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Autologous cells derived from different sources and administered using different regimens for 'no-option' critical lower limb ischaemia patients (Review)

Abdul Wahid SF, Ismail NA, Wan Jamaludin WF, Muhamad NA, Abdul Hamid MKA, Harunarashid H, Lai NM

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[Intervention Review]

Autologous cells derived from different sources and administered using different regimens for 'no-option' critical lower limb ischaemia patients

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ABSTRACT

Background

Revascularisation is the gold standard therapy for patients with critical limb ischaemia (CLI). In over 30% of patients who are not suitable for or have failed previous revascularisation therapy (the 'no-option' CLI patients), limb amputation is eventually unavoidable. Preliminary studies have reported encouraging outcomes with autologous cell-based therapy for the treatment of CLI in these 'no-option' patients. However, studies comparing the angiogenic potency and clinical effects of autologous cells derived from different sources have yielded limited data. Data regarding cell doses and routes of administration are also limited.

Objectives

To compare the efficacy and safety of autologous cells derived from different sources, prepared using different protocols, administered at different doses, and delivered via different routes for the treatment of 'no-option' CLI patients.

Search methods

The Cochrane Vascular Information Specialist (CIS) searched the Cochrane Vascular Specialised Register, the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE Ovid, Embase Ovid, the Cumulative Index to Nursing and Allied Health Literature (CINAHL), the Allied and Complementary Medicine Database (AMED), and trials registries (16 May 2018). Review authors searched PubMed until February 2017.

Selection criteria

We included randomised controlled trials (RCTs) involving 'no-option' CLI patients comparing a particular source or regimen of autologous cell-based therapy against another source or regimen of autologous cell-based therapy.

Data collection and analysis

Three review authors independently assessed the eligibility and methodological quality of the trials. We extracted outcome data from each trial and pooled them for meta-analysis. We calculated effect estimates using a risk ratio (RR) with 95% confidence interval (CI), or a mean difference (MD) with 95% CI.

Main results

We included seven RCTs with a total of 359 participants. These studies compared bone marrow-mononuclear cells (BM-MNCs) versus mobilised peripheral blood stem cells (mPBSCs), BM-MNCs versus bone marrow-mesenchymal stem cells (BM-MSCs), high cell dose versus low cell dose, and intramuscular (IM) versus intra-arterial (IA) routes of cell implantation. We identified no other comparisons in these studies. We considered most studies to be at low risk of bias in random sequence generation, incomplete outcome data, and selective outcome reporting; at high risk of bias in blinding of patients and personnel; and at unclear risk of bias in allocation concealment and blinding of outcome assessors. The quality of evidence was most often low to very low, with risk of bias, imprecision, and indirectness of outcomes the major downgrading factors.

Three RCTs (100 participants) reported a total of nine deaths during the study follow-up period. These studies did not report deaths according to treatment group.

Results show no clear difference in amputation rates between IM and IA routes (RR 0.80, 95% CI 0.54 to 1.18; three RCTs, 95 participants; low-quality evidence). Single-study data show no clear difference in amputation rates between BM-MNC- and mPBSC-treated groups (RR 1.54, 95% CI 0.45 to 5.24; 150 participants; low-quality evidence) and between high and low cell dose (RR 3.21, 95% CI 0.87 to 11.90; 16 participants; very low-quality evidence). The study comparing BM-MNCs versus BM-MSCs reported no amputations.

Single-study data with low-quality evidence show similar numbers of participants with healing ulcers between BM-MNCs and mPBSCs (RR 0.89, 95% CI 0.44 to 1.83; 49 participants) and between IM and IA routes (RR 1.13, 95% CI 0.73 to 1.76; 41 participants). In contrast, more participants appeared to have healing ulcers in the BM-MSC group than in the BM-MNC group (RR 2.00, 95% CI 1.02 to 3.92; one RCT, 22 participants; moderate-quality evidence). Researchers comparing high versus low cell doses did not report ulcer healing.

Single-study data show similar numbers of participants with reduction in rest pain between BM-MNCs and mPBSCs (RR 0.99, 95% CI 0.93 to 1.06; 104 participants; moderate-quality evidence) and between IM and IA routes (RR 1.22, 95% CI 0.91 to 1.64; 32 participants; low-quality evidence). One study reported no clear difference in rest pain scores between BM-MNC and BM-MSC (MD 0.00, 95% CI -0.61 to 0.61; 37 participants; moderate-quality evidence). Trials comparing high versus low cell doses did not report rest pain.

Single-study data show no clear difference in the number of participants with increased ankle-brachial index (ABI; increase of > 0.1 from pretreatment), between BM-MNCs and mPBSCs (RR 1.00, 95% CI 0.71 to 1.40; 104 participants; moderate-quality evidence), and between IM and IA routes (RR 0.93, 95% CI 0.43 to 2.00; 35 participants; very low-quality evidence). In contrast, ABI scores appeared higher in BM-MSC versus BM-MNC groups (MD 0.05, 95% CI 0.01 to 0.09; one RCT, 37 participants; low-quality evidence). ABI was not reported in the high versus low cell dose comparison.

Similar numbers of participants had improved transcutaneous oxygen tension (TcO_2) with IM versus IA routes (RR 1.22, 95% CI 0.86 to 1.72; two RCTs, 62 participants; very low-quality evidence). Single-study data with low-quality evidence show a higher TcO_2 reading in BM-MSC versus BM-MNC groups (MD 8.00, 95% CI 3.46 to 12.54; 37 participants) and in mPBSC- versus BM-MNC-treated groups (MD 1.70, 95% CI 0.41 to 2.99; 150 participants). TcO_2 was not reported in the high versus low cell dose comparison.

Study authors reported no significant short-term adverse effects attributed to autologous cell implantation.

Authors' conclusions

Mostly low- and very low-quality evidence suggests no clear differences between different stem cell sources and different treatment regimens of autologous cell implantation for outcomes such as all-cause mortality, amputation rate, ulcer healing, and rest pain for 'no-option' CLI patients. Pooled analyses did not show a clear difference in clinical outcomes whether cells were administered via IM or IA routes. High-quality evidence is lacking; therefore the efficacy and long-term safety of autologous cells derived from different sources, prepared using different protocols, administered at different doses, and delivered via different routes for the treatment of 'no-option' CLI patients, remain to be confirmed.

Future RCTs with larger numbers of participants are needed to determine the efficacy of cell-based therapy for CLI patients, along with the optimal cell source, phenotype, dose, and route of implantation. Longer follow-up is needed to confirm the durability of angiogenic potential and the long-term safety of cell-based therapy.

PLAIN LANGUAGE SUMMARY

Cell-based therapy using different sources and different treatment regimens for 'no-option' CLI patients

Background

Critical limb ischaemia (CLI) is characterised by severe leg pain on walking and at rest and hard-to-heal wounds, which may lead to disability and death. The procedure that aims to improve blood flow to the affected limb, known as 'revascularisation', is the gold standard therapy. However, 25% to 40% of people with CLI are not suitable for or have failed previous revascularisation therapy. Therefore, for these patients, the only option for relieving pain and stopping wound infection from spreading is limb amputation. These patients are commonly referred to as 'no-option' CLI patients.

Cell-based therapy is increasingly recognised as a promising novel treatment for CLI. Most of the data for this novel approach have been obtained from studies based on patients' own cells, also known as 'autologous cells'. However, current data on the efficacy of autologous cells are limited because available information about the sources used to obtain these cells (e.g. bone marrow, peripheral blood), the doses used (e.g. high or low cell dose), and the method of cell administration selected (e.g. cell injection into muscles or into blood vessels) is limited. In this review, we evaluated the efficacy and safety of autologous cell-based therapy derived from different sources and prepared as different treatment regimens for 'no-option' CLI patients.

Study characteristics and key results

We analysed the findings of seven randomised controlled trials (RCTs) involving 359 CLI patients revealed by our literature search, which was current to 16 May 2018.

We evaluated two main sources of stem cell treatment, namely, 'bone marrow-mononuclear cells (BM-MNCs)' and 'mobilised peripheral blood stem cells (mPBSCs)'. Limited data suggest that BM-MNCs or mPBSCs resulted in similar rates of limb amputation and death. Also, the two cell sources appeared to yield similar numbers of patients with improved rest pain, ulcer healing, and lower limb blood flow parameters as measured via the ankle-brachial index (ABI). However, data from one RCT show that mPBSC implantation resulted in improved transcutaneous oxygen tension (TcO_2) readings when compared to BM-MNC. Data from one RCT show no clear difference in amputation rates between patients receiving high cell dose and low cell dose, and no difference in clinical outcomes whether patients received cell doses via intramuscular or intra-articular routes. Study authors reported no significant short-term adverse effects attributed to autologous cell implantation.

Quality of the evidence

The quality of evidence for all outcomes varied but was mostly low to very low owing to limitations in study design and lack of data for several important outcomes. Taken together, there is insufficient high-quality evidence to assess the effects of using a particular source or treatment regimen of cell-based therapy for CLI in clinical practice. Larger trials with longer follow-up are needed to evaluate the long-term benefits and safety of various cell-based products for patients with CLI.

SUMMARY OF FINDINGS

Summary of findings for the main comparison. BM-MNCs compared to mPBSCs for critical lower limb ischaemia

Is BM-MNC implantation more effective than mPBSC implantation for reducing all-cause mortality, amputation rate, number of participants with any reduction in rest pain score, and number of healing ulcers and for improving lower limb perfusion in people with critical lower limb ischaemia?

Patient or population: people with critical lower limb ischaemia

Setting: hospital

Intervention: BM-MNCs

Comparison: mPBSCs

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	No. of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with mPBSCs	Risk with BM-MNCs				
All-cause mortality Assessed during in-patient stay	See comments.	See comments.	Not estimable	202 (2 RCTs)	See comments.	No deaths were reported by the 2 studies in this comparison (Huang 2007; Zhang 2009).
Amputation rate Assessed during in-patient stay	Study population		RR 1.54 (0.45 to 5.24)	150 (1 RCT)	⊕⊕⊖⊖ LOW[a,b]	
	53 per 1000	81 per 1000 (24 to 276)				
	Moderate**					
	53 per 1000	81 per 1000 (24 to 276)				
Wound/ulcer healing: number of participants with healing ulcers Assessed during in-patient stay	Study population		RR 0.89 (0.44 to 1.83)	49 (1 RCT)	⊕⊕⊖⊖ LOW[a,b]	
	407 per 1000	363 per 1000 (179 to 746)				
	Moderate**					

	407 per 1000	363 per 1000 (179 to 746)				
Reduction in rest pain: number of participants with any reduction in rest pain score Assessed during in-patient stay	Study population		RR 0.99 (0.93 to 1.06)	104 (1 RCT)	⊕⊕⊕⊖ MODERATE[a]	
	977 per 1000	968 per 1000 (909 to 1000)				
	Moderate**					
	977 per 1000	968 per 1000 (909 to 1000)				
Improvement in lower limb perfusion: number of participants with increased ABI Assessed at 4 weeks	Study population		RR 1.00 (0.71 to 1.40)	104 (1 RCT)	⊕⊕⊖⊖ LOW[a,b]	
	568 per 1000	568 per 1000 (403 to 795)				
	Moderate**					
	568 per 1000	568 per 1000 (369 to 744)				
Improvement in lower limb perfusion: TcO_2 reading in mmHg	Mean TcO_2 reading in mmHg was 4.68.	Mean TcO_2 reading in the intervention group was 1.7 mmHg more (0.41 more to 2.99 more).	-	150 (1 RCT)	⊕⊕⊖⊖ LOW[a,b]	The study included in this comparison reported the TcO_2 reading in mmHg, not the number of participants with improvement in lower limb perfusion, which was our preferred outcome.

*__The risk in the intervention group__ (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).

__ We took the median control group event rate for the outcome as "moderate risk".__

ABI: ankle-brachial index; BM-MNCs: bone marrow-mononuclear cells; CI: confidence interval; mPBSCs: mobilised peripheral blood stem cells; RCT: randomised controlled trial; RR: risk ratio; TcO_2: transcutaneous oxygen tension.

GRADE Working Group grades of evidence

High quality: we are very confident that the true effect lies close to that of the estimate of the effect

Moderate quality: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different

Low quality: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect

Very low quality: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect

[a]The single included study had unclear risk of selection bias and high risk of performance bias (patients and personnel were not blinded). Quality of evidence was downgraded by one level.
[b]The 95% CI is wide, as it ranges from substantial benefits favouring BM-MNCs to substantial benefits favouring mPBSCs. Quality of evidence was downgraded by one level.

Summary of findings 2. BM-MNCs compared to BM-MSCs for critical lower limb ischaemia

Is BM-MNC implantation more effective than BM-MSC implantation for reducing all-cause mortality, amputation rate, number of participants with any reduction in rest pain score, and number of healing ulcers and for improving lower limb perfusion in patients with critical lower limb ischaemia?

Patient or population: people with critical lower limb ischaemia
Setting: hospital
Intervention: BM-MNCs
Comparison: BM-MSCs

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with BM-MSCs	Risk with BM-MNCs				
All-cause mortality Assessed during inpatient stay	See comments.	See comments.	Not estimable	37 (1 RCT)	See comments.	No deaths were reported by the single study included in this comparison (Lu 2011).
Amputation rate Assessed during inpatient stay	See comments.	See comments.	Not estimable	37 (1 RCT)	See comments.	No amputations were reported by the single study included in this comparison (Lu 2011).
Wound/ulcer healing: number of participants with healing ulcers	Study population		RR 2.00 (1.02 to 3.92)	22 (1 RCT)	⊕⊕⊕⊖ MODERATE[a]	
	455 per 1000	909 per 1000 (464 to 1000)				
	Moderate**					

	455 per 1000	909 per 1000 (464 to 1000)				
Reduction in rest pain: rest pain score	Mean rest pain score was 1.4.	Mean rest pain score in the intervention group was similar (0) (0.61 fewer to 0.61 more).	-	37 (1 RCT)	⊕⊕⊕⊖ MODERATE[b]	The study included in this comparison reported the rest pain score, not the number of participants with improved rest pain, which was our preferred outcome.
Improvement in lower limb perfusion: ABI score	Mean ABI score was 0.12.	Mean ABI score in the intervention group was 0.05 more (0.01 more to 0.09 more).	-	37 (1 RCT)	⊕⊕⊖⊖ LOW[c,d]	The study included in this comparison reported the ABI score, not the number of participants with improvement in lower limb perfusion, which was our preferred outcome.
Improvement in lower limb perfusion: TcO_2 reading in mmHg	Mean TcO_2 reading in mmHg was 16.4.	Mean TcO_2 reading in the intervention group was 8 mmHg more (3.46 more to 12.54 more).	-	37 (1 RCT)	⊕⊕⊖⊖ LOW[d,e]	The study included in this comparison reported the TcO_2 reading in mmHg, not the number of participants with improvement in lower limb perfusion, which was our preferred outcome.

*__The risk in the intervention group__ (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).

__ We took the median control group event rate for the outcome as "moderate risk".__
ABI: ankle-brachial index; BM-MNCs: bone marrow mononuclear cells; BM-MSCs: bone marrow mesenchymal stem cells; CI: confidence interval; RCT: randomised controlled trial; RR: risk ratio; TcO_2: transcutaneous oxygen tension.

GRADE Working Group grades of evidence
High quality: we are very confident that the true effect lies close to that of the estimate of the effect
Moderate quality: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low quality: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect
Very low quality: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect

[a]The effect estimate of a single small study ranges from slight benefit to substantial benefit favouring BM-MSCs. Quality of evidence was downgraded by one level.
[b]The effect estimate of a single small study ranges from substantial benefit favouring BM-MNCs to substantial benefit favouring BM-MSCs. Quality of evidence was downgraded by one level.
[c]ABI score is not a clinical outcome, and its correlation with clinical symptoms and functions is unclear. Quality of evidence was downgraded by one level.
[d]The effect estimate of a single small study ranges from slight to substantial benefit for the intervention group in terms of the outcome measured (ABI score and TcO_2 reading, respectively). Quality of evidence was downgraded by one level for each outcome concerned.
[e]TcO_2 reading is not a clinical outcome, and its correlation with clinical symptoms and functions is unclear. Quality of evidence was downgraded by one level.

Summary of findings 3. High cell dose compared to low cell dose for critical lower limb ischaemia

Is high cell dose more effective than low cell dose for reducing all-cause mortality, amputation rate, number of participants with any reduction in rest pain score, and number of healing ulcers and for improving lower limb perfusion in patients with critical lower limb ischaemia?

Patient or population: people with critical lower limb ischaemia
Setting: hospital
Intervention: high cell dose
Comparison: low cell dose

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with low cell dose	Risk with high cell dose				
All-cause mortality Assessed during in-patient stay	See comments.	See comments.	Not estimable	16 (1 RCT)	See comments.	No deaths were reported by the single RCT included in this comparison (Losordo 2012).
Amputation rate Assessed during in-patient stay (Losordo 2012)	Study population		RR 3.21 (0.87 to 11.90)	16 (1 RCT)	⊕⊖⊖⊖ VERY LOW[a,b]	
	222 per 1000	713 per 1000 (193 to 1000)				
	Moderate**					
	222 per 1000	713 per 1000 (193 to 1000)				
Wound ulcer healing	See comments.	See comments.	See comments.	See comments.	See comments.	This outcome was not reported by the single RCT included in this comparison (Losordo 2012).
Reduction in rest pain	See comments.	See comments.	See comments.	See comments.	See comments.	This outcome was not reported by the single RCT included in this comparison (Losordo 2012).
Improvement in lower limb perfusion: ABI	See comments.	See comments.	See comments.	See comments.	See comments.	This outcome was not reported by the single RCT included in this comparison (Losordo 2012).

Improvement in lower limb perfusion: TcO_2	See comments.	See comments.	See comments.	See comments.	See comments.	This outcome was not reported by the single RCT included in this comparison (Losordo 2012).

*__The risk in the intervention group__ (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).
** __We took the median control group event rate for the outcome as "moderate risk".__
ABI: ankle-brachial index; CI: confidence interval; RCT: randomised controlled trial; RR: risk ratio; TcO_2: transcutaneous oxygen tension.

GRADE Working Group grades of evidence
High quality: we are very confident that the true effect lies close to that of the estimate of the effect
Moderate quality: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low quality: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect
Very low quality: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect

[a]The 95% CI of the effect estimate is extremely wide, covering both sides of the no-effect line. Quality of evidence was downgraded by two levels on the basis of imprecision.
[b]The single included study had unclear risk of selection bias and high risk of selection bias and unclear risk of performance and detection biases, attrition bias, and reporting bias. Quality of evidence was downgraded by one level.

Summary of findings 4. IM injection compared to IA injection for critical lower limb ischaemia

Is IM cell implantation more effective than IA cell implantation for reducing all-cause mortality, amputation rate, number of participants with any reduction in rest pain score, and number of healing ulcers and for improving lower limb perfusion in patients with critical lower limb ischaemia?

Patient or population: people with critical lower limb ischaemia
Setting: hospital
Intervention: IM injection
Comparison: IA injection

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with IA injection	Risk with IM injection				
All-cause mortality Assessed during in-patient stay	See comments.	See comments.	See comments.	95 (3 RCTs)	See comments.	Three RCTs with a total of 100 participants reported 9 deaths (Gu 2008; Klepanec 2012; Van Tongeren 2008).

						None of the 3 studies reported all-cause mortality according to treatment group.
Amputation rate Assessed during in-patient stay	Study population		RR 0.80 (0.54 to 1.18)	95 (3 RCTs)	⊕⊕⊕⊖ LOW[a,b]	
	500 per 1000	400 per 1000 (270 to 590)				
	Moderate**					
	636 per 1000	509 per 1000 (344 to 751)				
Wound/ulcer healing: number of participants with healing ulcer	Study population		RR 1.13 (0.73 to 1.76)	41 (1 RCT)	⊕⊕⊖⊖ LOW[b,c]	
	619 per 1000	700 per 1000 (452 to 1000)				
	Moderate**					
	619 per 1000	699 per 1000 (452 to 1000)				
Reduction in rest pain: number of participants with reduction in rest pain score	Study population		RR 1.22 (0.91 to 1.64)	32 (1 RCT)	⊕⊕⊖⊖ LOW[b,c]	
	765 per 1000	933 per 1000 (696 to 1000)				
	Moderate**					
	765 per 1000	933 per 1000 (696 to 1000)				
Improvement in lower limb perfusion: number of participants with increased ABI	Study population		RR 0.93 (0.43 to 2.00)	35 (1 RCT)	⊕⊖⊖⊖ VERY LOW[b,c,d]	
	444 per 1000	413 per 1000 (191 to 889)				
	Moderate**					

	444 per 1000	41 3 per 1000 (191 to 889)			
Improvement in lower limb perfusion: number of participants with improved TcO_2 reading	Study population		RR 1.22 (0.86 to 1.72)	62 (2 RCTs)	⊕⊖⊖⊖ VERY LOW[b,e,f]
	613 per 1000	748 per 1000 (527 to 1000)			
	Moderate**				
	603 per 1000	735 per 1000 (518 to 1000)			

*__The risk in the intervention group__ (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).
__ We took the median control group event rate for the outcome as "moderate risk".__
ABI: ankle-brachial index; CI: confidence interval; IA: intra-arterial; IM: intramuscular; RCT: randomised controlled trial; RR: risk ratio; TcO_2: transcutaneous oxygen tension.

