical power to assess the role of intramuscular mononuclear cell implantation in patients with CLI.

PLAIN LANGUAGE SUMMARY

Treatment of reduced blood flow to the legs using mononuclear cell therapy

Background

Critical limb ischaemia occurs when blood flow to the legs is reduced because of the worsening of peripheral arterial disease. Initially, patients experience cramping leg pain that limits walking (termed intermittent claudication), but over time some patients experience more severe symptoms including pain at rest, leg ulceration and gangrene. The available treatment options are very limited when the disease reaches this stage, especially when surgical or catheter revascularisation is not an option. A substantial proportion of these patients require amputation of the affected limb. A new therapy (mononuclear cell therapy using the patient's own cells) offers the possibility of an alternative treatment for patients, by supplying cells that could stimulate the formation of stable capillary vessels to improve the blood flow in the affected limb. These cells can be obtained from the bone marrow or from peripheral blood following subcutaneous injections (of granulocyte colony-stimulating factor) for five days. They are then treated in a laboratory and injected into the large muscle at the back of the lower leg.

Key results

The review authors identified only two small randomised controlled trials with a combined total of 57 participants testing the safety and effectiveness of this treatment. The findings were inconsistent. In one trial, pain at rest, pain-free walking distance, ankle brachial blood pressure index, and the number of amputations all clearly improved in the group receiving mononuclear cell implantation. In the other trial only the number of amputations showed a significant improvement in the treatment group compared with the control group. No deaths were reported during the study period.

Quality of the evidence

The two included studies differed from each other in how they obtained the cells for injection and assessed the clinical effects at different time points, up to three months in one trial, and six months in the other. They were classified as having a moderate risk of bias with unclear issues regarding their methods and the overall quality of the evidence was considered moderate.

BACKGROUND

Description of the condition

Peripheral arterial disease is a major health problem with a total disease prevalence of 3% to 10% that increases to 15% to 20% in individuals over the age of 70 years (Hirsch 2006). As the disease progresses, about 1% to 2% of patients develop critical limb ischaemia (CLI), which is characterised by chronic ischaemic rest pain, ischaemic ulcers, or gangrene (Norgren 2007).

The current mainstay treatment of CLI has been surgical or catheter-based revascularisation. However, a substantial number of patients remain for whom revascularisation would not be feasible because of the involvement of distal vessels. For patients with CLI who are not candidates for revascularisation, around a quarter will require a major amputation during the following year (Norgren 2007).

Description of the intervention

A novel approach to treating CLI has developed from the observation in animal models that mononuclear cells from the bone marrow improved capillary density in hindlimb ischaemia and promoted collateral vessel formation (Shintani 2001). Therefore, on the basis of these results in animals, clinical trials were started in order to test cell therapy with autologous bone marrow derived mononuclear cells (BMMNCs) in patients with ischaemic lower limbs.

At the current time, the procedure is as follows. Mononuclear cells are derived either directly from bone marrow aspiration (Higashi 2004; Tateishi-Yuyama 2002a) or through mobilisation into the peripheral blood using granulocyte colony-stimulating factor (G-CSF) (Huang 2004; Huang 2005a). In the first procedure, cells are usually collected (mostly under general anaesthesia) from the iliac crest. Thereafter the mononuclear cells are enriched away from other bone marrow cells in sterile conditions in a laboratory. For the G-CSF mobilisation procedure, following G-CSF administration for four to five days the cells are collected from a peripheral blood sample and then enriched in sterile conditions away from other blood cells. In both procedures, the enriched mononuclear cells are directly implanted into the gastrocnemius muscle of the ischaemic leg.

How the intervention might work

The exact mechanism of action of mononuclear cell implantation has not been fully elucidated. Marrow mononuclear cells have many of the characteristics of stem cells and have also been shown to secrete a number of angiogenic cytokines (Leibovich 1987; Prockop 1997). Therefore, it seems that marrow implantation into ischaemic limbs could enhance angiogenesis by supplying endothelial progenitor cells and providing multiple angiogenic factors or cytokines. These combined mechanisms may subsequently lead to the formation of stable capillary vessels and so reverse the ischaemic status of the affected limb.

Why it is important to do this review

Since mononuclear cell implantation has emerged as a novel intervention in clinical practice for CLI, it is important that a systematic review is undertaken in order to assess the safety and efficacy of this intervention. Therefore, we performed the current systematic review in order to investigate the potential therapeutic benefits of local intramuscular mononuclear cell implantation for patients with CLI.

OBJECTIVES

To determine the effectiveness and safety of local intramuscular transplantation of autologous adult bone marrow mononuclear cells (BMMNCs) as a treatment for critical limb ischaemia (CLI).

METHODS

Criteria for considering studies for this review

Types of studies

Randomised controlled trials.

Types of participants

Participants with a clinical diagnosis of critical limb ischaemia (CLI) who had been admitted to hospital for treatment. The participants were not candidates for open or endovascular revascularisation and did not show any evidence of improvement in response to best standard therapy in the previous four weeks. There was no age restriction.

Types of interventions

Studies involving the administration of autologous adult BMMNCs by direct infusion into the gastrocnemius muscle of ischaemic legs of patients as treatment for CLI.

Participants in the control treatment arm had either no intervention or conventional conservative therapy, for example bed rest, a pharmacological therapy, or administration of an inert placebo such as isotonic saline.

Types of outcome measures

Primary outcomes

1. All-cause mortality
2. Reduction in pain, assessed by analgesic requirements or a pain analogue scale
3. Progression of disease in terms of the incidence of amputation

Secondary outcomes

1. Progression of disease in terms of the incidence of surgical reconstruction or a non-surgical (radiological) intervention
2. Increase in ankle brachial index (ABI)
3. Increase in pain-free walking distance (PFWD)
4. Side effects and complications such as local or systemic inflammation, cardiovascular abnormalities and thromboembolic complications

Search methods for identification of studies

Electronic searches

For this update, the Cochrane Peripheral Vascular Diseases Group Trials Search Co-ordinator (TSC) searched the Specialised Register (last searched February 2014) and the Cochrane Central Register of Controlled Trials (CENTRAL; 2014, Issue 1). See Appendix 1 for details of the search strategy 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).

Searching other resources

We checked reference lists of papers and reports retrieved from the electronic searches to identify additional, potentially relevant studies.

Data collection and analysis

Selection of studies

One review author (KM) screened the titles and abstracts of references identified by the search and two review authors (BM and AR) independently assessed their eligibility for inclusion in the review. We resolved any disagreements by consensus or by discussion with the third author (KM). We obtained full versions of articles that potentially met the inclusion criteria based on the title or abstract and assessed these independently against the inclusion criteria. We recorded the reasons for exclusion of any study previously considered for inclusion in the 'Characteristics of excluded studies' table.

Data extraction and management

Two review authors (BM and ED) independently extracted and recorded the dichotomous and continuous data concerning the outcome measures on forms developed by the Cochrane Peripheral Vascular Diseases Group. Disagreements were resolved by the third author (RM) and, if necessary, we sought additional information from the study authors.

Assessment of risk of bias in included studies

The methodological quality of each included trial was assessed for both allocation concealment and for internal and external validity using the checklist provided by the Cochrane Peripheral Vascular Diseases Group. All three review authors independently assessed the methodological aspects of each trial and gave an allocation score of A (clearly concealed), B (unclear if concealed), C (not concealed), and a summary score of A (low risk of bias), B (moderate risk), or C (high risk). Trials scoring B were discussed and attempts were made to obtain further information from the authors. We resolved discrepancies by consensus or by discussion with the third author (RM). We only included trials scoring A or B in the review. We sought all information regarding the adequacy of the randomisation process, allocation concealment, blinding, intention-to-treat analysis and completeness of follow-up.

In addition, we used the 'Risk of bias' tool as described in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011) to assess allocation (selection bias), blinding (performance bias and detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias) and other potential sources of bias. We judged each item of bias to be either at low, unclear or high risk of bias according to the guidance by Higgins 2011.

Measures of treatment effect

If sufficient trials were available, we planned to use risk ratios (RRs) as the measure of effect for each dichotomous outcome. Where continuous scales of measurement were used to assess the effects of treatment, we planned to use mean difference (MDs). If different scales were used in the different studies, we planned to standardise the results, where possible, and then combine them (that is, using standardised mean difference).

Dealing with missing data

We sought missing data from authors, and data regarding patient demographics, and requested outcome measures by contacting the authors, where necessary. If some outcome data remained missing despite our attempts to obtain complete outcome data, we planned to exclude the trials from the analyses where there were data with more than 10% incomplete or missing entries for each variable.

Assessment of heterogeneity

If sufficient trials were available, we planned to explore and assess heterogeneity using the I^2 and Q statistics, and by subjective judgment of the comparability of patients, interventions and outcomes. We consider an I^2 greater than 30% or Q statistic with a P value less than 0.1 as indicative of heterogeneity. If there was significant heterogeneity among the studies, we planned to record and explore the reasons for such heterogeneity.

Assessment of reporting biases

If sufficient trials were available, we planned to use funnel plots to assess publication bias.

Data synthesis

If sufficient trials were available, we planned to use both random-effects and fixed-effect models for assessing the robustness of the results. If heterogeneity did not exist, we planned to use a fixed-effect model. If statistical but not clinical heterogeneity existed, we planned to use a random-effects model.

Subgroup analysis and investigation of heterogeneity

If sufficient trials were available, we planned to perform subgroup analyses, by the type of disease (atherosclerosis versus thromboangitis obliterans) and the type of cellular preparation (bone marrow derived versus peripheral blood derived).

Sensitivity analysis

If sufficient studies were identified, we planned to undertake sensitivity analyses to examine the robustness of the observed findings in relation to a number of factors including study quality and patient type.