GRADE Working Group grades of evidence
High quality: we are very confident that the true effect lies close to that of the estimate of the effect
Moderate quality: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low quality: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect
Very low quality: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect

[a]All three included studies had unclear risk of selection bias and high risk of performance biases (participants and personnel were not blinded). Quality of evidence was downgraded by one level.
[b]The 95% CI for the effect estimate from a single small study ranges from an effect size that clearly favours IM injection to an effect size that clearly favours IA injection. Quality of evidence was downgraded by one level on the basis of imprecision.
[c]The single included study had unclear risk of selection bias and high risk of performance bias (participants and personnel were not blinded). Quality of evidence was downgraded by one level.
[d]An improvement in ABI might not correlate strongly with an improvement in clinical symptoms and functions. Quality of evidence was downgraded by one level.
[e]The two included studies had unclear risk of selection bias and high risk of performance bias (participants and personnel were not blinded). Quality of evidence was downgraded by one level.
[f]An improvement in $TcPO_2$ reading might not correlate with an improvement in clinical symptoms and functions. Quality of evidence was downgraded by one level.

BACKGROUND

Description of the condition

Peripheral arterial disease (PAD) affects 3% to 10% of the population (Norgren 2010). It is a global health problem that is associated with significant morbidity and mortality attributed to intermittent claudication and critical limb ischaemia (CLI). Intermittent claudication is the most common presentation of PAD and is generally managed conservatively. Critical limb ischaemia, the severe form of PAD, is characterised by rest pain, ulceration, and gangrene. Revascularisation therapy via the surgical or endovascular approach is the gold standard treatment for severe PAD, provided with the aim of improving blood flow to the affected extremity. However, this treatment modality cannot be applied to over 30% of patients owing to excessive anaesthetic and operative risks and unfavourable vascular involvement (Sasajima 1997). Moreover, revascularisation therapy is likely to be futile in the presence of extensive atherosclerotic plaque and low rates of long-term vessel patency in severe PAD (Conrad 2011). Hence, many patients are reliant on medical therapy that may halt disease progression only temporarily, leaving limb amputation as the only remaining option for relief from pain or gangrene (Botti 2012). Of note, after one year from diagnosis, limb amputation is unavoidable in 30% of patients with CLI (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 or neovascularisation in patients with CLI who are not suitable for conventional treatments, to reduce physical disability, mortality, and socioeconomic burden.

Description of the intervention

Although initial clinical studies on cell-based therapy have been encouraging, current evidence from large-scale randomised controlled clinical trials (RCTs) comparing active treatment versus placebo is limited, leading to a previous Cochrane review concluding that there was "insufficient evidence to support cell therapy in clinical practice" (Moazzami 2014).

To date, the types of cell-based products used for implantation in CLI patients have been derived from bone marrow-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 (mPBSCs) (Huang 2005; Huang 2007; Ishida 2005), CD34 antigen-positive mononuclear cells (MNCs) (Inaba 2002; Kawamoto 2009), CD133 antigen-positive MNCs (Burt 2010), and BM-mesenchymal stem cells (BM-MSCs) (Dash 2009; Lu 2011).

Cell implantation procedures are generally safe and well tolerated, as has been described in extensive clinical studies involving patients with PAD that utilised stem cells derived from various sources (Benoit 2013; Liew 2016; Liu 2015; Sun 2015). Bone marrow is the most common source of stem cells in clinical trials involving cell-based therapy. However, mobilised stem cells from patients' peripheral blood after administration of G-CSF (mPBSCs) are now preferred over bone marrow stem cells owing to relative ease of collection and avoidance of anaesthesia and pain associated with bone marrow biopsy (Fadilah 2013). Apart from bone marrow and peripheral blood, mesenchymal stem cells (MSCs) can be isolated from various adult human tissues (e.g. adipose tissue, skeletal muscles, tendons, nerves, cartilages) or neonatal tissues (placenta and umbilical cord blood). The safety and efficacy of MSCs isolated from adipose tissue (AT-MSCs) in a small series of CLI patients have been reported (Lee 2012). However, apart from bone marrow, peripheral blood, and adipose tissue sources, MSCs derived from other human tissues have not been tested in CLI patients.

Mononuclear cells and stem cells derived from different sources may lead to 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 (modulation of immune response) properties. This may be attributed to the inherent biological properties of the stem cells or to changes to the cells that may occur during cell enrichment and culture. For example, G-CSF injection used to mobilise bone marrow-derived progenitor cells may significantly enhance the formation of several growth factors involved in vascular repair (Huang 2007). In addition, the apheresis procedure results in transient cleavage of chemokine receptors expressed on cell surfaces, causing uncoupling from the bone marrow stroma (Honold 2006). Implantation of cells into the lower limb of CLI patients can be performed via several routes including intramuscular, intra-arterial, or a combination of both, although it is yet unclear which method is superior (Gu 2008; Klepanec 2012; Van Tongeren 2008). Intramuscular administration is usually performed through multiple injections at the level of gastrocnemius muscles, and intra-arterial infusion is usually performed via the femoral artery. Limited data from two RCTs have not shown superiority of either route (Gu 2008; Klepanec 2012).

How the intervention might work

To date, the mechanisms by which implanted cells improve clinical outcomes in patients with PAD are still unclear. Experimental animal studies indicate that bone marrow-derived cells contribute to vascular and muscle regeneration by physically integrating into the tissues, by secreting growth factors, or by both means (Fadini 2007; Honold 2006). Adult bone marrow stem cells with angiogenic potential such as endothelial progenitor cells (EPCs) and MSCs have the capability to stimulate formation of new blood vessels by differentiating into endothelial cells and vascular smooth muscle (Schatteman 2004), and by stimulating endothelial cell proliferation and migration (Pittenger 1999; Reyes 2002). EPCs also exert direct angiogenic action through their ability to secrete paracrine mediators (Jarajapu 2010). Furthermore, MSCs support neo-angiogenesis by releasing soluble factors to stimulate EPC sprouting from pre-existing blood vessels (Cobellis 2010; Jarajapu 2010). Therefore, cell implantation 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, leading to reversal of ischaemia in the affected limb.

Why it is important to do this review

It is important to determine if different sources or methods of MNCs and stem cell preparations have different effects on clinical

outcomes following implantation into CLI patients; and whether a combination versus a single type of MNC or stem cell treatment improves ischaemic symptoms and survival among these patients. To date, data comparing the angiogenic potency of autologous cells derived from different sources are limited. Moreover, direct comparison of different autologous cell types shows conflicting results (Liew 2016). One study showed that BM-MNCs were associated with significant improvement in lower limb perfusion when compared to PB-MNCs (Tateishi-Yuyama 2002); another study showed similar outcomes in both treatment groups (Onodera 2011). Additionally, other sources of stem cells such as placenta or stored autologous cord blood might become available. It is not yet known whether cells from these sources would be as effective as cells derived from bone marrow or peripheral blood for treating CLI patients. Furthermore, thus far no safety data have been published by head-to-head RCTs comparing a particular cell-based therapy versus another type of cell-based therapy. The individual RCTs comparing cell treatment and non-cell treatment presented in previous reviews have not reported significant procedure-related complications or adverse biochemical and immunological effects related to cell implantation in CLI patients (Liu 2015; Teraa 2013; Wang 2014).

Up-to-date synthesised evidence on optimal cell sources, cell dose, and administration protocols for the treatment of 'no-option' CLI patients is required to guide clinical practice and direct future research. The current proposed meta-analysis aims to attain comprehensive insight into the optimal cell-based treatment program for patients with CLI.

OBJECTIVES

To compare the efficacy and safety of autologous cells derived from different sources, prepared using different protocols, administered at different doses, and delivered via different routes for the treatment of 'no-option' CLI patients.

METHODS

Criteria for considering studies for this review

Types of studies

We included randomised controlled trials (RCTs). We excluded cluster-randomised trials (owing to difficulties in adjusting for the unit of analysis) and cross-over studies (owing to a possible 'contaminating' effect of one intervention on another).

Types of participants

Study participants were adult patients with the diagnosis of CLI who were not candidates for revascularisation therapy and did not show any improvement in response to best standard medical therapy. We included in the review all causes of CLI such as atherosclerosis, Buerger's disease, acute embolism, and others. We applied no age restriction.

Types of interventions

Intervention: administration to CLI patients of autologous MNCs or stem cells obtained from a particular source, prepared using a particular protocol, administered at a particular dose, and delivered via a particular route.

Comparison: administration to CLI patients of autologous MNCs or stem cells obtained from any other source of MNCs or stem cells, prepared using any other protocol, administered at any other dose, and delivered via any other route.

We did not compare administration of autologous MNCs or stem cells to patients with CLI against no cell therapy, control, standard therapy, or best medical practice because this approach would overlap that used in another Cochrane review (Moazzami 2014).

Types of outcome measures

Primary outcomes

1. All-cause mortality
2. Amputation rate
3. Wound/ulcer healing as determined by the number of ulcers healed and the change in ulcer size

Secondary outcomes

1. Reduction in rest pain as assessed by a validated visual analogue scale (VAS) or analgesic requirement (rest pain score)
2. Improvement in lower limb perfusion as measured by improvement in ankle-brachial index (ABI)
3. Improvement in lower limb perfusion as measured by improvement in transcutaneous oxygen tension (TcO_2)
4. Improvement in ischaemic symptoms as assessed by improvement in pain-free walking distance (PFWD) and pain-free walking time (PFWT)
5. Improvement in vascularity and blood supply to the ischaemic limb as measured by the numbers of newly formed collaterals in the lower limbs
6. Adverse effects and safety
   a. Adverse effects included an inflammatory reaction at the stem cell implantation site (grade I to IV), cardiovascular abnormalities, or thromboembolic complications
   b. Safety was measured as the rate of adverse events and the rate of withdrawal

Search methods for identification of studies

Electronic searches

The Cochrane Vascular Information Specialist (CIS) first searched the following databases for relevant trials on 17 February 2017: the Cochrane Vascular Specialised Register; and the Cochrane Central Register of Controlled Trials (CENTRAL; 2017, Issue 1) in the Cochrane Library, via the Cochrane Register of Studies Online. See Appendix 1 for details of the search strategy used to search CENTRAL.

The CIS also searched the following trials databases on 17 February 2017 for details of ongoing and unpublished studies using the terms "(critical ischemia or critical ischaemia)" and "cell": the World Health Organization International Clinical Trials Registry (http://apps.who.int/trialsearch/); ClinicalTrials.gov (clinicaltrials.gov/); and the International Standard Randomized Controlled Trials Number (ISRCTN) Register (isrctn.com/).

The CIS performed a top-up search of the Cochrane Vascular Specialised Register, CENTRAL, MEDLINE Ovid, Embase Ovid, CINAHL, AMED, and trials registries, on 16 May 2018. See Appendix 2 for details of the search strategies used.

Review authors' searches

The review authors searched PubMed until February 2017 using the strategy shown in Appendix 3.

Searching other resources

To identify further eligible studies, we inspected the reference lists of relevant articles that we had retrieved via the search strategies outlined above and from relevant Cochrane reviews that assessed cell-based treatments as interventions.

Data collection and analysis

Selection of studies

Two review authors (NAI and MKAAH) independently screened the titles and abstracts of articles retrieved in the first round of the search, aiming to exclude articles that were clearly irrelevant. After completing the initial step of screening, we had obtained a list of articles that appeared to be relevant to our review. Two review authors (SFAW and NAM) independently assessed the short-listed articles in greater detail by using the abstract and full text to identify eligible articles. In instances of disagreements between review authors on article selection, a third review author (NML) acted as an arbiter.

We accepted published and unpublished studies in full article and abstract forms, as long as assessment of risk of bias was possible and relevant data were available. When required, we would contact authors of unpublished studies and studies available only as abstracts to request further information.

We screened for duplicate publications of the same trial, and we contacted the trial authors for clarification when necessary.

Data extraction and management

Three review authors (SFAW, NAI, and MKAAH) independently extracted and coded all data for each included study using a proforma designed specifically for this review. We extracted the following information from each study: study design, participants, setting, sample size, nature of intervention, comparison, outcomes, methods (unit of allocation and analysis), and results. We screened for duplicate entry of patients, when possible, by matching the initial number of patients recruited against the total number along each step in the conduct of the study. If we discovered a discrepancy (e.g. if the total number in a later stage of the study exceeded the initial number), we attempted to look for an explanation within the article (e.g. multiple enrolment of the same patient at different hospital admissions). We contacted study authors for clarification if necessary. We compared data in duplicate publications against data from all versions to avoid duplicate extraction.

We resolved disagreements among the review authors through discussion leading to a consensus.

Assessment of risk of bias in included studies

Three review authors (NAI, SFAW, and NML) independently assessed each included study using Cochrane's tool for assessing risk of bias to address six specific domains (Higgins 2011).

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 made a judgement on each of the criteria above as to whether the study was at high, low, or unclear risk of bias. We assessed blinding for each category of outcomes (objective and subjective) separately when possible. We completed a 'Risk of bias' table for each eligible study and resolved disagreement among review authors through discussion leading to a consensus. We presented an overall assessment of the risk of bias using the 'Risk of bias' graph and the 'Risk of bias' summary.

Measures of treatment effect

For dichotomous data (amputation rate and ulcer healing rate, numbers of patients with improvement in blood flow parameters, and number of limbs with new collaterals), we used risk ratio (RR) to measure outcome estimates on the same scale. For continuous data (rest pain score, ABI score, TcO_2 reading, PFWD, PFWT), we pooled measures at a similar time point using mean difference (MD). If pooled analyses were not possible, we reported results of the studies individually.

Unit of analysis issues

We used each individual patient as our unit of analysis when possible. However, some studies reported their results using the limbs as the unit of analysis without adjusting results to account for non-independence between two limbs of the same patient. None of the studies reported the number of patients with one or two limbs included in the analysis, making it impossible for us to adjust the results. We therefore reported the results of those studies unadjusted but undertook a sensitivity analysis when we encountered a mixture of studies with patients and limbs as the unit of analysis, to assess the impact of pooled results after exclusion of studies that reported results using the limbs as the unit of analysis.

We did not include cluster-RCTs and cross-over studies.

Dealing with missing data

We assessed the dropout rate of each study and determined whether an intention-to-treat analysis was performed. To assess whether the dropout rate was worrisome, we inspected event rates for intervention and comparison groups. We then used a 'worst-case scenario' method for the primary outcomes (Guyatt 1993). For instance, with negatively worded outcomes (such as mortality), for a trial that favoured the intervention group we assumed that all dropouts from the intervention group had developed the outcome, and that all dropouts from the comparison group had not developed the outcome. We then analysed the results to see if such an assumption changed the direction of the results (e.g. from favouring the intervention group to favouring the comparison group). If so, we considered the dropout rate to be worrisome and made a corresponding note in the table that corresponded to characteristics of the study and its accompanying risk of bias assessment table under the heading of 'Incomplete outcome data'. We made the reverse assumption when a trial favoured the comparison group.

Assessment of heterogeneity

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

1. Aetiology of the disease.
2. Baseline characteristics of participants (age, gender, race and ethnicity, comorbidity group).
3. Nature of the intervention (different regimens of implantation, different types of cells, different preparations and doses of cells implanted).
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 were defined as studies with unclear or no allocation concealment; studies in which participants, caregivers, or investigators were not blinded, or in which blinding was unclear).

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

Assessment of reporting biases

We specifically assessed publication bias in our review using a funnel plot if 10 or more studies were included in the analysis. If publication bias was implied by significant asymmetry of the funnel plot, we would have included a statement in our results with a corresponding note of caution in our discussion.

Data synthesis

We used Review Manager to perform meta-analysis of the included studies (RevMan 5.3). We used a fixed-effect model unless we found significant heterogeneity, in which case we employed the strategies as outlined in the previous section on assessment of heterogeneity. For data management, we followed the strategies detailed in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). Our primary data analyses followed the intention-to-treat principle, namely, we used the original number of participants allocated to each study arm as the denominator in subsequent analyses.

Subgroup analysis and investigation of heterogeneity

Had sufficient data been available, we would have performed the following subgroup analyses for studies describing patients:

1. with different severity of CLI (e.g. rest pain vs tissue loss);
2. with different aetiology of CLI (e.g. atherosclerosis obliterans (ASO) vs thromboangiitis obliterans (TAO));
3. with and without significant comorbidity (e.g. smoking, diabetes, hypercholesterolaemia, hypertension);
4. with different age groups;
5. with different genders;
6. with different races or ethnicities;
7. injected with cells obtained via different preparation techniques (e.g. fresh vs cultured, non-selected vs selected);
8. injected with different doses of cells;
9. injected with single versus a combination of cell-based products; and
10. implanted via different routes;

or when:

1. the intervention was administered with and without co-intervention; or
2. studies were undertaken in patients with different follow-up periods.

Sensitivity analysis

We performed sensitivity analysis for each outcome that we extracted and pooled from a mixture of studies with patients and limbs as the unit of analysis to assess the impact of the pooled results after exclusion of studies that reported results using the limbs as the unit of analysis.

Had sufficient data been available, we would have performed the following additional sensitivity analyses to assess the impact of excluding studies based on the following criteria.

1. Significant or worrisome 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 took the following criteria to indicate a significant risk of bias: studies with unclear or no allocation concealment; and studies in which participants, caregivers, or investigators were not blinded, or in which blinding was unclear.

'Summary of findings'

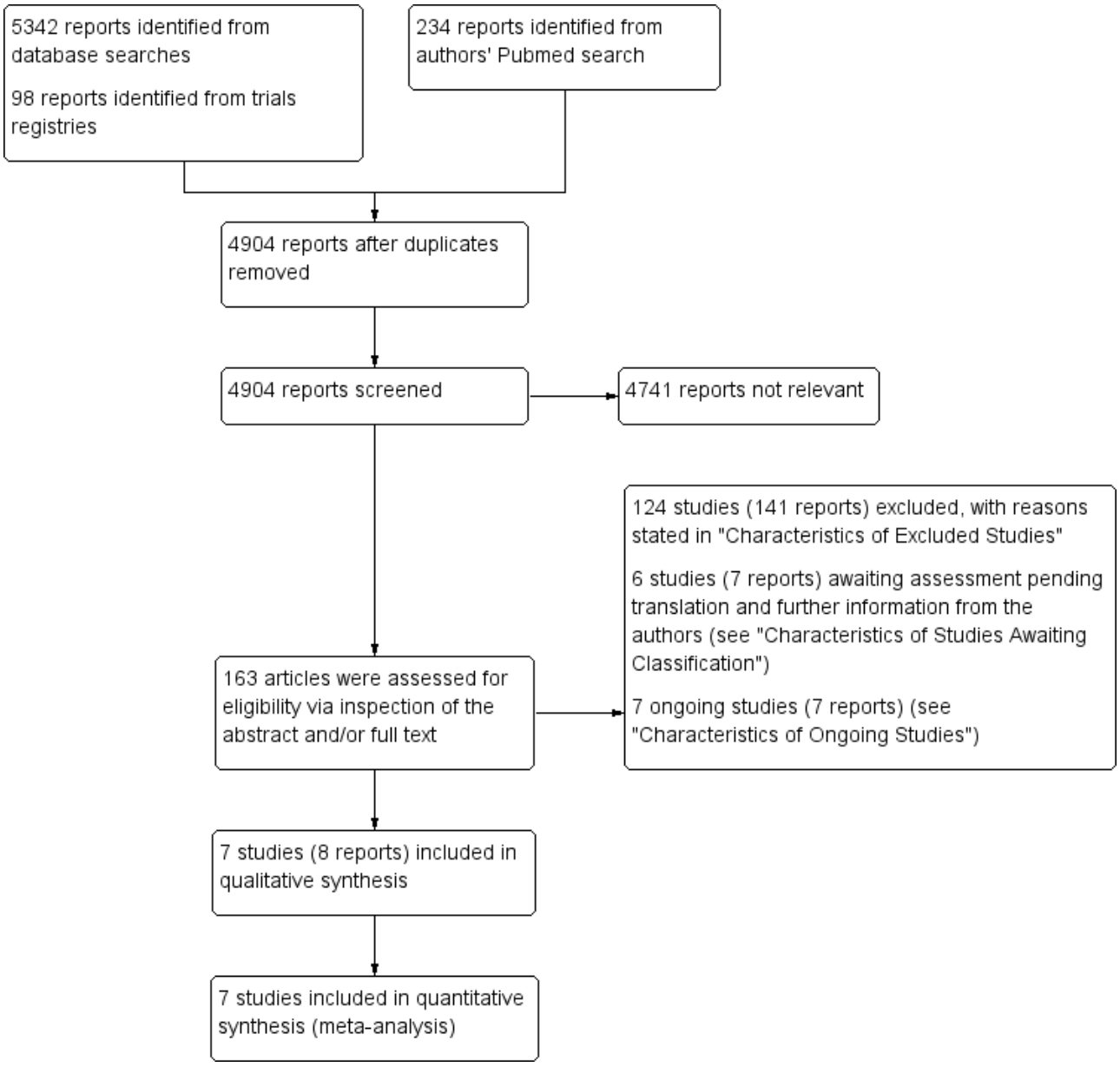
We presented in 'Summary of findings' (SoF) tables the main findings of this review concerning quality of evidence, magnitude of effects of the interventions examined, and sum of available data on the primary outcomes in the review, namely, all-cause mortality, amputation rate, and wound/ulcer healing, as well as the major secondary outcomes (i.e. reduction in rest pain and improvement in lower limb perfusion as measured by ABI and TcO_2) (Schünemann 2011), according to Higgins 2011. We used the web-based GRADEpro software (gdt.guidelinedevelopment.org) to generate the SoF table (Schünemann 2011a). In generating the SoF table, we took the median control group event rate for the outcome as showing 'moderate risk'.