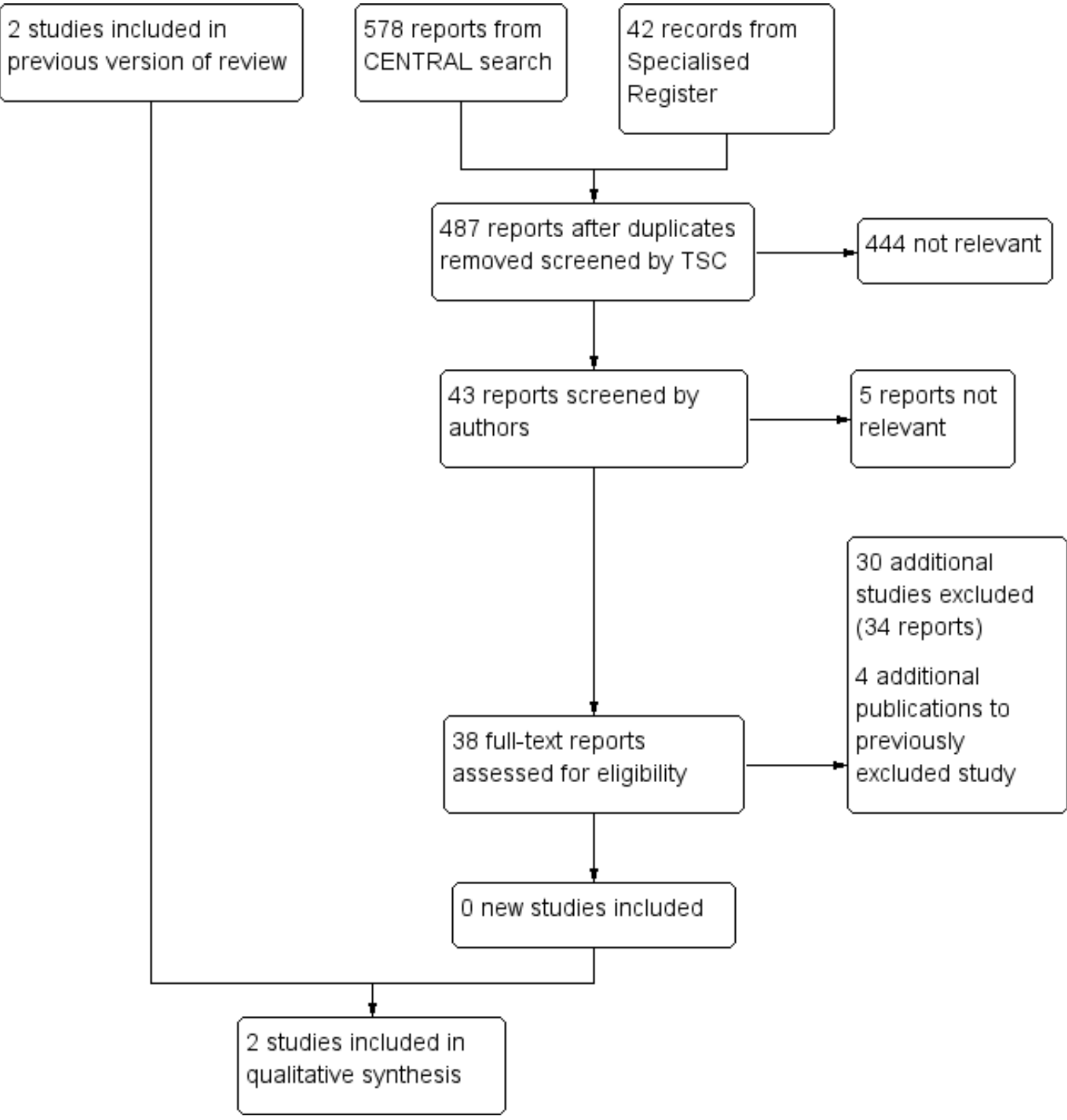
RESULTS

Description of studies

Results of the search

See Figure 1.

Figure 1. Study flow diagram.



Included studies

See the table Characteristics of included studies. We included two trials in the review (Barc 2006; Huang 2005). We did not include any new studies for this update.

One study compared the effect of intramuscular injections of bone marrow mononuclear cells (BMMNCs) versus control (the type of control therapy was described by the authors as standard conservative therapy, which was also given to the treatment group) in 29 patients with critical limb ischaemia (CLI) (Barc 2006). In the other study, following subcutaneous injections of G-CSF for five days, peripheral blood derived mononuclear cells (PBMNCs) were collected and transplanted by intramuscular injections into the patients' ischaemic lower limbs (Huang 2005). The effects were compared with prostaglandin E1 in a total of 28 randomised diabetic patients with CLI. The duration of follow-up differed between the two studies, with Barc 2006 following the patients for six months, while in the Huang 2005 study, the final assessment of patients took place after three months of intervention.

Excluded studies

See the table Characteristics of excluded studies.

For this update we excluded 30 additional studies (34 reports) (Benoit 2011; Choi 2012; Dash 2009; Debin 2008; Dong 2013; Doudar 2013; Dubsky 2013; Gupta 2013; Iafrati 2011; Kirana 2012; Klepanec 2011; Lasala 2011; Li 2013; Lu 2011; Madaric 2011; Mohammadzadeh 2013; NCT00922389; NCT00955669; NCT01049919; NCT01245335; Ozturk 2012; Pawan 2012; NCT00913900; Smadja 2012; Szabo 2013; Takagi 2011; Walter 2011; Wen 2010; Zafarghandi 2010; Zhang 2010) and added four additional reports to studies which had previously been excluded. This made a total of 63 excluded studies (Arai 2006; Bartsch T 2007; Benoit 2011; BONMOT; Capiod 2009; Chen 2009; Choi 2012; Cobellis 2008; Dash 2009; Debin 2008; Dong 2013; Doudar 2013; Dubsky 2013; Gu 2006; Gu 2007; Gu 2008; Gupta 2013; Gurunathan 2009; Hernandez 2007; Higashi 2010; Holzinger 1994; Huang 2007; Iafrati 2011; JUVENTAS; Kirana 2007; Kirana 2012; Klepanec 2011; Lasala 2011; Li 2013; Lu 2008; Lu 2011; Madaric 2011; Mohammadzadeh 2013; Napoli 2008; NCT00282646; NCT00498069; NCT00539266; NCT00595257; NCT00616980; NCT00904501; NCT00913900; NCT00922389; NCT00955669; NCT01049919; NCT01245335; Ozturk 2012; Pawan 2012; Perin 2011; RESTORE-CLI Trial; Sica 2006; Smadja 2012; Subramaniyam 2009; Szabo 2013; Takagi 2011; Tateishi-Yuyama 2002; Van Huyen 2008; Van Tongeren 2008; Walter 2011; Wen 2010; Zafarghandi 2010; Zhang 2009; Zhang 2010; Zhao 2008).

1. In 15 studies, two active interventions were compared and no control arm was present (Capiod 2009; Gu 2006; Gu 2007; Gu 2008; Hernandez 2007; Huang 2007; Klepanec 2011; Lasala 2011; Madaric 2011; Napoli 2008; Tateishi-Yuyama 2002; Van Tongeren 2008; Zafarghandi 2010; Zhang 2009).
2. Twenty studies investigated the effect of preparations of cells other than mononuclear cells. These included bone marrow cells (NCT00498069; NCT00539266), CD34 positive cells (Dong 2013; NCT00616980; NCT00922389; Szabo 2013), bone marrow concentrate (BONMOT; Gurunathan 2009; Iafrati 2011; NCT00595257; NCT01049919; NCT01245335), bone marrow mesenchymal stem cells (Chen 2009; Debin 2008; Gupta 2013; NCT00955669; Pawan 2012), adult stem cells (NCT00913900), bone marrow-derived aldehyde dehydrogenase bright cells (Perin 2011), expanded autologous bone marrow-derived tissue, Ixmyelocel-T and vascular repair cells (RESTORE-CLI Trial).
3. Seven studies did not include patients with CLI (Arai 2006; Bartsch T 2007; Holzinger 1994; Kirana 2007; Kirana 2012; Sica 2006; Subramaniyam 2009).
4. Fourteen studies were not randomised (Benoit 2011; Choi 2012; Cobellis 2008; Dash 2009; Doudar 2013; Dubsky 2013; Li 2013; Lu 2008; Mohammadzadeh 2013; Ozturk 2012; Smadja 2012; Takagi 2011; Van Huyen 2008; Wen 2010).
5. In five studies, cells were not administered intramuscularly and the intra-arterial route of administration was applied (Gu 2008; JUVENTAS; NCT00282646; NCT00904501; Walter 2011).
6. In one study, the active intervention arm consisted of BMMNCs co-administered with PBMNCs (Zhao 2008).
7. In one study, BMMNCs were co-administered with a selective 5-HT(2A) antagonist in the treatment arm (Higashi 2010).

Risk of bias in included studies

We awarded both of the included studies (Barc 2006; Huang 2005) a B grade for methodological quality when assessed for allocation concealment and for internal and external validity using the checklist provided by the Cochrane Peripheral Vascular Diseases Group.

See also the 'Risk of bias' tables for the included studies and Figure 2 and Figure 3.

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

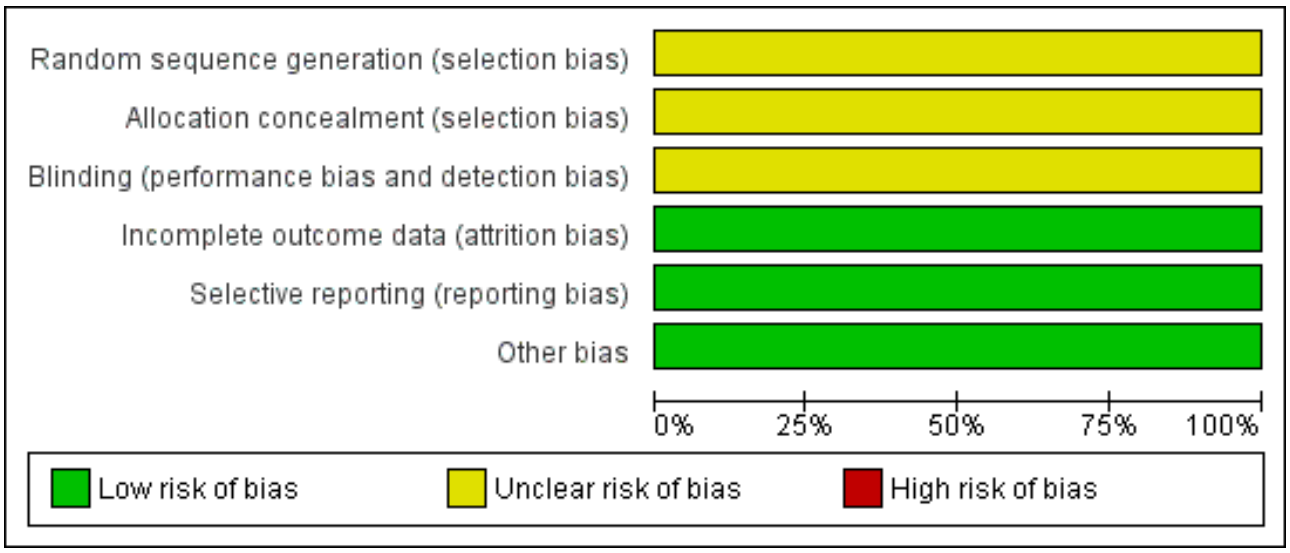


Figure 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

Allocation

While in both studies the method of treatment allocation was described as 'random', no description was given in the publications as to which methods were used to generate the random sequence.

Blinding

The blinding of all trial personnel (participants, clinicians and outcome assessors) to treatment allocation was unclear in both trials.

Incomplete outcome data

Both trials included all randomised participants in the analysis of outcome data and did not lose any participants during follow-up.

Selective reporting

We did not identify any reporting bias. All outcomes in the protocol of the studies have been described in the results.

Other potential sources of bias

We did not identify any other potential sources of bias in the included studies.