RESULTS

Description of studies

A summary of our search results is shown in Figure 1.

Figure 1. Study flow diagram.



Results of the search

We included in this review seven RCTs from eight published articles (Gu 2008; Huang 2007; Klepanec 2012; Losordo 2012; Lu 2011; Van Tongeren 2008; Zhang 2009). We excluded 124 studies (141 reports) on the basis of study design, characteristics of participants, types of interventions, characteristics of cell treatment, and absence of outcome data (see Characteristics of excluded studies). We identified six studies (seven reports) awaiting classification (Gurunathan 2009; Korymasov 2009; Molavi 2016; NCT00595257; NCT00987363; NCT02993809): three studies are clinical trial protocols without outcome data (NCT00595257; NCT00987363; NCT02993809), one pilot study provided preliminary reporting of outcome data based on small sample size (Molavi 2016), one article provided incomplete reporting of outcome data (Gurunathan 2009), and one article written in the Russian language could not be assessed fully by the time of writing the review, and we are still awaiting its translated full-text version (Korymasov 2009). (See Characteristics of studies awaiting classification.) We identified seven ongoing studies (seven reports) (NCT00311805; NCT00753025; NCT01257776; NCT01408381; NCT01446055; NCT01745744; NCT02454231. (See Characteristics of ongoing studies.) Recent review articles and meta-analyses did not yield additional relevant clinical trials. We did not identify new trials by scanning the reference lists of included clinical trials.

Included studies

Characteristics of included studies

We included seven RCTs that were conducted in four countries, including China (four studies), USA, Netherlands, and Slovakia (one study each). Six studies were single-centre RCTs, and one was a multi-centre RCT (see Characteristics of included studies).

Characteristics of participants

The included studies were conducted between 2003 and 2010 and enrolled a total of 359 participants. The number of participants recruited in the individual RCTs ranged from 16 in Losordo 2012 to 150 in Huang 2007. The mean age of participants ranged from 61.8 years to 69.8 years. Atherosclerosis obliterans and thromboangiitis obliterans were the two most common aetiologies of CLI. Most studies reported the baseline demographics of participants, including age and gender, comorbidities (diabetes mellitus, renal failure, cerebral and coronary artery disease), and severity of limb ischaemia as comparable between groups. One study recruited only diabetic patients (Lu 2011). In two studies, researchers used each of the two limbs as one experimental arm, resulting in administration of the two interventions to two different limbs of the same patient (Lu 2011). Median follow-up ranged from one to twelve months after cell implantation.

Characteristics of cell treatment

The included RCTs used essentially two sources of autologous stem cells: bone marrow and peripheral blood. Five of the seven included RCTs used mononuclear cells (MNCs) harvested and separated manually from bone marrow by density gradient centrifugation on Ficoll-Hypaque and implanted into affected limbs without prior manipulation; study authors denoted these as BM-MNCs. Three RCTs used progenitor cells isolated from patients' peripheral blood via leukapheresis following administration of 5 µg/kg/d of G-CSF for four or five days (termed 'mobilised PBSCs', or 'mPBSCs') for implantation (Huang 2007; Losordo 2012; Zhang 2009). One RCT further enriched the leukapheresis product (mPBSC) for CD34+ cells using a magnetic cell selection system before implantation of CD34+ cells (Losordo 2012). Bone marrow cells were cultured to generate mesenchymal stem cells (MSCs), and one RCT used these cells (BM-MSCs) (Lu 2011).

One RCT evaluated the effect of cell dose on clinical outcomes. In this study, researchers randomised patients and treated them with 1×10^6 (high dose) and 1×10^5 (low dose) autologous CD34+ cells/kg (Losordo 2012). No standard definition of high versus low cell dose is provided in the literature. However, we considered a standard definition not critical for determining clinical outcomes in the present review because only a single RCT performed this comparison, and pooled analysis was not feasible.

Intramuscular (IM) cell implantation was the route most commonly employed. Other methods of cell administration were intra-arterial (IA) - in Klepanec 2012 - and combined IM plus IA - in Van Tongeren 2008. IM cell implantation was usually performed via multiple injections into the gastrocnemius muscle, and IA cell infusion was usually done through the femoral artery of the affected lower limb.

Trials provided four major categories of interventions.

1. Comparison between BM-MNCs (104 participants) and mPBSCs (98 participants) in two RCTs (Huang 2007; Zhang 2009).
2. Comparison between BM-MNCs (20 participants) and BM-MSCs (21 participants) in one RCT (Lu 2011).
3. Comparison between high cell dose (9 participants) and low cell dose (7 participants) implanted into the affected limb in one RCT (Losordo 2012).
4. Comparison between IM (52 participants) and IA or IA plus IM (48 participants) cell implantation in three RCTs (Gu 2008; Klepanec 2012; Van Tongeren 2008).

Assessment of outcomes

For efficacy analysis, the participants in the seven included RCTs were pooled in groups according to source, type, dose, and route of delivery of MNCs/stem cells. We extracted the outcome data from individual RCTs and incorporated them into the meta-analysis according to the following groups: BM-MNCs versus mPBSCs, BM-MNCs versus BM-MSCs, high cell dose versus low cell dose, and IM implantation route versus IA infusion route.

Most included RCTs assessed four efficacy outcomes almost exclusively, including amputation rate (six studies), rest pain (six studies), ulcer healing (three studies), and ABI (six studies).

All RCTs assessed subjective symptoms (rest pain) and objective surrogate indexes of blood flow in the lower limbs (wound healing, ABI, TcO_2, PFWD, PFWT) before cell implantation and regularly thereafter, ranging from two weeks to twelve months. The researchers determined the number and nature of amputations at the end of the study. They determined wound or ulcer healing weekly by (1) ulcer healing rate (number of participants whose ulcers healed divided by the total number of participants with ulcers in a particular group) and (2) changes from baseline in ulcer size and area as measured by grid maps, acetate tracings, and digital planimetry. Trialists assessed rest pain using two types of numerical rating pain scales ranging from 0 (completely resolved without analgesics) to 4 points (severe pain unresolved with analgesics) (Lu 2011), or from 1 (least pain) to 10 points (greatest pain) on a visual analogue scale (VAS). They measured resting ABI using a laser Doppler at room temperature according to standard protocol. Study authors measured TcO_2 with a TCM400 Mk2 monitor (Klepanec 2012), and they assessed walking capacity reflected by a change in walking distance (metres), walking duration (minutes), and walking speed (miles per hour or mph) at a constant speed on a treadmill, or at the same ground with no inclination. Investigators determined collateral vessel formation of the lower limbs using magnetic resonance angiography (MRA) (Lu 2011), or via subtraction angiography (Klepanec 2012), before and at three to six months after cell implantation. They assessed angiographic scores of blood vessel images as +0 (no collateral formation), +2 (moderate collateral circulation), and +3 (abundant collateral circulation) (Drescher 2006; Klepanec 2012). They paid specific attention to detecting potential adverse effects resulting from cell harvesting and implantation during follow-up visits.

Excluded studies

In total, we excluded 141 reports of 124 studies on the basis of study design, patient characteristics, types of interventions, characteristics of cell treatment, and incomplete outcome data (see Characteristics of excluded studies).

1. Forty RCTs investigated effects of cell-based treatment versus standard medical therapy (SMT), including BM-MNCs versus SMT (Amann 2008; Arai 2006; Barć 2006; Benoit 2011; Dou 2015; Gu 2017;Guo 2018; Iafrati 2016; Li 2013; NCT00539266; NCT01049919; NCT01245335; Peeters 2016; Pignon 2017; Prochazka 2010; Tateishi-Yuyama 2002; Teraa 2014; Teraa 2015; Walter 2011; Zhou 2017), BM-MSCs versus SMT (Dash 2009; Debin 2008), concentrated bone marrow aspirate versus

SMT (Murphy 2017; NCT00434616; Wang 2017), peripheral blood-derived stem cell therapy versus SMT (Doudar 2013; Huang 2005; Mohammadzadeh 2013; NCT00922389; Niven 2017;Ohtake 2017; Ozturk 2012; Szabo 2013), Ixmyelocel-T versus SMT (Powell 2012), Rexmyelocel-T versus SMT (NCT03174522), CD133+ cells versus SMT (Zhang 2016), aldehyde dehydrogenase (ALDH) bright cells versus SMT (Perin 2017), vascular endothelial growth factor (VEGF) 165 gene-modified PB CD34+ cells versus SMT (Zhou 2017a), granulocyte macrophage-stimulating factor (GM-CSF) versus placebo (NCT03304821), and angiogenic cell precursors (ACPs) versus SMT (NCT01584986).

2. Forty-two studies were single-arm studies without a comparator/control arm. These studies investigated the safety and efficacy of bone marrow cells (Chochola 2008; Cobellis 2010; Duong 2008; Franz 2011; Gabr 2011; Heo 2016;Higashi 2004; Iso 2010; Kolvenbach 2010; Kondo 2018;Maione 2013; Malyar 2014; Matoba 2009; Motukuru 2008; Murphy 2011; NCT00306085; Nizankowski 2005; Ponemone 2017; Ruiz-Salmeron 2011; Saito 2007; Schiavetta 2012; Wester 2008; Yanishi 2017), PBSCs (Hoshino 2007; Ishida 2005; Kawamoto 2009; Kinoshita 2012; Lara-Hernandez 2010; Madaric 2016), PB-MNCs (Amato 2012; Moriya 2009), adipose tissue stem cells (Bura 2014; Darinskas 2017;Kondo 2016; Lee 2012), BM-MSCs plus BM-MNCs (Lasala 2010), BM-MSCs (Mohamed 2017), peripheral blood CD133+ cells (Arici 2015; Burt 2010), circulating blood-derived progenitor cells (Frogel 2017; Lenk 2005), and venous endothelial and smooth muscle cells, called MultiGeneAngio (MGA) (Grossman 2016).
3. Eleven studies were comparative non-randomised studies. These studies investigated the effects of BM-MNCs versus mPBSCs (Dubsky 2013; Gu 2007; Kamata 2007; Matsui 2003), BM-MNCs versus control (Bartsch 2007; Cobellis 2008; Idei 2011; Napoli 2008), PB-MNCs versus control (De Angelis 2014), circulating blood-derived progenitor cells versus control (Nemcova 2017), and low versus high cell dose used for implantation (Gu 2006).
4. In three studies, the study populations were not CLI patients (Bing 2009; Holzinger 1994; Subramaniyam 2009).
5. Nine studies used allogeneic mesenchymal stem cells (MSCs) instead of autologous MSCs (Das 2013; Du 2017;Gupta 2013; Gupta 2017; Majumdar 2015;NCT02336646; NCT03339973; Wang 2018;Wijnand 2018).
6. In one study, the agent used in the intervention group was not a cell-based product (Rajagopalan 2003).
7. Three studies investigated the therapeutic effects of growth factors (GM-CSF and G-CSF) instead of cell-based products (Choi 2012; Poole 2013; Zafarghandi 2010).
8. Two studies compared cell treatment versus treatment with non-cell-based products (Takagi 2011; Wang 2014).
9. Two studies investigated specialised cell-based products derived via specific isolation and culture methods (Kirana 2012; Perin 2011). Significant variation in the final product injected into participants precluded inclusion of these studies in our systematic review.
10. Three studies co-administered two different sources of autologous stem cells into the affected limb: BM-MNCs combined with mPBSCs (Zhao 2008), BM-MSCs with BM-MNCs versus BM-MNCs alone (Harunarashid 2016), and venous endothelial cells (ECs) combined with venous smooth muscle cells (SMCs) (Flugelman 2017).
11. One study compared the clinical outcomes of CLI patients treated with one type of stem cell (BM-MNC) that was separated via two different methods (Hernandez 2007).
12. One study compared the clinical outcomes of CLI patients treated with BM-MNCs versus mPBSCs by performing a pooled analysis using data from two previous cohort studies (Onodera 2011).
13. Two studies investigated the count and phenotype of cells used for implantation and did not study the clinical outcomes after cell implantation (Capiod 2009; Smadja 2012).
14. One study investigated the thrombogenicity of the transplanted cell and did not study clinical outcomes after cell implantation (Tournois 2015).
15. One study investigated the number of endothelial progenitor cells (EPCs) in PAD patients and did not study clinical outcomes after cell implantation (Afan 2015).
16. One study investigated the level of asymmetric dimethylarginine (ADMA) and changes in oxidative stress in patients with CLI after BM-MNC therapy and did not study clinical outcomes of cell treatment (Madaric 2017).
17. One study investigated the safety and efficacy of hyaluronic acid (HA) combined with BM-MNCs for patients with PAD (NCT03214887).

Risk of bias in included studies

See 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.

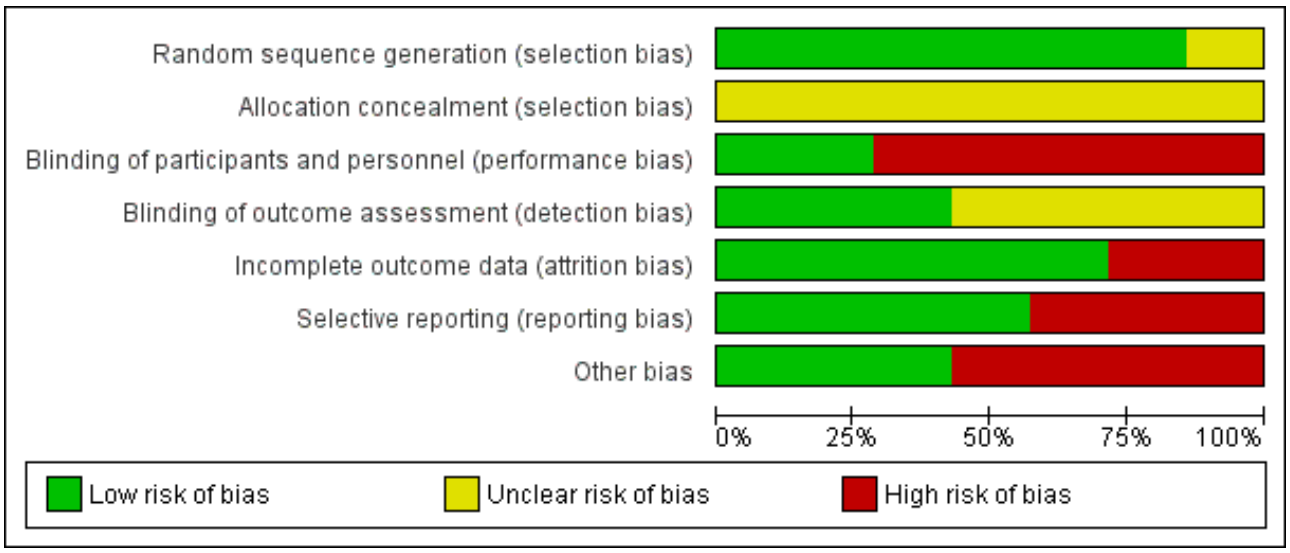
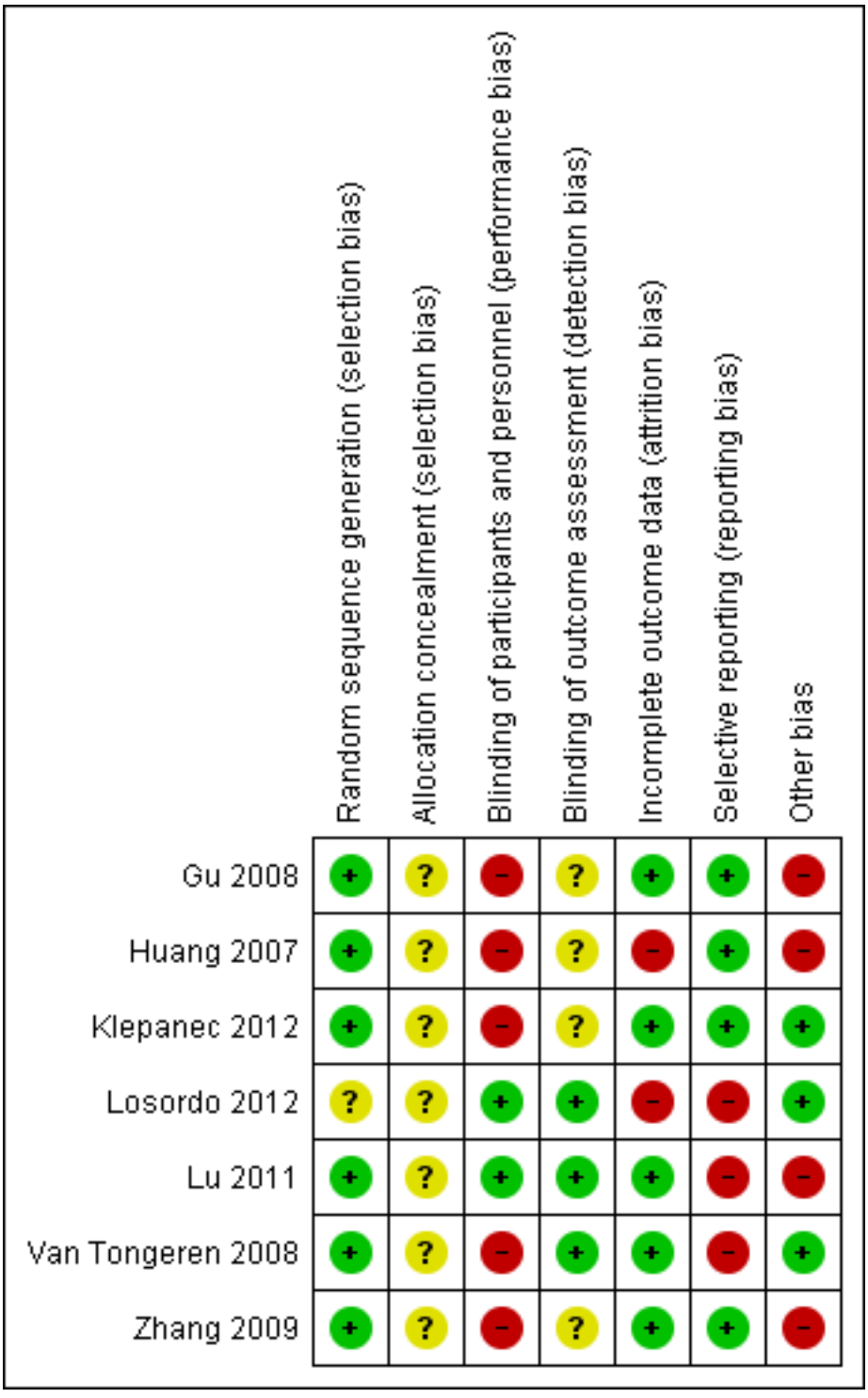


Figure 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.



Allocation

We judged six studies as having low risk of bias for random sequence generation (Gu 2008; Huang 2007; Klepanec 2012; Lu 2011; Van Tongeren 2008; Zhang 2009). One RCT had unclear risk of bias for random sequence generation, as study authors did not explicitly state the method of sequence generation used (Losordo 2012). We graded allocation concealment as causing unclear risk in all studies because papers provided insufficient information.

Blinding

Five studies had high risk of performance bias. In two RCTs, both physicians and patients were unblinded to the different techniques used in autologous cell collection (Huang 2007; Zhang 2009), and in

three studies, both physicians and patients were unblinded to the different routes of cell implantation employed (Gu 2008; Klepanec 2012; Van Tongeren 2008). We deemed the remaining two included studies to have low risk of performance bias (Losordo 2012; Lu 2011).

Four studies had unclear detection bias, as study authors did not mention whether outcome assessors were blinded to patients' assigned groups (Gu 2008; Huang 2007; Klepanec 2012; Zhang 2009). We deemed the remaining three included studies to have low risk of detection bias (Losordo 2012; Lu 2011; Van Tongeren 2008).

Incomplete outcome data

One study reported that six participants (two in one group and four in another) were excluded before the implantation and 10 participants (four in one group and six in another) discontinued after amputation (Huang 2007). Another study reported that eight participants (28.6%) did not complete the 12-month study period (Losordo 2012). We judged both studies to have high risk of attrition bias owing to unequal numbers of dropouts between groups and high overall dropout rates. The remaining studies had low risk of bias in this domain.

Selective reporting

We judged three studies as having high risk of reporting bias because study authors provided incomplete outcome information (Losordo 2012; Lu 2011; Van Tongeren 2008); we considered the remaining studies to have low risk of reporting bias.

Other potential sources of bias

We screened for other potential sources of bias including extreme baseline imbalance and unit of analysis issues. We identified four studies with unit of analysis issues (Gu 2008; Huang 2007; Lu 2011; Zhang 2009). We judged the remaining included studies to have low risk of other potential sources of bias. Cell doses for MNCs and stem cells cannot be equated, as < 0.01% of MNCs consisted of stem cells. Therefore, an analysis of cell doses would have been intrinsically biased.

Effects of interventions

See: **Summary of findings for the main comparison** BM-MNCs compared to mPBSCs for critical lower limb ischaemia; **Summary of findings 2** BM-MNCs compared to BM-MSCs for critical lower limb ischaemia; **Summary of findings 3** High cell dose compared to low cell dose for critical lower limb ischaemia; **Summary of findings 4** IM injection compared to IA injection for critical lower limb ischaemia

Primary outcomes

1. All-cause mortality

Three of the nine included RCTs involving a total of 100 CLI participants reported a total of nine deaths (9%) during the study follow-up period (Gu 2008; Klepanec 2012; Van Tongeren 2008). None of the included studies reported all-cause mortality according to treatment group. Causes of death were heart failure in four participants (Gu 2008; Klepanec 2012), myocardial infarction in two participants (Klepanec 2012; Van Tongeren 2008), and respiratory tract infection in three participants (Klepanec 2012; Van Tongeren 2008).

2. Amputation rate

Comparison 1: BM-MNCs vs mPBSCs

A single study in this comparison showed no clear difference in amputation rates between the two groups (risk ratio (RR) 1.54, 95% confidence interval (CI) 0.45 to 5.24; 150 participants; low-quality evidence) (Analysis 1.1) (Huang 2007).

Comparison 2: BM-MNCs vs BM-MSCs

Only a single study (37 participants) performed this comparison, reporting zero cases of amputation in both groups (Analysis 2.1) (Lu 2011).

Comparison 3: high cell dose vs low cell dose

The only study in this comparison showed no clear difference in amputation rates between high cell dose and low cell dose groups (RR 3.21, 95% CI 0.87 to 11.90; 16 participants; very low-quality evidence) (Analysis 3.1) (Losordo 2012).

Comparison 4: route of implantation: IM vs IA

Three studies involving a total of 95 CLI participants showed no clear difference in amputation rates between the different routes of cell implantation (RR 0.80, 95% CI 0.54 to 1.18; 95 participants; I^2 = 48%; low-quality evidence) (Analysis 4.1) (Gu 2008; Klepanec 2012; Van Tongeren 2008).

3. Wound/ulcer healing

Included studies reported wound/ulcer healing as the number of participants with improvement or healing of ulcers or change in ulcer size.

Comparison 1: BM-MNCs vs mPBSCs

The single study in this comparison showed no clear difference in numbers of participants with healing ulcers between the two treatment groups (RR 0.89, 95% CI 0.44 to 1.83; 49 participants; low-quality evidence) (Analysis 1.2) (Huang 2007).

For the change in ulcer size outcome, Huang 2007 reported no clear change between the two treatment groups (mean difference (MD) -1.06 cm^2, 95% CI -5.35 to 3.23; 49 participants) (Analysis 1.3).

Comparison 2: BM-MNCs vs BM-MSCs

The only study in this comparison showed that the number of participants with healing ulcers was higher in the BM-MSC group than in the BM-MNC group (RR 2.00, 95% CI 1.02 to 3.92; 22 participants; P = 0.04; moderate-quality evidence) (Analysis 2.2) (Lu 2011).

Comparison 3: high cell dose vs low cell dose

The single study in this comparison did not report on this outcome (Losordo 2012).

Comparison 4: route of implantation: IM vs IA

A single included study showed no clear difference in the numbers of participants with healing ulcers between IM and IA cell implantation (RR 1.13, 95% CI 0.73 to 1.76; 41 participants; low-quality evidence) (Analysis 4.2) (Klepanec 2012).

Secondary outcomes

1. Reduction in rest pain

Reduction in rest pain is reported either as the number of participants with reduction in rest pain score or as a mean reduction in rest pain score.

Comparison 1: BM-MNCs vs mPBSCs

Two studies involving a total of 202 CLI participants reported rest pain score of any magnitude at three to six months from baseline for participants treated with either BM-MNCs or mPBSCs (Huang 2007; Zhang 2009). We could not pool data obtained from Huang 2007 with data from Zhang 2009 because Huang 2007 reported rest pain data as mean reduction in rest pain score, but Zhang 2009 reported data as the number of participants with reduction in rest pain. Zhang 2009 showed no clear difference in pain relief between the two treatment groups (RR 0.99, 95% CI 0.93 to 1.06; 104 participants; moderate-quality evidence) (Analysis 1.4). In contrast, Huang 2007 showed a reduction in rest pain score 12 weeks after mPBSC injection (MD -0.57, 95% CI -0.90 to -0.24; 150 participants) (Analysis 1.5).

Comparison 2: BM-MNCs vs BM-MSCs

The only study in this comparison did not show a difference in pain relief in ischaemic limbs between the two groups (MD 0.00, 95% CI -0.61 to 0.61; 37 participants; moderate-quality evidence) (Analysis 2.3) (Lu 2011).

Comparison 3: high cell dose vs low cell dose

Losordo 2012 did not report on this outcome.

Comparison 4: route of implantation: IM vs IA

A single included study showed no difference in the numbers of participants with reduction in rest pain between IA and IM routes of implantation (RR 1.22, 95% CI 0.91 to 1.64; 32 participants; low-quality evidence) (Analysis 4.3) (Gu 2008).

2. Improvement in lower limb perfusion as measured by improvement in ankle-brachial pressure index (ABI)

Dormandy 2000 defined improvement in ABI as an increase in ABI values greater than 0.1 in the treated limb from baseline value according to the standard assessment of interventional therapy for PAD. Pooled estimates of ABI values extracted from individual RCTs are presented as RRs of the number of participants with improvement in ABI or MD of the ABI score after intervention from baseline.

Comparison 1: BM-MNCs vs mPBSCs

Two studies reported change in ABI at 4 and 12 weeks from baseline in 202 participants treated with either BM-MNCs or mPBSCs (Huang 2007; Zhang 2009). Pooled analysis was not feasible owing to differences in units used to report a change in ABI in these studies. Huang 2007 showed that improvement in ABI was better in the mPBSC group than in the BM-MNC group (MD -0.06, 95% CI -0.09 to -0.03; 150 participants; Analysis 1.7), and Zhang 2009 showed no improvement in ABI between the two treatment groups (RR 1.00, 95% CI 0.71 to 1.40; 104 participants; moderate-quality evidence; Analysis 1.6).

Comparison 2: BM-MNCs vs BM-MSCs

Only a single study performed this comparison. Lu 2011 showed that participants who received BM-MSCs had higher ABI than those given BM-MNCs (MD 0.05, 95% CI 0.01 to 0.09; 37 participants; low-quality evidence) (Analysis 2.4).

Comparison 3: high cell dose vs low cell dose

Losordo 2012 did not report on this outcome.

Comparison 4: route of implantation: IM vs IA

Gu 2008 did not show a clear difference in the number of participants with improvement in ABI following treatment via the IA or IM route of implantation (RR 0.93, 95% CI 0.43 to 2.00; 35 participants; very low-quality evidence) (Analysis 4.4).

Klepanec 2012, who reported ABI score, did not show a clear difference in ABI scores between different routes of implantation (MD -0.17, 95% CI -0.37 to 0.03; 27 participants) (Analysis 4.5).

3. Improvement in lower limb perfusion as measured by improvement in transcutaneous oxygen tension (TcO_2)

We have presented pooled estimates of TcO_2 values extracted from individual RCTs as RR of the number of participants with improvement in TcO_2 readings or mean difference (MD) in the TcO_2 reading from baseline.

Comparison 1: BM-MNCs vs mPBSCs

One study involving a total of 150 CLI participants reported an increase in TcO_2 readings in the mPBSC group compared with the BM-MNC group (MD 1.70 mmHg, 95% CI 0.41 to 2.99; 150 participants; low-quality evidence) (Analysis 1.8) (Huang 2007).

Comparison 2: BM-MNCs vs BM-MSCs

Only a single study performed this comparison (Lu 2011). This study showed an increase in TcO_2 readings in the BM-MSC group compared with the BM-MNC group (MD 8.00 mmHg, 95% CI 3.46 to 12.54; 37 participants; low-quality evidence) (Analysis 2.5).

Comparison 3: high cell dose vs low cell dose

Losordo 2012 did not report on this outcome.

Comparison 4: route of implantation: IM vs IA

Pooled analysis of two studies did not show a difference in the numbers of participants with improved TcO_2 readings between IM and IA cell implantation groups (RR 1.22, 95% CI 0.86 to 1.72; 62 participants; $I^2 = 0\%$; very low-quality evidence) (Analysis 4.6) (Gu 2008; Klepanec 2012).

4. Improvement in ischaemic symptoms as assessed by improvement in pain-free walking distance (PFWD) and pain-free walking time (PFWT)

Only two studies reported PFWD or PFWT (Huang 2007; Lu 2011).

Comparison 1: BM-MNCs vs mPBSCs

The only study that performed this comparison did not show a clear difference in mean PFWD between the two treatment groups (MD 33.05 metres, 95% CI -37.69 min to 103.79 min; 150 participants) (Analysis 1.9) (Huang 2007).

Comparison 2: BM-MNCs vs BM-MSCs

The only study that performed this comparison did not show a clear difference in mean PFWT between the two treatment groups (MD 0.6 min, 95% CI -0.01 min to 1.21 min; 37 participants) (Analysis 2.6) (Lu 2011).

5. Improvement in vascularity and blood supply to the ischaemic limb as measured by collateral vessel formation

CLI participants in the included studies were subjected to angiography before and at three to six months after cell implantation to identify new collateral vessel formation in treated and control limbs.

Comparison 1: BM-MNCs vs PBSCs

Huang 2007 and Zhang 2009 did not report on this outcome.

Comparison 2: BM-MNCs vs BM-MSCs

The only study that performed this comparison showed a higher number of participants with new collaterals at 24 weeks in the BM-MSC-treated group than in the BM-MNC-treated group (RR 1.98, 95% CI 1.12 to 3.49; 37 participants) (Analysis 2.7) (Lu 2011).

Comparison 3: high cell dose vs low cell dose

Losordo 2012 did not report on this outcome.

Comparison 4: route of implantation: IM vs IA

The only study in this comparison showed no difference in the numbers of participants with collateral formation between different routes of cell implantation (RR 0.91, 95% CI 0.40 to 2.11; 15 participants) (Analysis 4.7) (Gu 2008).

6. Adverse events and safety

None of the included RCTs reported adverse events for the intervention and control groups separately, hence we were unable to undertake meta-analyses for this outcome. Nonetheless, most of the studies (six out of seven RCTs) reported that in general, cell-based therapy was well tolerated without significant and severe adverse events related to bone marrow aspiration and cell implantation into the affected limb. The individual studies reported no infection and no biochemical or immunological reactions among participants who had received cell implantation. However, reported data on the long-term safety of cell-based therapy were lacking.

As pooled analyses were not possible for this outcome, we describe adverse effects reported by the individual studies according to the following comparisons.

Comparison 1: BM-MNCs vs mPBSCs

One study reported that cell implantation in both groups did not induce local inflammatory reaction or oedema of the lower limbs (Huang 2007). Study results show that the most common adverse events during G-CSF mobilisation in mPBSC were bone pain (13.2%; 10/76) and lassitude (5.3%; 4/76), but no participants were required to withdraw from the mobilisation procedure or required special treatment. No implantation-related complications were observed through electrocardiograms, liver and kidney function tests, and urine tests during the 12-week follow-up period. Researchers followed up 43 participants in the mPBSC group and 41 in the BM-MNC group over a 12-month period and noted no implantation-related complications during this time. Another study reported no significant adverse effects in all participants (Zhang 2009).

Comparison 2: BM-MNCs vs BM-MSCs

One of the three RCTs that used BM-MSCs reported detailed data on the safety profile of cell-based treatment (Lu 2011). Study authors observed minimal bleeding at the posterior iliac crest after bone marrow aspiration in one and two participants in the BM-MSC and BM-MNC groups, respectively. In addition, three limbs in the BM-MSC group and two in the BM-MNC group were reported as painful in the short term after cell implantation (Lu 2011).

Comparison 3: high cell dose vs low cell dose

One RCT reported 60 serious adverse events (SAEs) in 22 participants (78.6%), 59 of which occurred after IM cell implantation, and one during G-CSF mobilisation (Losordo 2012). Only two SAEs were considered possibly study-related: one participant developed moderate hypotension and another experienced worsening ischaemia of the implanted limb. One participant in the control (placebo) group developed acute myocardial infarction four to five months after randomisation. Adverse events did not necessitate withdrawal of participants from the study.

Comparison 4: route of implantation: IM vs IA

Individual studies reported no adverse events related to implantation of cells and no difference in the occurrence of adverse events among participants who received cell treatment via IM and IA routes, indicating that both routes of cell delivery were safe and feasible (Gu 2008; Klepanec 2012; Van Tongeren 2008). Eight deaths due to cardiovascular and respiratory events were not related to cell implantation. Meta-analysis was not feasible, as these studies did not provide comparative adverse events data for the different routes of cell implantation.

Subgroup analysis

We were unable to perform any subgroup analyses for each outcome describing participants with different severity and aetiology of CLI, participants with or without significant comorbidity, and participants of different age groups, gender, and ethnicity, as we could not obtain separate data according to these pre-specified criteria from the included studies under each comparison.

Sensitivity analysis

We were unable to perform sensitivity analyses for significant dropout rates and significant methodological issues identified in the assessment of risk of bias, as insufficient data were available.

We conducted sensitivity analyses for the relevant outcomes to identify if pooled estimates of outcomes would change with inclusion and exclusion of studies in which limbs rather than participants were used as units of analysis.

Outcome: amputation rate

Comparison: IM versus IA implantation: results show no substantial change after one study that used limbs as the unit of analysis - Gu 2008 - was excluded (before exclusion: RR 0.80, 95% CI 0.54 to 1.18; three RCTs, 95 participants; I^2 = 48%; after exclusion: RR 0.86, 95% CI 0.59 to 1.26; two RCTs, 60 participants; I^2 = 61%) (Analysis 4.1).

Outcome: improvement in lower limb perfusion as measured by improvement in transcutaneous oxygen tension (TcO_2)

Number of participants with improved TcO_2 reading

Comparison: IM versus IA implantation: results show no substantial change after one study that used limbs as the unit of analysis - Gu 2008 - was excluded (before exclusion: RR 1.22, 95% CI 0.86 to 1.72; two RCTs, 62 participants; I^2 = 0%; after exclusion: RR 1.19, 95% CI 0.63 to 2.26; one RCT, 27 participants) (Analysis 4.6).

DISCUSSION

Summary of main results

The main findings of the review show no clear differences in almost all comparisons between different cell sources and implantation regimens for the major clinical outcomes of all-cause mortality, amputation rate, improvement in rest pain, number of participants with healing ulcer, and improvement in ankle-brachial index (ABI). However, underpowered analyses for all comparisons precluded any clear and firm conclusions.

Of note, most of the included trials were not specifically designed to compare adverse events or long-term safety between the various sources and types of cell-based regimens. Hence review authors found a paucity of comparative adverse event data between treatment groups, confounded by short follow-up duration. Nonetheless, the randomised controlled trials (RCTs) included in this review support the short–term safety and feasibility of autologous implantation of stem cells obtained from bone marrow or peripheral blood into ischaemic limbs. The most common adverse events following bone marrow harvesting were short-term episodes of slight pain, bleeding, and haematoma at the bone marrow aspiration site that did not require specific intervention. Apart from granulocyte colony-stimulating factor (G-CSF)-induced transient bone pain that did not require special treatment, mobilisation of mobilised peripheral blood stem cells (mPBSCs) was well tolerated. Researchers reported no local adverse reactions at the cell implantation site. Individual studies reported no infection and no biochemical or immunological reactions among participants following cell implantation.

Although each individual RCT described no difference in the occurrence of adverse events related to route of implantation and cell dose, the small sample size and lack of comparative data in these studies precluded any convincing conclusions on the overall safety of cell-based therapy. In addition, evidence on the long-term safety profile of different types of cell-based products, namely, bone marrow-mononuclear cells (BM-MNCs), bone marrow-mesenchymal stem cells (BM-MSCs), mPBSCs, and peripheral blood-mononuclear cells (PB-MNCs), is lacking.

Overall completeness and applicability of evidence

In terms of the applicability of our review, the current mainstay of treatment for critical limb ischaemia (CLI) has been endovascular or surgical revascularisation and pharmacological therapy. However, the presence of significant comorbidities and distal vessel disease renders many patients unsuitable for revascularisation, often resulting in amputation (Liew 2016). Exploring new strategies for revascularisation therapy is thus of major importance. The most recent published clinical practice guideline did not include cell-based therapy as one of the limb salvage treatment modalities (NGC 2012). Currently, cell-based therapy is at a preliminary stage of use or is being utilised mainly in research.

Each RCT included in this review contained a small sample size ranging from 16 to 150 participants and a relatively short follow-up period (range 1 to 12 months). The number of included RCTs was very small (seven), and comparisons for each outcome included only one to three studies.

Moreover, substantial variation in patient selection processes was evident, even within the relatively small population group (Sultan 2014). In particular, the criteria used to define 'CLI' and 'no-option' patients lacked uniformity. Trials examined varying degrees of lower limb ischaemia, ranging from intermittent claudication to non-healing ulcers and gangrene. Differences in the severity of peripheral arterial disease (PAD) may partly explain the differences in changes in blood flow parameters between these studies. The review includes a range of aetiologies for CLI, namely, atherosclerosis, Buerger's disease, acute embolism, and others. However, subanalysis for outcomes according to severity and aetiology of CLI was not possible, as individual RCTs did not provide such data. Risk factors such as diabetes mellitus and smoking that are known to cause worsening and progression of lower limb ischaemia were inhomogeneous between the included studies. For example, one RCT included only diabetic patients (Lu 2011), and another RCT excluded patients with poorly controlled diabetes mellitus, or those with proliferative diabetic retinopathy (Huang 2007).

Lack of uniformity in assessment, analysis, and reporting of efficacy endpoints between the individual RCTs precluded combining efficacy data for meta-analysis, so much so that the findings of this systematic review are based in part on results derived from one or two studies. For example, there were differences in the methods and units used to calculate, document, and report objective outcomes such as ABI and ulcer healing. Investigators used several different scoring systems to measure and interpret subjective outcomes including patient-perceived rest pain and pain-free walking distance (PFWD) and pain-free walking time (PFWT) in individual RCTs. Moreover, the timing of assessment of outcomes varied between studies. These important variations in efficacy data between studies resulted in insufficient data that could be pooled for meta-analysis. This was particularly important for comparisons performed with low number of patients in each arm.