Effects of interventions

In the present review, we did not perform a meta-analysis because the two included studies differed from each other in various aspects, including type of cell administration (bone marrow mononuclear cells (BMMNCs) versus peripheral blood derived mononuclear cells (PBMNCs) and different outcome assessment points.

Therefore, we presented and discussed the data separately for the included studies (Barc 2006; Huang 2005). In future updates of the review, as new studies emerge and data from ongoing studies become available, it may be possible to add further comparisons and, where appropriate, combine findings in a meta-analysis.

All-cause mortality

No deaths were reported during the study period in either Barc 2006 or Huang 2005.

Reduction in pain assessed by analgesic requirements or a pain analogue scale

Both studies included pain assessments as outcomes of their study and demonstrated that following mononuclear implantation, pain was decreased in the transplant group.

In the Barc 2006 trial, ischaemic pain was assessed with a visual analogue pain scale with 10 levels, where 0 was no pain at all and 10 was the most severe pain experienced. While the pain was shown to decrease in the treatment group (from 4.81 ± 0.87 to 1.27 ± 1.34, P < 0.001) such a decrease was also observed in the control group (from 5.12 ± 0.083 to 2.37 ± 2.13, P = 0.007) and no significant difference was observed between the groups (P = 0.37).

Huang 2005 investigated rest pain on a rating scale ranging from 0 points for the best result (complete relief of pain with no use of analgesics) to 4 points for the worst result. The scores for rest pain decreased from 3.86 ± 0.36 to 1.07 ± 0.92 points in the treatment group (P < 0.001). Statistically significant reductions in pain were also observed in the control group with pain decreasing from 3.79 ± 0.43 to 2.86 ± 1.17 points (P = 0.013). However, the reduction in rest pain was significantly greater in the treatment group compared with the control group (P < 0.001). The proportion of patients who reported recovering normal sleep appeared higher in the treatment group than in the control group (11 out of 14 versus 6 out of 14). However, the difference was not statistically significant (P = 0.29).

Progression of disease in terms of the incidence of amputation

Both studies examined the rate of amputations during their study period.

In the Huang 2005 study, none of the patients in the treatment group (n = 14) received any amputation, while in the control group five of 14 patients had to receive a lower limb amputation, which showed a significant difference in the proportion of patients undergoing amputation between the two groups (0/14 (0%) versus 5/14 (36%), P = 0.007).

In the Barc 2006 study, three of the 14 patients underwent amputation in the treatment group; two below the knee and one femoral amputation. In the control group, seven of 15 patients received amputations; two below the knee and five femoral amputations. The treatment group showed a significantly lower proportion of participants undergoing amputation compared with the control group (P = 0.026).

Progression of disease in terms of the incidence of surgical reconstruction or non-surgical (radiological) intervention

Progression of disease in terms of the incidence of surgical reconstruction or non-surgical (radiological) intervention was not reported by either Barc 2006 or Huang 2005.

Increase in ankle brachial index (ABI)

The ABI was measured before and after treatment in both trials.

According to the authors of the Barc 2006 study, no significant differences were observed between the two groups during the follow-up period, but numerical results for the ABI were not reported and the authors were unable to provide us with this information. In the Huang 2005 study, the mean ABI increased from 0.50 ± 0.21 at baseline to 0.63 ± 0.25 (P < 0.001) in the treatment group. In contrast, the mean ABI increased from 0.49 ± 0.25 at baseline to 0.51 ± 0.28 (P = 0.223) in the control group. The increase in ABI was significantly greater in the treatment group compared with the control group (P = 0.01). Huang 2005 also reported a significantly higher proportion of patients experiencing an increase in ABI (defined as an increase of ABI of more than 0.1) in the treatment group compared with the control group (65.2% versus 16.7%, P < 0.001).

Increase in pain-free walking distance (PFWD)

Only one study evaluated the effect of intramuscular mononuclear cell implantation on PFWD (Huang 2005). In this study, the treatment group experienced significant increases in PFWD (from 0.0 ± 0.0 m to 306.4 ± 289.1 m, P = 0.001). The control group also showed an increase in PFWD but the increase was not statistically significant (0.0 ± 0.0 m to 78.6 ± 142.3 m, P = 0.06). Finally, a statistically significant difference in change in PFWD was evident between the two arms of the trial (P = 0.007).

Side effects

Side effects of the treatment were assessed in only one study (Huang 2005). In this study, evaluations during a 12-week period included measurement of the electrocardiogram or dynamic electrocardiogram, ultrasound cardiogram, function of the liver and kidney or routine blood and urine parameters. No abnormalities or side effects were detected.

DISCUSSION

Summary of main results

For many years, bone marrow mononuclear cells (BMMNCs) or progenitor cells have been used for the treatment of patients with various haematological malignancies, but their role in the treatment of other diseases including critical limb ischaemia (CLI) has not been addressed exclusively. In the present review, very few trials were available to address the current uncertainties regarding the clinical application of intramuscular injection of BMMNCs in the treatment of lower limb ischaemia.

Regarding clinical efficiency, the two trials achieved inconsistent results. In the Huang 2005 study both subjective (rest pain, pain free walking distance (PFWD)) and objective (ankle brachial index (ABI), rate of amputations) parameters showed significant improvements in patients undergoing mononuclear cell implantation compared with control. However in the Barc 2006 study the only parameter which showed significant improvements in the treatment arm compared with the control group was the rate of amputation. Other parameters either showed no improvement in the treatment arm or, if any improvements were present, they did not differ from those seen in the control group.