The present systematic review is not able to demonstrate differences in efficacy and safety between the various sources and types of cell-based products for the following reasons. Most published trials on this topic compared cell-based treatment versus conventional, non-cell-based therapy. Our search revealed limited published RCTs that performed head-to-head comparisons between cells derived from different sources, prepared via different processing protocols, and administered through different doses and routes. We analysed two RCTs comparing BM-MNCs versus mPBSCs (Huang 2007; Zhang 2009), as well as one RCT comparing BM-MNCs versus BM-MSCs (Lu 2011). For the comparison BM-MNCs versus mPBSCs, meta-analysis was not feasible because of differences in the units used to report outcomes between the two cell treatment groups.

Limited evidence suggests clear differences in any clinical outcome whether cells were administered at high or low cell doses. Our

analysis based on data from a single study shows no clear difference in amputation rates between patients who received high and low doses of CD34+ cells. However, this finding should be interpreted cautiously because of the small sample size and the phenotype of cells used in this study. This study used mobilised PB CD34+ cells (Losordo 2012), whereas many studies examining cell therapy for CLI patients had used unselected bone marrow-derived cells (BM-MNCs). Of note, different cell sources and phenotypes may contain different proportions of stem cells with angiogeneic potential.

Most included trials implanted the cells into the gastrocnemius muscle (intramuscular (IM) route). Only three RCTs involving 100 participants directly compared the efficacy of IM and IA routes of cell administration (Gu 2008; Klepanec 2012; Van Tongeren 2008). Meta-analysis of these trials failed to show superiority of either route; however, the accuracy of this finding is confounded by the small sample size.

All-cause mortality from three RCTs was 9%. However, in view of baseline high risk factors among CLI patients and the limitations of the individual RCTs included in our review, it is not possible to attribute the cause of death to cell treatment. Of note, all RCTs included in this review reported no significant adverse effects related to cell-based treatment. However, none of these trials performed head-to-head comparison of safety data between treatment groups. Moreover, the long-term safety of cell-based therapy could not be determined, as the follow-up period for most RCTs was relatively short, ranging from one to twelve months. Head-to-head comparisons between CLI patients treated with different cell-based regimens and given long-term follow-up are warranted to confirm the safety and durability of the efficacy of cell-based therapy for these patients.

Additionally, the relatively short follow-up period of the included studies has limited the usefulness of this review in informing practice.

Quality of the evidence

Overall, we noted a mixed quality of evidence for different outcome-comparison combinations, and we concluded that overall the quality of evidence presented was very low to moderate (Summary of findings for the main comparison; Summary of findings 2; Summary of findings 3; Summary of findings 4). Major factors that led to downgrading of the quality of evidence were risk of bias issues, imprecision, and indirectness of evidence. Major methodological limitations in most studies included unclear randomisation methods and/or allocation concealment, as well as lack of blinding of participants and insufficient outcome data. It is important to note that single studies or small numbers of studies with limited participants contributed to all outcomes, resulting in underpowered analyses with wide 95% confidence intervals. Overall, high-quality evidence needed to assess the effects of utilisation of a particular source or treatment regimen of cell-based therapy for CLI in clinical practice is lacking.

Potential biases in the review process

We performed a comprehensive search covering multiple major databases to look for published and unpublished studies in full text or abstract format, and we screened for relevant studies among 4904 citations. We believe that the scope of our search was sufficiently complete.

It is possible that we may not have identified still unpublished trials. In particular, small studies reporting negative findings may not have been published as full articles. Moreover, several phase 3 trials are currently ongoing. We identified seven ongoing RCTs (NCT00311805; NCT00753025; NCT01257776; NCT01408381; NCT01446055; NCT01745744; NCT02454231), and we discovered six clinical trials classified as 'awaiting classification' (Gurunathan 2009; Korymasov 2009; Molavi 2016; NCT00595257; NCT00987363; NCT02993809), the eligibility criteria of which have yet to be determined. Results of these trials will likely provide efficacy data on cell-based therapy derived from different sources, administered via different doses, and delivered via different routes.

We have excluded a substantial number of clinical trials related to cell-based treatment for patients with PAD, as most of these were single-arm studies (42 trials) or were non-randomised (11 trials). To date, the number of high-quality trials conducted to compare different types of cell products and treatment regimens is limited.

Four RCTs reported their results using limbs rather than patients as the unit of analysis and reported unadjusted results (Gu 2008; Huang 2007; Lu 2011; Zhang 2009). We could not obtain sufficient data from the primary studies to adjust for clustering effects, and this might have led to a biased estimate in our results for certain limb-related outcomes.

Researchers described one RCT as a "controlled clinical case analysis", and indicated that patients were randomly assigned to two groups (BM-MNCs vs mPBSCs) (Zhang 2009). Study authors did not mention the true nature of randomisation. Ideally we would have performed a sensitivity analysis to investigate the effects of randomisation on estimates of outcome; however, this was the only study reporting these effects on outcomes, so we could not perform a sensitivity analysis.

Our sensitivity analyses performed to assess the impact of excluding such studies show that in most cases, these exclusions did not change the pooled estimates substantially.

We were unable to perform sensitivity analyses for significant or worrisome dropout rates and significant methodological issues identified during assessment of risk of bias, as insufficient data were available.

Agreements and disagreements with other studies or reviews

To date, apart from the current systematic review, limited published meta-analyses have included RCTs that directly compared the effectiveness of cell-based products derived from different sources, prepared using different regimens, and delivered via different routes. Most published meta-analyses compared cell therapy versus conventional treatment (non-cell-based therapy) and also included non-RCTs. Direct comparison of the efficacy of different cell sources and types reported by individual RCTs has yielded inconsistent results. Hence, the efficacy and safety of different cell-based products remain to be determined.

Three previous meta-analyses involving 510 (twelve RCTs), 276 (seven RCTs), and 373 (seven RCTs) PAD patients, respectively, underlined the promising results of cell-based therapy compared to conventional therapy (non-cell-based therapy) (Liu 2012; Teraa

2013; Wen 2011). In contrast to the current review, these previously published meta-analyses combined and analysed patients treated with cells from different sources and different types of cells (BM-MNCs, BM-MSCs, mPBSCs, Ixmyelocel-T) and collectively reported them as bone marrow-derived cell therapy.

Two recent meta-analyses attempted to compare outcomes for patients receiving different cell types versus non-cell-based therapy (Liew 2016; Liu 2015). In Liu 2015, the subgroup analysis based on stem cell sources showed significant improvement in amputation rates, ulcer healing, and ABI in patients implanted with bone marrow-derived cells or peripheral blood-derived cells compared to controls. However, in contrast to our review, Liu 2015 included non-randomised studies in its meta-analysis (Cobellis 2008; Dubsky 2013). Review authors compared clinical outcomes of cell treatment groups versus non-cell treatment groups and did not perform head-to-head comparisons between cells derived from different sources or implanted via different implantation regimens. Furthermore, review authors pooled and analysed outcome data from studies using different types of cells, for example, the bone marrow cell group consisted of patients treated with allogeneic MSCs (Gupta 2013), BM-MNCs (Walter 2011), and tissue repair cells (Powell 2012), and the peripheral blood cell group consisted of patients treated with VesCell - as in Szabo 2013 - and with mPBSCs (Huang 2005; Mohammadzadeh 2013; Ozturk 2012).

Liew 2016 concluded that patients treated with PB-MNCs and bone marrow concentrate had a reduced rate of major amputation compared to those given other cell types (i.e. PB-MNCs, BM-MSCs, BM-MNCs). However, in contrast to our analysis, review authors did not perform head-to-head comparisons between a particular cell-based product and another cell-based product. Furthermore, review authors arrived at their conclusions upon analysing pooled data from studies that used different types of cells, namely, mPBSCs, VesCell (Szabo 2013), and CD133+ cells (Raval 2014), and they combined and analysed data collectively as PB-MNCs, while pooling, combining, and analysing collectively as BM-MSCs allogeneic MSCs (Gupta 2013), autologous MSCs (Lu 2011), and tissue repair cells (Powell 2012). As with meta-analysis performed by Liu 2015, differences in the types of cell products used for implantation may have had an important impact on clinical outcomes.

A large meta-analysis involving almost 700 'no-option' CLI patients from 37 clinical trials comparing the efficacy of BM-MNC and mPBSC treatment included uncontrolled and non-randomised trials, rendering the results to have high risk of bias (Fadini 2010).

A Cochrane review that was first published in 2011 and was updated in 2014 includes two small RCTs involving 57 patients comparing IM autologous BM-MNCs and conventional therapy; the review authors concluded that evidence was insufficient to support intramuscular cell treatment in patients with CLI (Moazzami 2014). Review authors did not perform a meta-analysis because the two studies included in that review differed with regards to types of cells implanted (BM-MNCs vs mPBSCs) and assessment of outcomes (Barć 2006; Huang 2005); hence the conclusion regarding efficacy of cell treatment compared to conventional treatment was based on data extracted from individual studies with very small sample sizes.

Previous meta-analyses reported that autologous bone marrow-derived stem cells and G-CSF-mobilised PBSCs (mPBSCs) given via IM or IA implantation into the ischaemic limbs appeared to be relatively safe, and side effects were generally mild and transient (Liew 2016; Liu 2015; Sun 2015; Teraa 2013). Only two RCTs previously reported in detail comparisons of adverse events between cell treatment and non-cell treatment groups (Li 2013; Teraa 2014). These studies showed no significant differences in the incidence of adverse events between autologous BM-MNCs and control interventions. Adverse events most commonly reported in the BM-MNC group were short-term episodes of slight pain, bleeding, and haematoma at the bone marrow aspiration site that did not require specific intervention. Adverse events most commonly noted in the mPBSC group were bone pain (13.2%) and lassitude (5.3%) associated with G-CSF administration, which were transient and did not require discontinuation from G-CSF therapy and PBSC mobilisation (Huang 2007). In conclusion, most published clinical trials in people with CLI have presented a reassuring short-term safety profile but sparse data on the long-term safety of cell-based therapy.

AUTHORS' CONCLUSIONS

Implications for practice

Evidence of mostly low and very low quality shows no clear differences between different sources, cell doses, and implantation routes for cell-based therapy among patients with CLI in terms of mortality, amputation rate, rest pain, ulcer healing, and lower limb perfusion. Cell therapy appeared to be well tolerated without serious adverse events arising from cell harvesting from bone marrow or peripheral blood and cell implantation into the ischaemic limb. Overall the efficacy and long-term safety of autologous cells derived from different sources, prepared using different protocols, administered at different doses, and delivered via different routes for treatment of 'no-option' CLI patients, remain to be confirmed. Underpowered analyses have precluded any firm conclusions that may influence practice.

Implications for research

Authors of the current review could not draw definitive conclusions on the efficacy of different sources, types, and treatment regimens of cell-based therapy for CLI patients, and high-quality clinical trials are urgently needed to answer these clinical questions. In particular, the long-term safety of cell-based products derived from different sources, prepared using different protocols, and administered at different doses and via different routes, needs to be determined. Future clinical trials should consider the following factors.

1. Larger sample size with adequate blinding of study population and personnel.
2. Head-to-head comparison between cell-based treatment derived from different sources via different cell preparation protocols, routes of cell delivery, cell doses, and implantation regimens.
3. Determination of clinical endpoints according to underlying aetiologies, risk factors (smoking, diabetes, age group, and gender), and severity of PAD, with separate analyses and reporting of results according to these subgroups.
4. Long-term follow-up to evaluate the durability of angiogenic potential and the long-term safety of cell-based therapy.
5. Use of angiogenic growth factors, cell carriers, and other adjunctive therapies in combination with cell-based treatment.

6. Comparison of the safety and efficacy of allogeneic cell therapy versus autologous cell therapy.
7. Comparison of the cost of therapy amongst different cell-based treatment modalities.

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CHARACTERISTICS OF STUDIES

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

Gu 2008

Methods	Study design: stated as randomised Method of randomisation: not stated Blinding: not stated Exclusions post randomisation: not stated Losses to follow-up: not stated
Participants	Country: China No. of participants: 32, 16 in intramuscular (IM) group, 16 in intra-arterial (IA) group Mean age (SD): 69.5 (total) Gender: 20 male, 12 female Thirty-two patients (35 lower limbs) who were admitted into the Department of Vascular Surgery of Xuanwu Hospital in Beijing, for chronic ischaemic limbs from March 2003 to April 2004, were studied. Follow-up period: not stated
Interventions	Group 1 (16 participants with 18 affected limbs) received implantation of autologous BM-MNCs by IM injection into affected limbs. Group 2 (16 participants with 17 affected limbs) received implantation of autologous BM-MNCs by IA injection into affected limbs.
Outcomes	Clinical symptoms, physical examinations, and other vascular assessments 1. Improvement in rest pain 2. Improvement in sense of coldness 3. ABI 4. TcO_2 5. Amputation rate 6. Intermittent claudication 7. Collateral vessel formation by angiographic analysis
Notes	
Risk of bias	

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* Indicates the major publication for the study

Gu 2008 *(Continued)*

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "The 32 patients with lower limb ischaemia were divided into two groups by randomisation"
Allocation concealment (selection bias)	Unclear risk	Not mentioned
Blinding of participants and personnel (performance bias) All outcomes	High risk	Although not clearly stated, blinding appears very unlikely, as one group had injection into the muscles, while the other had injection from a percutaneous retrograde contralateral femoral.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	It was not stated whether the outcome assessor was blinded to participants' assigned groups.
Incomplete outcome data (attrition bias) All outcomes	Low risk	There were no dropouts.
Selective reporting (reporting bias)	Low risk	We did not identify any reporting bias.
Other bias	High risk	We noted unit of analysis issues, as the outcome of ABI was reported with the lower limbs rather than patients as the unit of analysis, and study authors did not adjust their data to account for the effect of clustering.

Huang 2007

Methods	Study design: stated as randomised Method of randomisation: not stated Blinding: not stated Exclusions post randomisation: not stated Losses to follow-up: not stated
Participants	Country: China No. of participants: 150, 76 in mPBSC group, 74 in BM-MNC group Mean age (SD): 67 (9) in mPBSC group, 67 (11) in BM-MNC group Gender: 47 male, 29 female in mPBSC group; 51 male, 23 female in BM-MNC group Patients qualified for cell implantation in group A (active treatment) and group B (control) if they were diagnosed with LASO, with no improvement after a at least 3 months of treatment with adapted drugs, including urokinase, prostaglandin E1, heparin, or pentoxifylline, as described previously. Requisite haemodynamic deficits included resting ABI < 0.9 in the affected limb on 2 consecutive examinations performed at least 1 week apart. Patients with proliferative retinopathy, evidence of malignant disorder during the past 5 years, hypercoagulable states, gangrene above the ankle, and/or severe coronary, cerebral, and renal vascular disease were excluded Follow-up period: 12 months

Huang 2007 *(Continued)*

Interventions	Randomised (1:1) to implantation of mPBSCs (group A, active treatment, 76 participants) or BM-MNCs (group B, control, 74 participants) Route of delivery: IM implantation
Outcomes	Primary outcomes 1. ABI 2. Rest pain 3. PFWD 4. TcO_2 5. Ulcers 6. Amputation rate
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	They were randomised (1:1) to implantation of mPBSCs or BM-MNCs.
Allocation concealment (selection bias)	Unclear risk	Not mentioned
Blinding of participants and personnel (performance bias) All outcomes	High risk	Although not clearly stated, blinding appeared very unlikely, as 1 group underwent bone marrow aspiration, and the other group received subcutaneous G-CSF followed by apheresis.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	It was not stated whether the outcome assessor was blinded to participants' assigned groups.
Incomplete outcome data (attrition bias) All outcomes	High risk	Six participants (2 from the intervention group and 4 from the comparison group) were excluded before implantation, and 10 participants (4 from the intervention group and 6 from the comparison group) discontinued owing to limb amputation after implantation. Although total loss to follow-up (10.6%) was not by itself a major concern, the unequal numbers of withdrawals between the 2 groups posed a concern; therefore this study was accorded high risk for incomplete outcome data.
Selective reporting (reporting bias)	Low risk	We did not identify any reporting bias.
Other bias	High risk	There were unit of analysis issues as some outcomes (ulcer healing, ABI) were reported using the limbs rather than patients as the unit of analysis, and the study authors did not adjust their data to account for the effect of clustering.

Klepanec 2012

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

Klepanec 2012 *(Continued)*

	Blinding: not stated Exclusions post randomisation: not stated Losses to follow-up: not stated
Participants	Country: Slovakia No. of participants: 41, 20 in intra-arterial (IA) group, 21 in intramuscular (IM) group Mean age (SD): 66 (11) in IA group, 66 (10) in IM group Gender: 18 male, 2 female in IA group; 17 male, 4 female in IM group Between October 2009 and August 2010, 41 patients with advanced CLI (Rutherford category 5 or 6) after failed or impossible revascularisation were randomised to application of 40 mL of bone marrow concentrate via the local IM route (n = 21) or via selective IA infusion (n = 20). Inclusion criteria 1. Over 18 years of age with ischaemic skin lesions (ulcers or gangrene) with a CLI Rutherford category of 5 or 6 according to the TransAtlantic Intersociety Consensus (TASC) classification (minor or major tissue loss) 2. CLI defined by ABI ≤ 0.4, or ankle systolic pressure < 50 mmHg, or toe systolic pressure < 30 mmHg, or transcutaneous oxygen pressure (TcO_2) < 30 mmHg 3. No option for endovascular or surgical revascularisation assessed by a vascular surgeon and an intervention radiologist 4. Failed revascularisation defined as no change in clinical status with best standard care 4 weeks after endovascular or surgical revascularisation Exclusion criteria 1. Life expectancy < 6 months 2. Evidence of malignancy during previous 5 years 3. Proliferative retinopathy 4. Critical coronary artery disease or unstable angina pectoris 5. End-stage kidney disease and on dialysis 6. Bone marrow disease (e.g. myelodysplastic syndrome, severe anaemia, leucopaenia, thrombocytopaenia) Follow-up period: 6 months
Interventions	This study compared the therapeutic effects of IM (Group A) and IA (Group B) delivery of bone marrow cells (BMCs).
Outcomes	Primary endpoints: limb salvage and wound healing Secondary endpoints: changes in TcO_2, Rutherford category, quality-of-life questionnaire (EQ5D), ABI, amputation rate, pain score (0–10)
Notes	This study was sponsored by a grant from European Regional Development Funding (ITMS code: 26240220023).

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "…..randomised to application of 40 ml of bone marrow concentrate via the local IM route (n = 21) or via selective IA infusion (n = 20)"

Klepanec 2012 *(Continued)*

Allocation concealment (selection bias)	Unclear risk	Not mentioned
Blinding of participants and personnel (performance bias) All outcomes	High risk	Blinding appeared very unlikely, as in Group A cells were administered into the muscles of affected limbs, and in Group B IA injection of cells was undertaken from a percutaneous retrograde contralateral femoral approach or an antegrade femoral approach at the site of arterial occlusion of the affected limb.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	It was not stated whether the outcome assessor was blinded to assigned participant groups.
Incomplete outcome data (attrition bias) All outcomes	Low risk	All participants were accounted for, for all outcomes.
Selective reporting (reporting bias)	Low risk	We did not identify any reporting bias.
Other bias	Low risk	None identified

Losordo 2012

Methods	Study design: prospective double-blind randomised placebo-controlled clinical pilot study Method of randomisation: not stated Blinding: double-blind Exclusions post randomisation: not stated Losses to follow-up: not stated
Participants	Country: United States No. of participants: 28, 7 in low-dose group, 9 in high-dose group, 12 in control group Mean age (SD): 61.8 (13.9) in low-dose group, 69.7 (10.9) in high-dose group, 67.1 (14.2) in control group Gender: 5 male, 2 female in low-dose group; 8 male, 1 female in high-dose group; 6 male, 6 female in control group Inclusion criteria: male or female patients aged ≥ 21 years with Rutherford category 4 or 5 CLI and no suitable revascularisation options; demonstrated atherosclerosis with stenosis (> 70%) or occlusion (100%) of a major vessel and absolute ankle pressure in the affected limb < 60 mmHg or reduced toe pressure < 40 mmHg or abnormal photoplethysmography; diagnosis of microvascular insufficiency Exclusion criteria: thromboangiitis obliterans (Buerger's disease) allowed if arterial insufficiency in the lower extremity was the result of a non-atherosclerotic disorder, including but not limited to advanced scleroderma (CREST syndrome); advanced CLI (Rutherford category 6); expected amputation within 4 weeks of screening; clinical evidence of sepsis; advanced AV block or New York Heart Association class III or class IV heart failure; myocardial infarction within 3 months; clinically successful aortic or lower extremity arterial surgery; percutaneous revascularisation; lumbar sympathectomy within 3 months preceding screening Follow-up period: 12 months
Interventions	Route of delivery: IM injection

Losordo 2012 *(Continued)*

	Treatment groups: 1. Low dose (1×10^5 auto-CD34+ cells/kg, 7 participants) 2. High dose (1×10^6 auto-CD34+ cells/kg, 9 participants) 3. Control: 12 participants
Outcomes	Safety 1. Primary endpoint of this exploratory study was safety of the IM injection of auto-CD34+ cells 2. Follow-up period: at 2, 4, 6, 8, and 12 weeks and at 6 and 12 months Efficacy 1. Assessment of limb salvage, occurrence of amputation, nature of amputation (toe or trans-metatarsal, below or above the knee, preserving or not preserving function), and time to amputation were recorded during the 12-month follow-up period.
Notes	Baxter Healthcare Corporation. This study was supported in part by grants from the NIH (HL-53354, HL-77428, HL-63414, HL-80137, HL95874, HLPO1-108795, HL-57516). The control group is not included in the analysis of this review.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Quote: "A total of 28 subjects were randomised 1:1:1…" Comments: the method of sequence generation was not explicitly stated.
Allocation concealment (selection bias)	Unclear risk	Not mentioned
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Investigator, subject, study site personnel, core laboratory(ies), blinded study statistician, and all sponsor and clinical research organisation personnel remained blinded to all treatments.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Investigator, subject, study site personnel, core laboratory(ies), blinded study statistician, and all sponsor and clinical research organisation personnel remained blinded to all treatments.
Incomplete outcome data (attrition bias) All outcomes	High risk	Eight participants (28.6%) (4 in the high-dose group and 4 in the control group) did not complete the 12-month study period.
Selective reporting (reporting bias)	High risk	Rest pain, ABI, and ulcer size cannot be incorporated into the meta-analysis, as data provided were insufficient.
Other bias	Low risk	We did not identify any other potential sources of bias.

Lu 2011

Methods	Study design: double-blind randomised controlled trial Method of randomisation: randomisation table

Lu 2011 *(Continued)*

	Blinding: double-blind Exclusions post randomisation: not stated Losses to follow-up: 4 of the 41 enrolled participants left this trial because 3 died from sudden cardiac death and 1 from severe pulmonary infection within 4 weeks after they were enrolled into the trial.
Participants	Country: China No. of participants: 41 Mean age: 64 Gender: 19 male, 22 female Inclusion criteria 1. Age from 40 to 70 years 2. Type 2 diabetic patients 3. Bilateral CLI (ABI from 0.30 to 0.60) 4. At least 1 foot ulcer Exclusion criteria 1. Dry gangrene above the ankle or moist gangrene 2. Malignant tumour 3. Severe coronary, cerebral, and renal vascular disease Follow-up period: 24 weeks
Interventions	3 groups: BM-MSCs 20 limbs BM-MNCs 21 limbs Normal saline (NS) 41 limbs
Outcomes	Primary outcome: safety and feasibility of treatment, defined as improvements in rest pain, PFWT, ulcer healing rate, limb salvage rate, ABI, and TcO_2 and enhancement of vessel formation as judged by the MRA
Notes	This study was supported by the Clinical Research Fund of Southwest Hospital, Third Military Medical University. The study authors thank all patients and investigators who participated in this study.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Participants were randomised to groups A and B via a randomisation table.
Allocation concealment (selection bias)	Unclear risk	It was not stated whether allocation was performed independently from sequence generation.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	The procedures of this clinical trial are presented, and patients and investigators were blinded to the treatments.
Blinding of outcome assessment (detection bias)	Low risk	Study authors stated that "..... investigators were blinded to the treatments".

Lu 2011 *(Continued)*

All outcomes		
Incomplete outcome data (attrition bias) All outcomes	Low risk	There were no dropouts in the study.
Selective reporting (reporting bias)	High risk	Data for ABI in the control group were not provided.
Other bias	High risk	There were unit of analysis issues, as the allocation was made at the level of the legs rather than the participants, and study authors did not adjust their data to account for the effect of clustering.

Van Tongeren 2008

Methods	Study design: stated as randomised Method of randomisation: random number table Blinding: not stated Exclusions post randomisation: not stated
Participants	Country: Netherlands No. of participants: 27, 12 in IA + IM group, 15 in intramuscular (IM) group Mean age (SD): 66.9 (16.3) in IA + IM group, 69.8 (12.4) in IM group Gender: 9 male, 3 female in IA + IM group; 10 male, 5 female in IM group Technical options for revascularisation by percutaneous transluminal angioplasty (PTA) or reconstructive surgery were extensively evaluated and disqualified. Patients were eligible for implantation of autologous bone marrow-derived mononuclear cells if they suffered from CLI (ischaemic rest pain or ulcers), or if they had persistent (> 12 months) profound disabling claudication and a maximum walking distance of 100 metres. Life expectancy should be at least 1 year. Patients with a history of malignant disease in the 5 years before treatment were excluded. Mean follow-up: 24 ± 8 months
Interventions	27 participants were treated with combined IA + IM (n = 12) or sole IM (n = 15) administration of autologous BMCs.
Outcomes	1. Amputation rate 2. PFWD 3. ABI 4. Pain score 5. Limb salvage 6. Collateral vessels
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement

Van Tongeren 2008 *(Continued)*

Random sequence generation (selection bias)	Low risk	The method of administration was randomly assigned to participants via a random number table.
Allocation concealment (selection bias)	Unclear risk	Information was insufficient to enable an evaluation on whether allocation was made independently from sequence generation.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Blinding appears very unlikely, as 1 group had injection into the muscles, and the other had injection from a percutaneous retrograde contralateral femoral.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Two evaluating radiologists and 2 vascular surgeons were blinded for preprocedural and postprocedural angiographies.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Two participants (1 in each group) were excluded. One participant died early in the study, and another did not attend follow-up.
Selective reporting (reporting bias)	High risk	Apart from amputation rate, the study author did not report other key outcomes in sufficient detail.
Other bias	Low risk	None identified

Zhang 2009

Methods	Study design: controlled clinical case analysis Method of randomisation: not stated Blinding: not stated Exclusions post randomisation: not stated
Participants	Country: China No. of participants: 52, 30 in BM-MNC group, 22 in PBSC group Mean age (SD): 69 (6) total Gender: 27 male, 25 female 52 patients with diabetic lower limb ischaemia Follow-up period: not stated
Interventions	Treatment group (30): bone marrow stem cell Intervention (22): peripheral blood stem cell
Outcomes	1. Percentage of pain relief 2. Percentage of cold relief 3. Percentage of intermittent claudication relief 4. ABI
Notes	The article was reported in Chinese and was translated into English for the purposes of the current review.

Zhang 2009 *(Continued)*

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "...., were randomly divided into two groups....."
Allocation concealment (selection bias)	Unclear risk	Not mentioned
Blinding of participants and personnel (performance bias) All outcomes	High risk	Blinding appears very unlikely, as 1 group had blood drawn from the bone marrow, and the other had blood drawn from the peripheral blood.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	It was not stated whether the outcome assessor was blinded to participants' assigned groups.
Incomplete outcome data (attrition bias) All outcomes	Low risk	All patients are accounted for for all outcomes.
Selective reporting (reporting bias)	Low risk	We did not identify any reporting bias.
Other bias	High risk	There were unit of analysis issues as allocation was made at the level of the legs rather than patients, and study authors did not adjust their data to account for the effect of clustering.

ABI: ankle-brachial pressure index.
AV: atrioventricular.
BM-MNCs: bone marrow-mononuclear cells.
BM-MSCs: bone marrow-mesenchymal cells.
BMCs: bone marrow cells.
CLI: critical limb ischaemia.
CREST: calcinosis, Raynaud phenomenon, oesophageal dysmotility, sclerodactyly, and telangiectasia.
EQ5D: EuroQoL–5 Dimensional forma.
G-CSF: granulocyte colony-stimulating factor.
IA: intra-arterial.
IM: intramuscular.
LASO: limb arteriosclerosis obliterans.
mPBSCs: mobilised peripheral blood stem cells.
MRA: magnetic resonance angiography.
NS: normal saline.
PB-MNCs: peripheral blood-mononuclear cells.
PBSCs: peripheral blood stem cells.
PFWD: pain-free walking distance.
PFWT: pain-free walking time.
RCT: randomised controlled trial.
SD: standard deviation.
TASC: TransAtlantic Intersociety Consensus.
TBI: toe-brachial index.
TcO_2: transcutaneous oxygen tension.
VAS: visual analogue scale.

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

Study	Reason for exclusion
Afan 2015	This study investigated the number of endothelial progenitor cells (EPCs) in PAD patients and did not study clinical outcomes after cell implantation.
Amann 2008	This is a randomised, double-blind, placebo-controlled phase 3 study for autologous bone marrow cell transplantation in critical limb ischaemia.
Amato 2012	This is a pilot single-arm study investigating the therapeutic effects of peripheral blood mononuclear cells (PB-MNCs) in CLI patients.
Arai 2006	This is an RCT investigating BM-MNCs plus conventional drug therapy compared with conventional treatment in PAD patients.
Arici 2015	This is a prospective single-centre non-randomised single-arm study assessing the safety and effectiveness of highly purified CD133+ autologous stem cells in CLI.
Bartsch 2007	This is a non-randomised study investigating the therapeutic effects of IM + IA implantation of BM-MNCs in CLI patients.
Barć 2006	This is an RCT investigating BM-MNC therapy compared with conventional therapy for CLI patients.
Benoit 2011	This is an RCT investigating BMCs compared with sham injection in CLI patients.
Bing 2009	This study investigated the effects of BM-MSCs on patients with diabetic foot only, not CLI patients.
Bura 2014	This study investigated the feasibility and safety of intramuscular injections of autologous cultured adipose-derived stroma/stem cells and included no control group.
Burt 2010	This study investigated the safety and feasibility of autologous CD133+ cells and included no control group.
Capiod 2009	This study compared the characteristics of bone marrow and peripheral blood mononuclear cells for CLI and did not report clinical outcomes.
Chochola 2008	This is a single-arm study investigating the safety and feasibility of IA BM-MNC injection in CLI patients.
Choi 2012	This is an RCT conducted to determine the effectiveness of G-CSF in stimulating angiogenesis in patients with CLI. Abstract only
Cobellis 2008	This is a non-randomised study investigating the long-term effects of repeated autologous transplantation of bone marrow cells in patients affected by peripheral arterial disease.
Cobellis 2010	This is a case report showing the beneficial effects of vascular endothelial growth factor secreted from stromal cells in supporting endothelial cell functions in a patient with CLI.
Darinskas 2017	This is a pilot study using stromal vascular fraction cells for the treatment of CLI.
Das 2013	This is a phase 1 single-arm study investigating the safety and efficacy of allogeneic BM-MSCs in CLI patients.
Dash 2009	This is an RCT investigating BM-MSCs compared with standard wound care in patients with lower limb ischaemia.
De Angelis 2014	This is a prospective non-randomised study investigating the efficacy of peripheral blood mononuclear cells (PM-MNCs) in patients with CLI.

Study	Reason for exclusion
Debin 2008	This is a randomised trial investigating the efficacy and safety of autologous BM-MSCs compared with control interventions in patients with lower limb ischaemia.
Dou 2015	This study assessed the long-term effectiveness and safety of autologous BM-MNC transplantation compared with standard medical therapies in patients with critical diabetic lower arteriosclerosis obliterans (ASO).
Doudar 2013	This is a randomised trial investigating the safety and efficacy of PB-MNCs compared with control interventions in patients with chronic lower limb ischaemia.
Du 2017	This clinical trial studied the clinical efficacy of implanted umbilical cord MSCs combined with bone marrow stem cells compared with implanted bone marrow stem cells for treatment of lower limb ischaemia.
Dubsky 2013	This non-randomised study compared the effects of BM-MNCs vs mPBSCs vs standard medical therapy for diabetic patients with CLI.
Duong 2008	This is a multi-centre phase 1 non-randomised study including a single group treated with BM-MNCs.
Flugelman 2017	Study participants received venous endothelial cells (ECs) combined with venous smooth muscle cells (SMCs).
Franz 2011	This single-arm study investigated the therapeutic effects of IM + IA implantation of BM-MNCs in CLI.
Frogel 2017	This is a pilot open-label study of treatment progenitor cells derived from peripheral blood for CLI patients.
Gabr 2011	This is a single-arm prospective study using intramuscular injection of unfractionated autologous BM-MNCs in CLI patients.
Grossman 2016	This is a phase 1 safety, dose-escalating, non-randomised, open-label study of autologous, fully differentiated venous endothelial and smooth muscle cells called MultiGeneAngio (MGA) for claudication due to peripheral artery disease.
Gu 2006	This is a non-randomised study comparing cell dosage for treatment of CLI patients.
Gu 2007	This is a non-randomised study comparing BM-MNCs vs mPBSCs for treatment of CLI patients.
Gu 2017	Study participants receive 2 injections of G-CSF (300 μg) before BM-MNC transplantation.
Guo 2018	In this retrospective study, participants were treated with smoking cessation and either aspirin (100 mg/d) alone or aspirin and ABMMNC injection according to participant preference.
Gupta 2013	This randomised placebo-controlled phase 1/2 study assessed the safety and efficacy of allogeneic BM-MSCs in CLI patients.
Gupta 2017	This phase 2, non-randomised, multi-centre, dose-finding study assessed the efficacy of allogeneic BM-MSCs given to CLI patients with Buerger's disease.
Harunarashid 2016	Study participants received both BM-MSCs with BM-MNCs or BM-MNCs alone.
Heo 2016	This single-arm study used autologous BM-MNCs for CLI.

Study	Reason for exclusion
Hernandez 2007	This non-randomised study investigated the therapeutic effects of BM-MNC implantation in CLI patients.
Higashi 2004	This phase 1 single-arm clinical trial was conducted to determine the effect of autologous bone marrow-mononuclear cell (BM-MNC) implantation on endothelial dysfunction in patients with limb ischaemia.
Holzinger 1994	This study investigated the therapeutic effects of BM-MNC implantation in patients with chronic arterial occlusive disease or venous post-thrombotic syndrome, not CLI.
Hoshino 2007	This single-arm study investigated the therapeutic effects of G-CSF mobilised PBSCs in diabetic patients on haemodialysis with limb ischaemia.
Huang 2005	This randomised study was conducted to investigate the efficacy of PB-MNCs vs control interventions in patients with CLI.
Iafrati 2016	This randomised placebo-controlled study was conducted to assess the safety of autologous bone marrow concentrate in patients with CLI.
Idei 2011	This non-randomised trial was undertaken to investigate the effects of bone marrow-mononuclear cells (BM-MNCs) implanted in patients with CLI.
Ishida 2005	This single-arm study was conducted to investigate the therapeutic effects of G-CSF mobilised PBSCs in CLI patients.
Iso 2010	This single-arm study investigated the therapeutic effects of BM-MNCs in CLI patients.
Kamata 2007	This non-randomised study compared the therapeutic effects of BM-MNCs and PB-MNCs in ischaemic digits of patients with connective tissue diseases.
Kawamoto 2009	This single-arm study investigated the safety and feasibility of CD34+ cell therapy in CLI patients.
Kinoshita 2012	This study investigated the therapeutic effects of G-CSF mobilised PBSCs in CLI and included no control group.
Kirana 2012	This study used specialised cultured cell products (CD90+ tissue repair cells) that have been expanded from autologous bone marrow.
Kolvenbach 2010	This single-arm study investigated the safety and feasibility of intraoperative stem cell treatment in combination with bypass surgery and/or interventional treatment in CLI patients.
Kondo 2016	This single-arm, non-randomised, open-label, historically controlled study was designed to assess the safety and feasibility of intramuscular injection of ADRCs for treatment of patients with CLI.
Kondo 2018	This retrospective, observational, non-controlled study included no-option CLI patients who had BM-MNC implantation performed.
Lara-Hernandez 2010	This prospective pilot study investigated the safety and efficacy of therapeutic angiogenesis in patients with CLI.
Lasala 2010	This phase 1 non-randomised single-arm study assessed the safety and efficacy of combined BM-MNCs plus BM-MSCs in CLI patients.
Lee 2012	This study investigated the safety and effects of adipose tissue-derived stem cells and included no control group.

Study	Reason for exclusion
Lenk 2005	This single-arm study investigated the safety and therapeutic effects of circulating blood-derived progenitor cells in CLI patients.
Li 2013	This single-blinded study investigated the effects of BM-MNCs compared with control interventions in patients with CLI.
Madaric 2016	This study investigated factors predictive of the effects of BMCs on progression of advanced CLI in patients with CLI receiving autologous BMCs (SmartPreP2) by local intramuscular (n = 32) or intra-arterial (n = 30) application.
Madaric 2017	This study investigated levels of asymmetrical dimethylarginine (ADMA) and changes in oxidative stress in patients with CLI after BM-MNC therapy and did not study the clinical outcomes of cell treatment.
Maione 2013	This pilot non-randomised non-controlled phase 1 study assessed the effects of bone marrow cells in CLI patients.
Majumdar 2015	This phase 2 study investigated allogeneic mesenchymal stromal cells in CLI.
Malyar 2014	This non-randomised single-arm study evaluated the effects of autologous BM-MNCs in severe PAD.
Matoba 2009	This single-arm study investigated the therapeutic effects of BM-MNCs in CLI patients.
Matsui 2003	Study participants with bilateral ischaemic limbs received both autologous mononuclear bone marrow and peripheral blood mononuclear cells.
Mohamed 2017	This is a phase 1b, non-randomised, uncontrolled, open-label, dose-escalation study.
Mohammadzadeh 2013	This study investigated the efficacy and safety of transplanted mPBSCs compared with control interventions in diabetic patients with CLI.
Moriya 2009	This study is a retrospective analysis of the therapeutic effects of PB-MNCs for limb ischaemia.
Motukuru 2008	This single-arm study investigated the efficacy of IM implantation of BM-MNCs in CLI.
Murphy 2011	This non-randomised single-arm study assessed the safety and efficacy of autologous BM-MNCs.
Murphy 2017	This double-blinded, placebo-controlled trial was designed to assess the safety and efficacy of autologous concentrated bone marrow aspirate vs placebo.
Napoli 2008	This non-randomised study compared the effects of BM-MNCs plus antioxidant/L-arginine vs antioxidant/L-arginine only.
NCT00306085	This is a non-randomised study.
NCT00434616	This multi-centre randomised placebo-controlled double-blind clinical study investigated autologous BMC implantation vs saline injection.
NCT00539266	This clinical trial compared BM-MNCs vs placebo in patients with limb ischaemia.
NCT00922389	This clinical trial compared peripheral blood-derived stem cells vs standard therapy in patients with CLI.
NCT01049919	This clinical trial compared autologous concentrated bone marrow aspirate vs placebo in patients with CLI.