Safety parameters were assessed in only one trial (Huang 2005), which did not show any side effects or deaths following BMMNC therapy. No deaths were reported in Barc 2006 or Huang 2005.

Overall completeness and applicability of evidence

The two included trials involved a very small number of participants and there were substantial variations in both the treatment strategies and the follow-up duration, which precluded any combination of the trial results. Since only two studies with low numbers of patients were finally evaluated we highly doubt the completeness and applicability of the evidence presented in this review. Further studies are needed in order to reach a definite conclusion.

In addition, it should be emphasised that in order to make the review more complete and applicable to clinical practice, the implantation of other cell types as well as other routes of administration will be captured in future updates of this systematic review.

Quality of the evidence

Both studies in the present review were classified as B (moderate risk of bias) and had unclear issues regarding their methods. Therefore, according to the GRADE approach (GRADE 2004), the overall quality of the evidence in the present study would be considered as moderate.

Potential biases in the review process

In the present review, additional information regarding the control group for Barc 2006 was retrieved through direct contact with the study authors. There are a number of ongoing studies in the field, but none of them fulfilled the criteria for inclusion in our review. Selection of studies and extraction of data were performed independently by two authors in order to minimise the probability of introducing bias.

Agreements and disagreements with other studies or reviews

To date, no review has evaluated the effect of intramuscular mononuclear therapy for patients with lower limb ischaemia.

AUTHORS' CONCLUSIONS

Implications for practice

The limited data from the published trials suggest that there is very little evidence to support this practice. Therefore evidence from larger randomised controlled trials is needed in order to provide adequate statistical power to assess the role of intramuscular mononuclear cell implantation in patients with critical limb ischaemia (CLI).

Implications for research

Further well-conducted randomised double-blind trials with high quality methodological assessments should be performed. Key outcomes of these new studies should be amputation-free survival, incidence of surgical reconstruction and non-surgical (radiological) interventions, assessment of pain reduction, and assessments of side effects and complications of the treatment. Sufficient numbers of patients should be included to provide statistically powerful information. Also, studies should be conducted in order to determine factors including the optimal dose of bone marrow mononuclear cells (BMMNCs) infused, the route of cell delivery and the exact mechanism of action of the intervention. Moreover, the effects of implantation of other cell types and comparisons between them, as well as other routes of administration should be addressed. Finally, longer durations of follow-up and standardisation of outcome assessment methods are needed in future studies.

ACKNOWLEDGEMENTS

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Barc 2006

Methods	Study design: stated as randomised
	Method of randomisation: not stated

Barc 2006 *(Continued)*

	Exclusions post-randomisation: not stated Losses to follow-up: not stated
Participants	Country: Poland Participants: 29 randomised Mean age: not stated Sex: not stated Inclusion criteria: 1. patients with CLI with risk of amputation; 2. presence of rest pain and/or necrosis lasting longer than 12 weeks; 3. no progress after eight weeks of conventional therapy; 4. ABI < 0.5 in two independent examinations performed during the seven-day interval pre-inclusion; 5. peripheral type of atherosclerosis and no possibility for operative therapy, confirmed in angiogram. Exclusion criteria: 1. age lower than 18 years; 2. need for urgent amputation; 3. reasons for ischaemia other than atherosclerosis; 4. cancer; 5. absence of conscious consent; 6. lack of understanding of the idea of the therapy by patient; 7. poor general condition (life expectancy less than six months)
Interventions	Treatment group: Type of cells: BMMNC Route of delivery: intramuscular injections Control group: Not stated in the paper but described by the authors as standard conservative therapy which was also given to the treatment group
Outcomes	Primary outcomes: 1. ABI; 2. photographic documentation of ischaemic ulcerations and necrosis; 3. subjective parameters (feeling of pain in VAS, quality of life in WHO scale). Secondary outcomes: not stated Outcome assessment points: baseline and months 1, 3 and 6
Notes	

Barc 2006 *(Continued)*

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	The study is described as randomised but details are unclear
Allocation concealment (selection bias)	Unclear risk	Not mentioned
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Not mentioned
Incomplete outcome data (attrition bias) All outcomes	Low risk	All patients are accounted for
Selective reporting (reporting bias)	Low risk	We did not identify any reporting bias
Other bias	Low risk	We did not identify any other potential sources of bias

Huang 2005

Methods	Study design: stated as randomised Method of randomisation: not stated Exclusions post-randomisation: not stated Losses to follow-up: none
Participants	Country: China Participants: 28 randomised Mean age: 71 years in treatment group, 70 years in control group Sex: male and female Inclusion criteria: Diabetic patients with CLI who suffered from persistent, recurring rest pain requiring analgesia and an ankle systolic pressure 50 mmHg and/or toe systolic pressure 30 mmHg, and/or ulceration, gangrene, or non-healing wounds of the foot with ankle systolic pressure 50 mmHg or toe systolic pressure 30 mmHg
Interventions	Treatment group: Type of cells: PBMNC Route of delivery: intramuscular injections G-CSF details: 600 µg/day recombinant human G-CSF by subcutaneous injection for five days Control group: An intravenous injection of 90 - 200 µg/day prostaglandin E1