Study	Reason for exclusion
NCT01245335	This clinical trial compared bone marrow aspirate concentrate vs placebo in patients with CLI.
NCT01584986	This randomised open-label clinical study assessed the safety and efficacy of autologous angiogenic cell precursors (ACPs) compared with SMT.
NCT02336646	This randomised clinical trial compared allogeneic MSCs vs placebo in patients with CLI.
NCT03174522	This clinical trial compared Rexmyelocel-T vs placebo in patients with CLI.
NCT03214887	This clinical trial investigated the safety and efficacy of hyaluronan (HA) combined with BM-MNCs for PAD.
NCT03304821	This double-blind placebo-controlled randomised clinical study examined whether 3 weeks of 3-times-a-week injection of GM-CSF would improve measures of ischaemia in patients with intermittent claudication compared with placebo.
NCT03339973	This clinical trial is investigating the efficacy and safety of 1 dose of allo-APZ2-PAOD administered intramuscularly into the affected lower leg of patients with peripheral arterial occlusive disease.
Nemcova 2017	This study was conducted to evaluate serum levels of angiogenic cytokines in diabetic patients with CLI treated by ACT vs patients treated by PTA.
Niven 2017	This pilot open-label study examined treatment with progenitor cells for peripheral vascular disease.
Nizankowski 2005	This non-randomised single-arm study investigated the safety and therapeutic effects of BM-MNC implantation in CLI patients.
Ohtake 2017	This prospective phase 1/2 interventional clinical trial examined autologous granulocyte colony-stimulating factor (G-CSF)-mobilised CD34+ cell transplantation in HD patients with CLI.
Onodera 2011	This non-randomised trial compared BM-MNCs vs mPB-MNCs in CLI patients.
Ozturk 2012	This prospective randomised study evaluated the therapeutic effects of mobilised peripheral blood mononuclear cells vs control interventions in type 2 diabetic patients with CLI.
Peeters 2016	This study investigated quality of life between BM-MNCs and placebo in patients with severe limb ischaemia.
Perin 2011	This study used specialised cell products (aldehyde dehydogenase bright cells) that had been isolated from autologous bone marrow via immunomagmetic beads and cell sorters.
Perin 2017	This study randomised participants 1:1 to receive aldehyde dehydogenase bright (ALDHbr) cells or placebo.
Pignon 2017	This multi-centre randomised controlled double-blind clinical trial compared BM-MNCs vs control interventions in patients with CLI.
Ponemone 2017	This open-label single-arm feasibility study evaluated the safety and therapeutic effectiveness of autologous bone marrow cell (aBMC) concentrate.
Poole 2013	This RCT investigated the effects of granulocyte macrophage colony-stimulating factor (GM-CSF), not cell therapy, in patients with intermittent claudication

Study	Reason for exclusion
Powell 2012	This RCT assessed the safety and efficacy of patient-specific multi-cellular cell products (Ixmyelocel-T) that had been expanded from autologous bone marrow in an automated closed-culture system.
Prochazka 2010	This randomised study investigated major limb amputation between participants given autologous bone marrow cells compared to standard care for CLI and foot ulcer.
Rajagopalan 2003	This study assessed the effects of vascular endothelial growth factor, not cell therapy, in PAD patients.
Ruiz-Salmeron 2011	This non-randomised single-arm study demonstrated neoangiogenesis after intra-arterial infusion of autologous BM-MNCs in diabetic patients with CLI.
Saito 2007	This single-arm study investigated the therapeutic effects of IM implantation of BM-MNCs in CLI patients.
Schiavetta 2012	This multi-centre prospective not-controlled phase 2 study examined bone marrow stem cells for no-option CLI patients.
Smadja 2012	This phase 1 non-randomised study assessed the numbers of endothelial progenitor cells in peripheral blood and bone marrow cells. Results include no clinical outcome data.
Subramaniyam 2009	This study included patients with intermittent claudication and excluded patients with CLI or rest pain. Also, patients received subcutaneous GM-CSF, not stem cell treatment.
Szabo 2013	This RCT assessed the safety and efficacy of a specialised cultured cell product (VesCell) that had been expanded from autologous peripheral blood.
Takagi 2011	This study compared the therapeutic effects of autologous bone marrow-derived stem cells vs basic fibroblast growth factor.
Tateishi-Yuyama 2002	This RCT investigated BM-MNCs vs placebo and BM-MNCs vs PB-MNCs in CLI patients.
Teraa 2014	This study investigated BM-MNCs vs placebo in patients with CLI.
Teraa 2015	This randomised double-blind placebo-controlled clinical trial compared BM-MNCs vs placebo in patients with limb ischaemia.
Tournois 2015	This phase 1 and 2 clinical trial examined bone marrow-CTPs (cell therapy products) and CTPs obtained by cytapheresis (peripheral blood-CTPs).
Walter 2011	This multi-centre phase 2 trial with a double-blind randomised design evaluated the safety and feasibility BM-MNCs vs control interventions in patients with CLI.
Wang 2014	This RCT assessed the efficacy and safety of the combination of mPBSC and *Panax notoginseng* saponins (PNS) for treatment of CLI.
Wang 2017	This multi-centre randomised double-blind placebo-controlled trial was designed to assess the efficacy of intramuscular injections of cBMA for promoting amputation-free survival in patients with poor-option CLI.
Wang 2018	Study participants received intramuscular injections of allogeneic MSCs (CHAMP; n = 16) or autogenous concentrated bone marrow aspirate.
Wester 2008	This pilot study assessed the effects of autologous BM-MNCs in CLI patients.

Study	Reason for exclusion
Wijnand 2018	This study used allogeneic mesenchymal stem cells for CLI.
Yanishi 2017	This single-arm study used BM-MNCs for CLI.
Zafarghandi 2010	This study investigated the therapeutic effects of granulocyte colony-stimulating factor (G-CSF) administration following implantation of autologous BM-MNCs for patients with lower limb ischaemia. Both control and intervention groups received BM-MNCs.
Zhang 2016	This prospective non-randomised trial evaluated the efficacy and immune-regulatory impact of intra-arterial infusion of autologous CD133+ cells for diabetic patients with PAD.
Zhao 2008	Study participants received both autologous mononuclear bone marrow and peripheral blood mononuclear cells.
Zhou 2017	This randomised open parallel-control clinical study compared BM-MNC with SMT.
Zhou 2017a	This prospective single-centre open-label randomised controlled clinical trial compared peripheral blood CD34+ cells transfected with vascular endothelial growth factor 165 (VEGF165) vs SMT.

aBMC: autologous bone marrow cell.
ABMMNCs:autologous bone marrow-derived mononuclear cells.
ACPs: angiogenic cell precursors.
ACT: autologous cell therapy.
ADRCs: adipose-derived regenerative cells.
ALDHbr: aldehyde dehydrogenase bright.
ASO: arteriosclerosis obliterans.
BMCs: bone marrow cells.
BM-MNCs: bone marrow-mononuclear cells.
BM-MSCs: bone marrow-mesenchymal stem cells.
cBMAs: concentrated bone marrow aspirate.
CLI: critical limb ischaemia.
CTPs: cell therapy products.
ECs: endothelial cells.
EPCs: endothelial progenitor cells.
G-CSF: granulocyte colony-stimulating factor.
GM-CSF: granulocyte-macrophage colony-stimulating factor.
HA: hyaluronan.
HD: haemodialysis.
IA: intra-arterial.
IM: intramuscular.
MGA: MultiGeneAngio.
mPB-MNCs: mobilised peripheral blood mononuclear cells.
mPBSCs: mobilised peripheral blood stem cells.
MSCs: mesenchymal stem cells.
PAD: peripheral arterial disease.
PB-MNCs: peripheral blood mononuclear cells.
PBSCs: peripheral blood stem cells.
PM-MNCs: peripheral blood mononuclear cells.
PNS: *Panax notoginseng* saponins.
PTA: percutaneous transluminal angioplasty.
RCT: randomised controlled trial.
SMCs: smooth muscle cells.
SMT: Standard Medical Treatment.
VEGF: vascular endothelial growth factor.

Characteristics of studies awaiting assessment *[ordered by study ID]*

Gurunathan 2009

Methods	Multi-centre RCT
Participants	Sixty patients with lower limb CLI as per the Rutherford classification 4 or 5 were randomised to 2 arms
Interventions	IM injections (n = 30) IA + IM injections (n = 30)
Outcomes	1. TcO_2 2. ABI 3. Pain assessment (VAS)
Notes	Abstract, incomplete reporting of outcome data

Korymasov 2009

Methods	Randomised double-blind placebo-controlled study
Participants	Patients were subdivided into 3 groups, each consisting of 14 participants.
Interventions	Group 1: participants received autologous progenitor cells CD133+ Group 2: participants were given the leucocytic fraction of the marrow (CD34+) Group 3: participants (comparison group) received normal saline as a placebo
Outcomes	Clinical outcomes were evaluated according to the Rutherford scale, which revealed that group 1 and group 2 participants given the cellular material exhibited a statistically significant improvement in their clinical condition. Distance of pain-free walk was noted to statistically significantly increase in group 2 and group 2 participants.
Notes	This article was written in the Russian language could not be assessed fully, as we are still awaiting its translated full-text version.

Molavi 2016

Methods	Allocation: randomised controlled trial Endpoint classification: safety study Intervention model: parallel assignment Masking: open label Primary purpose: treatment
Participants	Inclusion criteria: 1. Ischaemic lower limb based on TASC guideline 2. Rutherford score: 2, 3 3. ABI < 0.6 4. Absolute ankle pressure < 60 mmHg

Molavi 2016 *(Continued)*

	5. Both genders 6. Age: 20 to 62 years Exclusion criteria: 1. EF < 30% 2. Creatinine > 2 3. HbA1c > 8% 4. Bone marrow disorders: leukaemia 5. Cognitive disorders 6. Infections 7. MI with ST elevation during past month 8. Malignancy 9. Immunological or rheumatological disorders
Interventions	1. 4 times injection of bone marrow-derived mononuclear stem cells 2. Mononuclear stem cell implantation by 1 intramuscular injection to the ischaemic lower limb
Outcomes	Primary outcome measures: side effects Secondary outcome measures: 1. PFWD 2. ABI 3. Size and depth of ulcer 4. Amputation 5. VAS 6. Wagner stage
Notes	

NCT00595257

Methods	Allocation: randomised Intervention model: parallel assignment Masking: open label Primary purpose: treatment
Participants	Inclusion criteria: 1. Diagnosis of critical limb ischaemia per protocol (see diagnostic criteria #2) with regard to the study limb. Existence of a PVD with clinical presentation corresponding to Rutherford category 4 or category 5 as defined in the reporting standards adopted by the Society of Vascular Surgeons (Table 1) 2. Meets at least 1 of the following diagnostic criteria in the study limb: ankle artery occlusion pressure absolute < 50 mmHg or ABI < 0.4; toe artery occlusive pressure < 40 mmHg or TBI (< 0.4); TcO_2 < 20 mmHg lying down breathing room air, if available 3. No reasonable open surgical or endovascular revascularisation option as determined by the treating vascular specialist. Factors that may contribute to the determination of inoperability may include anatomical considerations. No outflow targets. No appropriate conduit (i.e. vein for bypass). Long segment occlusions or calcified lesions that predict poor outcomes with endovascular approaches. High-risk medical conditions. Unstable cardiac disease. Renal insufficiency, history of prior failed revascularisation attempts. Unsuitability must be confirmed by 2 qualified physi-

NCT00595257 *(Continued)*

	cians. The attending vascular surgeon will provide the primary assessment. The confirmatory opinion must come from a fully licensed physician (not a resident). If anatomical considerations are invoked, the second physician may be a vascular surgeon, an interventional radiologist, a cardiologist, or a vascular medicine specialist. If medical comorbidity is deemed the high-risk aspect, the confirmatory opinion may be obtained from an internist, a family physician, a cardiologist, a vascular medicine specialist, a nephrologist, or a vascular surgeon. 4. Age > 18 years and ability to understand planned treatment 5. Has read and signed the IRB/IEC-approved informed consent form 6. Following medication(s) prescribed; must have a 1-month stable baseline of appropriate/maximally tolerated therapy before enrolment: Plavix/aspirin therapy, anticoagulation therapy, cholesterol-lowering agent, and/or blood pressure medication 7. Haematocrit ≥ 28.0%, white blood cell count ≤ 14,000, platelet count ≥ 50,000, INR ≤ 1.6 unless on Coumadin, or PTT < 1.5× control (to avoid bleeding complications). Patients on Coumadin will be corrected before the procedure and must have INR < 1.6 at the time of randomisation/surgery. Exclusion criteria: 1. Life expectancy < 6 months due to concomitant illnesses 2. History of bone marrow diseases (especially NHL, MDS) that prohibit transplantation 3. Terminal renal failure with existing dependence on dialysis 4. Known active malignancy or results outside of normal limits from the following tests: PAP, chest X-ray, PSA, mammogram, haemoccult unless follow-up studies reveal patient to be cancer free 5. Poorly controlled diabetes mellitus (HbA1C > 10%) 6. Medical risk that precludes anaesthesia (conscious sedation), or ASA Class 5 7. Life-threatening complications of ischaemia necessitating immediate amputation 8. Uncorrected iliac artery occlusion on index side 9. Extensive necrosis of the index limb or other condition that makes amputation inevitable (Rutherford category 6) 10. Active clinical infection treated by antibiotics within 1 week of enrolment 11. Treatment with immunosuppressant drugs (including prednisone > 5 mg per day) 12. Female who is pregnant or nursing, or of child-bearing potential and is not using a reliable birth control method 13. Underwent a major cardiovascular surgical procedure (carotid endarterectomy, open arterial aneurysm or bypass surgery, or coronary artery bypass surgery) or an adverse cardiovascular event (stroke or MI) within the 30 days before randomisation
Interventions	Experimental: Injection of BMAC into ischaemic limb. Intervention: device: centrifuge, laboratory, tabletop (SmartPReP2 BMAC System) Active comparator: injection and infusion of BMAC into ischaemic lower limb. Intervention: device: centrifuge, laboratory, tabletop (SmartPReP2 BMAC System)
Outcomes	Primary outcome measures: avoidance of amputation (time frame: 60 days) Secondary outcome measures: measurement of haemodynamic response (time frame: 60 days)
Notes	Clinical trial protocol without outcome data. According to ClincialTrials.gov, this study has been completed.

NCT00987363

Methods	Allocation: randomised Endpoint classification: safety/efficacy study Intervention model: parallel assignment

NCT00987363 *(Continued)*

	Masking: open label Primary purpose: treatment
Participants	Inclusion criteria: 1. Type 1 or 2 diabetes mellitus 2. Grade II-III Rutherford-Becker peripheral vascular disease affecting at least 1 limb 3. Arterial obstruction(s) located at infrapopliteal level 4. No options of endoarterial or surgical revascularisation 5. Life expectancy > 2 years 6. Unlikelihood of major amputation of the leg during the next 12 months 7. Normal analytical parameters in blood: leucocytes > 3000/microL, neutrophils > 1500/microL, Hb > 10 mg/dL, platelets > 100,000/microL, AST and ALT < 2.5 standard value, creatinine < 2.5 mg/dL 8. Written informed consent 9. Negative pregnancy test when applicable Exclusion criteria: 1. History of neoplasm or haematological disease 2. Uncontrolled high blood pressure (> 180/110) 3. Severe cardiac insufficiency (NYHA IV) or ejection fraction < 30% 4. Malignant ventricular arrhythmia 5. Deep venous thrombosis during the past 3 months 6. Active bacterial infection 7. Treatment with hyperbaric oxygen, vasoactive drugs, COX-II inhibitors or antiangiogenic agents 8. Body mass index > 40 9. Alcoholism 10. Proliferative retinopathy 11. HIV, HBV, or HCV viral infection 12. Stroke or myocardial infarction during the past 3 months
Interventions	1. Group 1 (15 participants): no cell therapy. 2. Group 2 (15 participants): 1×10^8 mononuclear cells of bone marrow 3. Gropu 3 (15 participants): 5×10^8 mononuclear cells of bone marrow 4. Group 4 (15 participants): 1×10^9 mononuclear cells of bone marrow 5. Cell therapy medicinal product shall be administered in all cases intra-arterially.
Outcomes	Primary outcome measures: 1. Number of adverse events 2. AngioRNM and/or AngioTC changes Secondary outcome measures: 1. Clinically objective improvement in the ischaemic limb 2. Ankle-brachial index, transcutaneous oxygen tension, degree of Rutherford-Becker, greater ulcer size and perimeter calf muscle
Notes	Clinical trial protocol without outcome data. According to ClincialTrials.gov, this study has been completed.

NCT02993809

Methods	Allocation: randomised

NCT02993809 *(Continued)*

	Intervention model: parallel assignment Masking: open label Primary purpose: treatment
Participants	Inclusion criteria: limb ischaemia patients (e.g. arteriosclerosis obliterans, diabetic critical limb ischemia, thromboangiitis obliterans) 1. 18 to 80 years (adult, senior) 2. Ability to understand and comply with study requirements and provide written informed consent before any procedures 3. Meets at least 1 of the following diagnostic criteria for the index limb: ABI < 0.7 mmHg, $TcPO_2$ < 40 mmHg or non-healing ulcer due to local arterial compromise with no opportunity for revascularisation 4. No improvement after conservative treatment and not suitable for surgical bypass surgery because no outflow tract of diseased vessel can be found by imaging 5. Despite having good outflow artery, elderly and frail patients cannot tolerate revascularisation or interventional surgery 6. Unlikelihood of major amputation of the leg during the next 12 months 7. Expected life span more than 2 years Exclusion criteria: 1. Pregnant or lactating 2. Diabetic individual with poorly controlled blood glucose levels (defined as HbA1c > 7% and/or proliferative retinopathy) 3. Decompensated cardiac, renal, or liver disease 4. Confirmed malignant tumour 5. Serious heart, liver, kidney, and lung failure or poor general condition with inability to undergo bone marrow harvesting and transplantation 6. Known or suspected disease of the immune system or osteomyelitis 7. Inability to sign informed consent form and to comply with the schedule of the study 8. Reason to suspect that the patient is forced to join the study 9. Any other condition, in the opinion of the investigator, that would render the patient unsuitable for the study
Interventions	1. Experimental: BM-ECs and PRPE (multi-point of intramuscular injections into ischaemic limbs; injections composed of bone marrow-derived endothelial cells (BM-ECs) and platelet-rich plasma extract (PRPE)) 2. Active comparator: BM-ECs (intramuscular injection of bone marrow-derived endothelial cells only)
Outcomes	Primary outcome measure: 1. Survival without major amputation Secondary outcome measures: 1. Perfusion rate in treated tissue by measure of ABI 2. Perfusion rate in treated tissue by $TcPO_2$ 3. Perfusion rate in treated tissue by digital subtraction angiography 4. Wound size 5. Wound stage 6. Pain intensity 7. Thermography

NCT02993809 *(Continued)*

Notes	According to ClincialTrials.gov, this study is not yet open for participant recruitment.

ABI: ankle-brachial pressure index.
ALT: alanine aminotransferase.
AngioRNM: magnetic resonance angiography.
AngioTC: computed tomography angiography.
ASA: American Society of Anesthesiologists.
AST: aspartate aminotransferase.
BM-ECs: bone marrow endothelial cells.
BM-MNCs: bone marrow-mononuclear cells.
BMACs: bone marrow cells.
BMT: bone marrow transplant.
CLI: critical limb ischaemia.
COX: cyclo-oxygenase.
EF: ejection fraction.
G-CSF: granulocyte colony-stimulating factor.
Hb: haemoglobin.
HbA1c: glycosylated haemoglobin.
HBV: hepatitis B virus.
HCV: hepatitis C virus.
HIV: human immunodeficiency virus.
IA: intra-arterial.
IM: intramuscular.
INR: international normalised ratio.
IRB/IEC: institutional review board/independent ethics committee.
MDS: myelodysplastic syndrome.
MI: myocardial infarction.
NHL: non-Hodgkin lymphoma.
NYHA: New York Heart Association.
PAP: Papanicolaou test.
PB-MNCs: peripheral blood-mononuclear cells.
PFWD: pain-free walking distance.
PRPE: platelet-rich plasma extract.
PSA: prostate-specific antigen.
PTT: partial thromboplastin time.
PVD: peripheral vascular disease.
RCT: randomised controlled trial.
TASC: TransAtlantic Intersociety Consensus.
TBI: toe-brachial index.
TcO_2: transcutaneous oxygen tension.
$TcPO_2$: transcutaneous oxygen.
VAS: visual analogue scale.