Huang 2005 *(Continued)*

Outcomes	Primary outcomes: 1. rest pain on rating scale ranged from 0 points for the best (complete relief of pain with no use of analgesics) to four points for the worst result; 2. assessment of pain-free walking distance used a constant speed on the same road in our hospital; 3. ABI; 4. blood flow (height of wave amplitude) of 10 toes; 5. blood perfusion of lower limbs of the patients in the transplant group by laser Doppler; 6. angiographic analysis. Secondary outcomes: not stated Outcome assessment points: baseline and week 12
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	The study is described as randomised but details are unclear
Allocation concealment (selection bias)	Unclear risk	Not mentioned
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Not mentioned
Incomplete outcome data (attrition bias) All outcomes	Low risk	All patients are accounted for
Selective reporting (reporting bias)	Low risk	We did not identify any reporting bias
Other bias	Low risk	We did not identify any other potential sources of bias

ABI: ankle brachial index
BMMNC: bone marrow derived mononuclear cell
PBMNC: peripheral blood derived mononuclear cell
CLI: critical limb ischaemia
G-CSF: granulocyte colony-stimulating factor
VAS: visual analogue scale
WHO: World Health Organization

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Arai 2006	The study does not include direct infusion of cells into the gastrocnemius muscle and patients are not diagnosed as CLI

Study	Reason for exclusion
Bartsch T 2007	The study investigates the combined effect of intra-arterial and intramuscular transplantation of autologous mononuclear bone marrow stem cells and patients are not diagnosed as having CLI
Benoit 2011	The study is not randomised
BONMOT	The study is an ongoing RCT investigating the effects of autologous bone marrow concentrate, not BMMNCs
Capiod 2009	The study compared the effect of transplantation of BMMNCs or G-CSF-mobilised PBMNCs and no control group is included
Chen 2009	The study included patients with diabetic foot not CLI and patients received autologous bone marrow mesenchymal stem cells, not mononuclear cells
Choi 2012	The study is not randomised
Cobellis 2008	The study is not randomised
Dash 2009	The study is not randomised
Debin 2008	The study investigated the effects of bone marrow mesenchymal stem cells, not BMMNCs
Dong 2013	The study investigated the effects of purified CD34+ cells, not BMMNCs
Doudar 2013	The study is not randomised
Dubsky 2013	The study is not randomised
Gu 2006	The study compared the effect of transplantation of autologous mononuclear bone marrow cells in patients with mild or severe ischaemia and no control group is included
Gu 2007	The study compared the effect of autologous bone marrow stem cell implantation and autologous peripheral blood stem cell implantation and no control group is present
Gu 2008	The study compared the effect of intra-arterial transplantation with that of intramuscular transplantation and no control group is included
Gupta 2013	The study investigates the effects of allogeneic bone marrow derived mesenchymal stem cell not BMMNC
Gurunathan 2009	The study is an ongoing RCT investigating the effects of autologous bone marrow concentrate, not BMMNCs
Hernandez 2007	The study compared the effect of transplantation of autologous mononuclear bone marrow sorted on a blood cell separator or isolated by density gradient on Ficoll Hypaque. No control group is included
Higashi 2010	The study investigates the combined effect of BMMNC implantation and sarpogrelate, a selective 5-HT(2A) antagonist
Holzinger 1994	The study investigates patients with chronic skin ulcers causes by chronic arterial occlusive disease or venous post-thrombotic syndrome, not CLI
Huang 2007	The study compared the effect of transplantation of BMMNCs or G-CSF-mobilised peripheral blood mononuclear cells and no control group is included

Study	Reason for exclusion
Iafrati 2011	The study investigates bone marrow aspirate concentrate not BMMNC
JUVENTAS	The study is an ongoing RCT investigating the effects of intra-arterial injection of BMMNCs
Kirana 2007	The study investigates patients with diabetic foot ulcers not CLI
Kirana 2012	The study investigates patients with diabetic foot ulcers not CLI
Klepanec 2011	The study compares intra-arterial and intramuscular mesenchymal stem cells therapy with no control group
Lasala 2011	The study investigates the transplantation of an autologous bone marrow-derived combination stem cell product
Li 2013	The study is not randomised
Lu 2008	The study is not randomised
Lu 2011	The study compares bone marrow mesenchymal stem cells with bone marrow-derived mononuclear cells with no control group
Madaric 2011	The study compares intra-arterial and intramuscular application of autologous bone marrow cells transplantation
Mohammadzadeh 2013	The study is not randomised
Napoli 2008	The study investigates the combined effect of intra-arterial injection of autologous bone marrow cells with oral treatment with antioxidants and L-arginine in comparison to oral treatment with antioxidants and L-arginine alone
NCT00282646	The study is an ongoing RCT investigating the effects of intra-arterial injection of autologous BMMNCs versus placebo
NCT00498069	The study is an ongoing RCT investigating the effects of bone marrow cells, not BMMNCs
NCT00539266	The study is an ongoing RCT investigating the effects of bone marrow cells, not BMMNCs
NCT00595257	The study is an ongoing RCT investigating the effects of autologous bone marrow concentrate, not BMMNCs
NCT00616980	The study is an ongoing RCT investigating the effects of peripheral blood derived stem cells, not BMMNCs
NCT00904501	The study is an ongoing RCT investigating the effects of intra-arterial injection of BMMNCs
NCT00913900	The study is an ongoing RCT investigating the effects of adult stem cells
NCT00922389	The study is an ongoing RCT investigating the effects of CD34 positive cells, not BMMNCs
NCT00955669	The study is an ongoing RCT comparing autologous mesenchymal stem cells and mononuclear cells, not BMMNCs
NCT01049919	The study is an ongoing RCT investigating the effects of autologous concentrated bone marrow aspirate, not BMMNCs

Study	Reason for exclusion
NCT01245335	The study is an ongoing RCT investigating the effects of bone marrow aspirate concentrate, not BMMNCs
Ozturk 2012	The study is not randomised
Pawan 2012	The study investigates adult bone marrow derived allogeneic mesenchymal stem cells
Perin 2011	The study investigates autologous therapy with bone marrow-derived aldehyde dehydrogenase bright cells
RESTORE-CLI Trial	The study is an ongoing RCT investigating the effects of vascular repair cells, not BMMNCs
Sica 2006	The study investigates patients with peripheral atherosclerosis not CLI
Smadja 2012	The study is not randomised
Subramaniyam 2009	The study investigates patients with intermittent claudication, not CLI
Szabo 2013	The study investigates the effects of autologous stem cell therapy not BMMNCs
Takagi 2011	The study is not randomised
Tateishi-Yuyama 2002	The study compared the effect of transplantation of BMMNCs or PBMNCs and no control group is included
Van Huyen 2008	The study is not randomised
Van Tongeren 2008	The study compared the effect of combined intra-arterial plus intramuscular transplantation with exclusive intramuscular injections and no control group is included
Walter 2011	The study compared the effect of intra-arterial route of transplantation
Wen 2010	The study is not randomised
Zafarghandi 2010	The study compared the effect of transplantation of BMMNCs with G-CSF-mobilised PBMNCs and no control group is included
Zhang 2009	The study compared the effect of transplantation of BMMNCs with G-CSF-mobilised PBMNCs and no control group is included
Zhang 2010	The study only included patients with diabetes
Zhao 2008	The study investigated the effect of combined PBMNCs and BMMNCs injection versus placebo

BMMNC: bone marrow derived mononuclear cell
CLI: critical limb ischaemia
G-CSF: granulocyte colony-stimulating factor
PBMNC: peripheral blood derived mononuclear cell
RCT: randomised controlled trial

APPENDICES

Appendix 1. CENTRAL search strategy

#1	MeSH descriptor: [Arteriosclerosis] this term only	893
#2	MeSH descriptor: [Limb Salvage] explode all trees	66
#3	MeSH descriptor: [Arteriosclerosis Obliterans] this term only	72
#4	MeSH descriptor: [Atherosclerosis] this term only	460
#5	MeSH descriptor: [Arterial Occlusive Diseases] this term only	784
#6	MeSH descriptor: [Intermittent Claudication] this term only	748
#7	MeSH descriptor: [Ischemia] this term only	790
#8	MeSH descriptor: [Peripheral Vascular Diseases] this term only	566
#9	atherosclero* or arteriosclero* or PVD or PAOD or PAD	18781
#10	(arter* or vascular or vein* or veno* or peripher*) near (occlus* or steno* or obstruct* or lesio* or block*)	8821
#11	peripheral near/3 dis*	3626
#12	claudic*	1576
#13	isch* or CLI	19471
#14	#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	43367
#15	MeSH descriptor: [Transplantation, Autologous] explode all trees	1281
#16	MeSH descriptor: [Bone Marrow Transplantation] explode all trees	1319
#17	MeSH descriptor: [Leukocytes, Mononuclear] explode all trees	5032
#18	mononuclear:ti,ab,kw	2110
#19	bone near marrow:ti,ab,kw	4967
#20	autologous near cell*:ti,ab,kw	1857
#21	BMC:ti,ab,kw	479
#22	#15 or #16 or #17 or #18 or #19 or #20 or #21	12934
#23	#14 and #22 in Trials	578

WHAT'S NEW

Date	Event	Description
2 October 2014	New citation required but conclusions have not changed	Search rerun. No new studies included. Thirty additional studies excluded. Minor edits made to the review text. New authors have joined the review team. No change to conclusions.
2 October 2014	New search has been performed	Search rerun. No new studies included. Thirty additional studies excluded.

CONTRIBUTIONS OF AUTHORS

KM: conceived, designed, co-ordinated and wrote the protocol; selected trials for inclusion, assessed methodological quality, extracted data, analysed data and wrote the review.
BM: selected trials for inclusion, assessed methodological quality, extracted data, analysed data and wrote the review.
AR: selected trials for inclusion, assessed methodological quality, extracted data, analysed data and wrote the review.
SN: selected trials for inclusion, assessed methodological quality, extracted and analysed data, and wrote the review.
ED: selected trials for inclusion, assessed methodological quality, extracted data, analysed data and wrote the review.

DECLARATIONS OF INTEREST

KM: none known.
BM: none known.
AR: none known.
SN: none known.
ED: none known.

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- No sources of support supplied

External sources

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DIFFERENCES BETWEEN PROTOCOL AND REVIEW

In addition to the assessment of the methodological quality according to the checklist from the Cochrane Peripheral Vascular Diseases Group, we also assessed the risk of bias using the recommended 'Risk of bias' tool as described in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). In order to reflect clinical importance, we considered 'incidence of amputation' and 'increase in ankle brachial index (ABI)' as primary and secondary outcomes, respectively, in the final review.

INDEX TERMS

Medical Subject Headings (MeSH)

Alprostadil [administration & dosage]; Amputation [statistics & numerical data]; Ankle Brachial Index; Bone Marrow Transplantation [methods]; Granulocyte Colony-Stimulating Factor [administration & dosage]; Injections, Intramuscular; Ischemia [etiology] [*therapy]; Leg [*blood supply]; Leukocytes, Mononuclear [*transplantation]; Pain Measurement; Peripheral Vascular Diseases [complications]; Randomized Controlled Trials as Topic; Transplantation, Autologous; Vasodilator Agents [administration & dosage]

MeSH check words

Adult; Humans