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

NCT00311805

Trial name or title	Autologous CD34+ stem cell injection for severe intermittent claudication (leg pain)
Methods	Allocation: randomised Endpoint classification: safety/efficacy study Intervention model: parallel assignment Masking: double-blind (patient, investigator) Primary purpose: treatment
Participants	Inclusion criteria:

NCT00311805 *(Continued)*

	1. Males or females 21 years of age or older 2. Infra-inguinal atherosclerosis with a stenosis or occlusion of a major vessel in the affected limb(s) of 1 or more of the following arteries: superficial femoral, popliteal, or 1 or more infrapopliteal arteries, which is/are non-reconstructable 3. Symptoms of severe intermittent claudication in at least 1 lower limb persisting for at least 6 months (Rutherford class 3) 4. Diagnosis of PAD in at least 1 lower limb secondary to atherosclerosis, for at least 6 months Exclusion criteria: 1. Successful aortic or lower extremity arterial surgery, angioplasty, or lumbar sympathectomy within 3 months preceding screening 2. Iliac disease amenable to revascularisation 3. Judged to be a suitable candidate for surgical or percutaneous revascularisation in the limb in which treatment is proposed 4. CLI with Rutherford Symptom Score of 4, 5, or 6 5. Arterial insufficiency in the lower extremity as the result of a non-atherosclerotic disorder
Interventions	Biological: autologous stem cells (CD34+), intramuscular injections
Outcomes	Primary outcome measures: safety of intramuscular administration of CD34-positive cells (time frame: all) Secondary outcome measures: functional improvement (time frame: week 12, month 6, month 12)
Starting date	April 2006
Contact information	
Notes	Primary completion date: December 2012 (final data collection date for primary outcome measure)

NCT00753025

Trial name or title	Autologous bone marrow for lower extremity ischemia treating
Methods	Allocation: randomised Endpoint classification: safety/efficacy study Intervention model: parallel assignment Masking: quadruple (participant, care provider, investigator, outcomes assessor) Primary purpose: treatment
Participants	Inclusion criteria: 1. Obliterating lower extremity atherosclerosis IIB stage (on Fontaine classification) 2. Painless walking distance of 10 to 50 m 3. Pulse absence on aa dorsalis pedis, tibialis posterior, poplitea 4. Absence of ischaemia in rest and necrotic changes 5. Mainly distal form of disease (lesion of a superficial femoral artery, a popliteal artery, anticnemion arteries) according to an angiography that testifies to impossibility of reconstructive operation performance 6. After lumbar sympathectomy and tibial bone osteoperforations executed previously 7. Heavy smokers Exclusion criteria:

NCT00753025 *(Continued)*

	1. Insulin-dependent diabetes 2. Myocardial infarction or stroke within the past year 3. Idiopathic hypertension III stage 4. Anaemia and other diseases of blood 5. Decompensation of chronic diseases that are contraindications to any surgical operation 6. HIV infection 7. A virus hepatitis 8. Oncological disease 9. Chemotherapy in the anamnesi
Interventions	Biological: injection of isolated CD 133+ cells
Outcomes	Primary outcome measure: increase in painless walking distance
Starting date	September 2008
Contact information	
Notes	Primary completion date: September 2008 (final data collection date for primary outcome measure)

NCT01257776

Trial name or title	Human adipose derived mesenchymal stem cells for critical limb ischemia (CLI) in diabetic patients
Methods	Allocation: randomised Endpoint classification: safety/efficacy study Intervention model: parallel assignment Masking: none (open label) Primary purpose: treatment
Participants	Inclusion criteria: 1. Diabetes, type 1 or 2 2. Critical limb ischaemia (Rutherford Becker Class II, III, or IV) of at least 1 limb 3. No options for target limb revascularisation Exclusion criteria: 1. Cancer antecedent in the past 2 years 2. Current limb infection or limb gangrene
Interventions	Biological: autologous adipose-derived mesenchymal stem cells
Outcomes	Primary outcome measures: angiographic assessment of neovasculogenesis (angiogenesis plus arteriogenesis) (time frame: 6 months) Secondary outcome measures: ankle-brachial index (time frame: 1 month, 6 months, 12 months); University of Texas Classification at target limb (time frame: 1 month, 6 months, 12 months)
Starting date	December 2010
Contact information	

NCT01257776 *(Continued)*

Notes	Primary completion date: August 2015 (final data collection date for primary outcome measure)

NCT01408381

Trial name or title	Intra-arterial Infusion of autologous bone marrow mononuclear cells in non-diabetic patients with critical limb ischaemia (CLI)
Methods	Allocation: randomised Endpoint classification: safety/efficacy study Intervention model: parallel assignment Masking: none (open label) Primary purpose: treatment
Participants	Inclusion criteria: 1. Patients of both sexes aged ≥ 18 and ≤ 89 years 2. Non-diabetic 3. Infrapopliteal atherosclerotic vascular disease with severe to severe claudication or Rutherford-Becker grade I-3, 2, 3, in at least 1 lower limb. Chronic critical ischaemia of the lower limb is defined as persistent/recurring pain requiring analgesia and/or non-healing ulcers present > 4 weeks, with no evidence of improvement with conventional therapies and/or walking test (stress test) between 1 and 6 minutes - 2 exercise tests separated by at least 2 weeks and/or ankle-brachial index at rest < 0.8 4. Inability to undergo endovascular or surgical revascularisation as recommended by the TransAtlantic Intersociety Consensus (TASC) 5. Failure of the revascularisation surgical procedure performed at least 30 days before, either persistently or at entry into the critical ischaemia phase 6. Life expectancy > 2 years 7. Not expected major amputation in the limb to be treated the next 6 months after inclusion 8. Normal laboratory parameters, defined by: a. Leucocytes ≥ 3000 b. Neutrophils ≥ 1500 c. Platelets ≥ 100,000 d. Aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ≤ 2.5 standard range institution e. Creatinine ≤ 2.5 mg/dL 9. Written informed consent to participate in the study 10. Women of child-bearing potential must have negative results on a pregnancy test following standard procedures for each hospital performed at the time of inclusion in the study and must agree to use a medically approved method of contraception throughout the duration of the study Exclusion criteria: 1. History of malignancy or haematological disease (myeloproliferative disease, myelodysplastic syndrome, or leukaemia) 2. Uncontrolled hypertension (defined as blood pressure > 180/110 on more than 1 occasion) 3. Severe heart failure (New York Heart Association IV) 4. Malignant ventricular arrhythmias or unstable angina 5. Diagnosis of deep vein thrombosis in the previous 3 months 6. Active infection or gangrene wet day infusion of mononuclear bone marrow cells 7. Corporal mass index (BMI) > 40 kg/m^2

NCT01408381 *(Continued)*

	8. Diagnosis of alcoholism at the time of inclusion 9. Proliferative retinopathy
Interventions	Biological: intra-arterial infusion of autologous bone marrow mononuclear cells
Outcomes	Primary outcome measures: adverse events (time frame: 6 months) Secondary outcome measures: 1. Ankle-brachial index (time frame: 1 month, 3 months, 6 months) 2. Transcutaneous oxygen pressure (TcO_2) (time frame: 1 month, 3 months, 6 months) 3. Greater ulcer size (time frame: 1 month, 3 months, 6 months) (ulcer diameter will be recorded) 4. Degree of Rutherford-Becker (time frame: 1 month, 3 months, 6 months) 5. Perimeter calf muscle (time frame: 1 month, 3 months, 6 months) 6. Presence of faster opacity in infrapopliteal vessels at 6 months compared with the basal situation of the patient (time frame: 1 month, 3 months, 6 months)
Starting date	April 2006
Contact information	
Notes	Primary completion date: December 2012 (final data collection date for primary outcome measure)

NCT01446055

Trial name or title	Safety and efficacy study of autologous BM-MNC processed by two methods for treating patients with chronic limb ischemia
Methods	Allocation: randomised Endpoint classification: safety/efficacy study Intervention model: parallel assignment Masking: single-blind (patient) Primary purpose: treatment
Participants	Inclusion criteria: 1. Fontaine stage 2 to 4 or resting ABI < 0.7 2. Age between 20 and 80 years 3. Signed informed consent, voluntary participants 4. Diagnosis of lower extremity arterial occlusive disease, or diabetic lower limb ischaemia, or Buerger's disease Exclusion criteria: 1. Poorly controlled diabetes (HBA1c > 7.0%) and proliferative retinopathy (III to IV stage) 2. Malignancy history in the past 5 years or serum level of tumour markers elevated more than doubled 3. Severe heart, liver, kidney, respiratory failure or poor general condition and cannot tolerate BM-MNC implantation 4. Serious infections (such as cellulitis, osteomyelitis, etc.) or gangrene such that a major amputation cannot be avoided 5. Aortic or iliac or common femoral artery occlusion

NCT01446055 *(Continued)*

	6. Pregnant female, or reproductive age female, who wants to give birth throughout the course of the study 7. Life expectancy less than 1 year
Interventions	Experimental: autologous BM-MNC is enriched with ResQ process (an automatic cell separator). Then the cell product is implanted into the ischaemic limbs of a patient Active comparator: a conventional method based on Ficoll cell separation is used to process bone marrow
Outcomes	Primary outcome measures: • Cell treatment-related adverse event: temperature, pulse, respiration, blood pressure, routine analysis of blood and urine, liver function (ALT: alanine aminotransferase, AST; aspartate transferase), renal function (blood urea nitrogen, creatinine), function of coagulation (APTT, prothrombin time, fibrinogen, thrombin time), ECG (electrocardiography), local inflammatory response, cell treatment-related death, cell treatment-related unexpected amputation. Secondary outcome measures: • Ulcer size • Rest pain score. • Cold sensation score • Claudication distance (m) • Resting ABI • Resting TcO_2 (mmHg) • Collateral vessel score • Amputation rate • Skin microcirculation measurement • Resting TBI
Starting date	October 2011
Contact information	Contact: Yongquan Gu, MD; 13910002909; gu-yq@263.net
Notes	The recruitment status of this study is unknown because the information has not been verified recently on clinical trials.gov.

NCT01745744

Trial name or title	Application of cell regeneration therapy with mesenchymal stem cells from adipose tissue in critical chronic ischaemic syndrome of lower limbs (CLI) in non-diabetic patients
Methods	Allocation: randomised Endpoint classification: safety/efficacy study Intervention model: parallel assignment Masking: open label Primary purpose: treatment
Participants	Inclusion criteria: • Patients of both sexes aged ≥ 18 and ≤ 89 years • Non-diabetic

NCT01745744 *(Continued)*

- Infrapopliteal atherosclerotic vascular disease of severe grade with either severe claudication or Rutherford-Becker grade II-III-IV, of at least 1 lower limb. Critical ischaemia of the lower limb is defined as persistent/recurrent pain requiring analgesia and/or non-healing present ulcers > 4 weeks, with no evidence of improvement with conventional therapies and/or walking test (stress test) between 1 and 6 minutes in 2 exercise tests separated by at least 2 weeks and/or ankle-brachial index at rest < 0.8
- Inability of surgical or endovascular revascularisation as recommended by the TransAtlantic Intersociety Consensus (TASC)
- Failure in the revascularisation surgery performed at least 30 days before, with persistence or entry into critical ischaemia phase
- Life expectancy > 2 years
- Not expected major amputation in the limb to be treated in the next 12 months after inclusion
- Normal biochemical parameters defined by leucocytes ≥ 3000, neutrophils ≥ 1500, platelets ≥ 100,000, aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ≤ 2.5 standard range institution, creatinine ≤ 2.5 mg/dL
- Written informed consent to participate in the study
- Women of child-bearing potential must have negative results on a pregnancy test done at the time of inclusion in the study and agree to use a medically approved method of contraception for the duration of the study

Exclusion criteria:

- History of malignancy or haematological disease (myeloproliferative disease, myelodysplastic syndrome, or leukaemia) in the past 2 years
- Uncontrolled hypertension (defined as blood pressure > 180/110 on more than 1 occasion)
- Severe heart failure (New York Heart Association Class IV) or ejection fraction of the left ventricle < 30%
- Malignant ventricular arrhythmias or unstable angina at the time of infusion
- Diagnosis of deep vein thrombosis in the previous 3 months
- Active infection or wet gangrene at day of infusion of mesenchymal stem cells from adipose tissue
- Concomitant therapy including hyperbaric oxygen. This allowed the use of antiplatelet agents
- Body mass index > 40 kg/m^2
- Diagnosis of alcoholism at the time of inclusion
- Untreated proliferative retinopathy
- Concomitant disease that reduces life expectancy to less than a year
- Predicted impossibility to obtain a biopsy providing 10 g of adipose tissue
- Human immunodeficiency virus, hepatitis B virus, or hepatitis C virus
- Difficulty in monitoring
- Stroke or myocardial infarction within the past 3 months
- Anaemia (haemoglobin < 7.9 mg/dL)
- Leucopaenia
- Thrombocytopaenia (< 100,000 platelets/uL)
- Pregnant women or women of child-bearing age who do not have adequate contraception
- Participated in a clinical trial within the past 3 months before inclusion in this clinical trial

Interventions	Experimental: low dose • Infusion of mesenchymal stem cells from adipose tissue: 0.5 × 10^6 cells/kg patient weight Experimental: high dose • Infusion of mesenchymal stem cells from adipose tissue: 1 × 10^6 cells/kg patient weight No intervention: control • Conventional treatment

NCT01745744 *(Continued)*

Outcomes	Primary outcome measures: numbers of adverse events and serious adverse events Secondary outcome measures: evolution of chronic critical ischaemia parameters: ankle-brachial index, transcutaneous oxygen tension, degree of Rutherford-Becker, larger ulcer size (as Texas classification), twin perimeter, scores for pain and intermittent claudication (walking test)
Starting date	February 2011
Contact information	
Notes	This study is ongoing but is not recruiting participants.

NCT02454231

Trial name or title	Monocentric trial: stem cell emergency life threatening limbs arteriopathy (SCELTA)
Methods	Allocation: randomised Endpoint classification: safety/efficacy study Intervention model: parallel assignment Masking: open label Primary purpose: treatment
Participants	Inclusion criteria: • Men and women older than 40 years of age with a diagnosis of CLI due to atherosclerosis of the lower extremities, as defined by the presence of persistent rest pain requiring systemic and continued analgesic treatment in the past 15 days and/or the presence of trophic lesions imputable to the occluding arteriopathy, ankle-brachial Index (ABI) < 0.40 (with systolic ankle pressure < 50 to 70 mmHg), toe-brachial index (TBI) < 0.40 (with big toe systolic pressure < 30 to 50 mmHg), and transcutaneous oxygen pressure (TcO_2) < 30 mmHg • Eligible for treatment and enrolled only after demonstration that intravascular or surgical revascularisation was not possible, as revealed by echography and angio-CAT, or when the patient refused to undergo surgical treatments and after written informed consent was obtained Exclusion criteria: • Age < 40 • Not atherosclerotic CLI • Myocardial infarction occurrence within 6 months • Cardiac failure of III-IV class NYHA • Ejection fraction < 40% • Arterial hypertension (> 160/100 mmHg) uncontrolled despite usage of 2 antihypertensive drugs • Presence of current or chronic severe infectious disease • Osteomyelitis • Diabetes with glycate haemoglobin > 7.5 • Proliferative diabetic retinopathy • Haemorrhagic disorders • Non-atherosclerotic arteriopathy • Chronic airway insufficiency (pO_2 < 65 mmHg, pCO_2 > 0.50 mmHg) • Renal failure (creatinine > 2 mg/dL) • Contraindications or intolerance to contrast media for radiological imaging
Interventions	Experimental: peripheral blood EPC injection

NCT02454231 *(Continued)*	Active comparator: bone marrow MNC injection
Outcomes	Primary outcome measures: • Safety as measured by evaluation of any adverse event temporarily correlated with treatment • Changes in ischaemic leg perfusion from baseline Secondary outcome measures: • Improvement in mean values of transcutaneous partial oxygen tension (TcO_2) • Improvement in mean values of ankle-brachial pressure index (ABI) • Improvement in vessel anatomical status • Improvement in leg perfusion • Improvement in vessel anatomical status • Quality of life improvement • Improvement in rest pain • Improvement in trophic limb lesions • Reduction in number of major amputations • Improvement in microvascular anatomy
Starting date	September 2009
Contact information	Contact: Enrico Maggi, professor; +39 055/2751802; enrico.maggi@unifi.it Contact: Francesco Annunziato, professor; +39 0552758337; francesco.annunziato@unifi.it
Notes	Primary completion date: May 2015 (final data collection date for primary outcome measure)

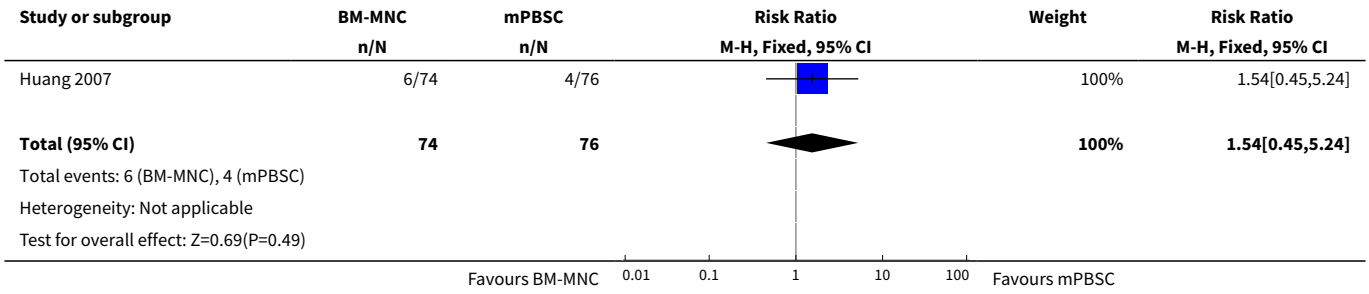
ABI: ankle-brachial pressure index.
ALT: alanine aminotransferase.
APTT: activated partial thromboplastin time.
AST: aspartate aminotransferase.
BM-MNCs: bone marrow-mononuclear cells.
BMI: body mass index.
CLI: critical limb ischaemia.
ECG: electrocardiography.
EPC: endothelial progenitor cell.
HbA1c: glycosylated haemoglobin.
HIV: human immunodeficiency virus.
IM: intramuscular.
MNC: mononuclear cell.
NYHA: New York Heart Association.
PAD: peripheral arterial disease.
PFWD: pain-free walking distance.
pCO_2: partial pressure of carbon dioxide.
pO_2: partial pressure of oxygen.
RCT: randomised controlled trial.
TASC: TransAtlantic Intersociety Consensus..
TBI: toe-brachial index.
TcO_2: transcutaneous oxygen tension.

DATA AND ANALYSES

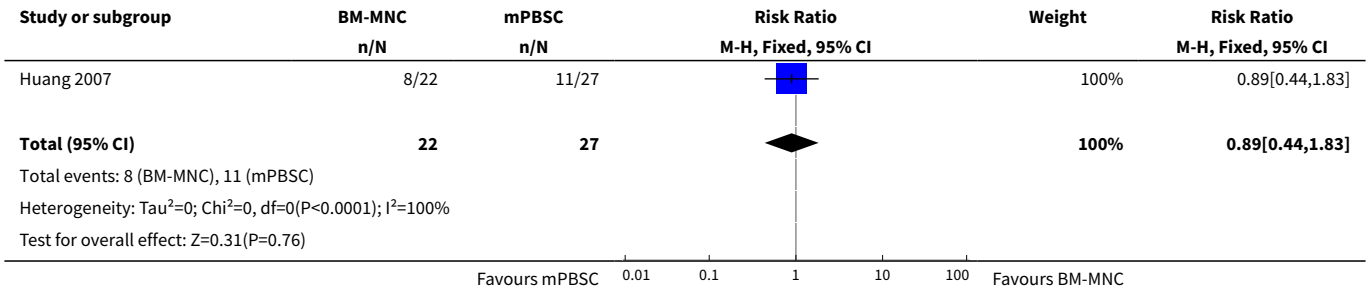
Comparison 1. BM-MNCs vs mPBSCs

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Amputation rate	1	150	Risk Ratio (M-H, Fixed, 95% CI)	1.54 [0.45, 5.24]
2 Wound/ulcer healing: number of participants with healing ulcers	1	49	Risk Ratio (M-H, Fixed, 95% CI)	0.89 [0.44, 1.83]
3 Wound/ulcer healing: change in ulcer size	1	49	Mean Difference (IV, Fixed, 95% CI)	-1.06 [-5.35, 3.23]
4 Reduction in rest pain: number of participants with any reduction in rest pain score	1	104	Risk Ratio (M-H, Fixed, 95% CI)	0.99 [0.93, 1.06]
5 Reduction in rest pain: rest pain score	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
5.1 12 weeks	1	150	Mean Difference (IV, Fixed, 95% CI)	-0.57 [-0.90, -0.24]
6 Improvement in lower limb perfusion: number of participants with increased ABI	1	104	Risk Ratio (M-H, Fixed, 95% CI)	1.00 [0.71, 1.40]
7 Improvement in lower limb perfusion: ABI score	1	150	Mean Difference (IV, Fixed, 95% CI)	-0.06 [-0.09, -0.03]
8 Improvement in lower limb perfusion: TcO_2 reading in mmHg	1	150	Mean Difference (IV, Fixed, 95% CI)	1.70 [0.41, 2.99]
9 Improvement in ischaemic symptoms: PFWD in metres at 12 weeks	1	150	Mean Difference (IV, Fixed, 95% CI)	33.05 [-37.69, 103.79]

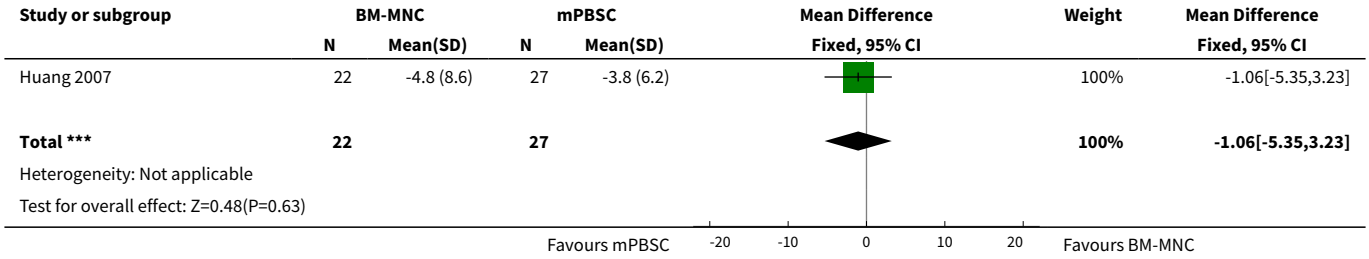
Analysis 1.1. Comparison 1 BM-MNCs vs mPBSCs, Outcome 1 Amputation rate.



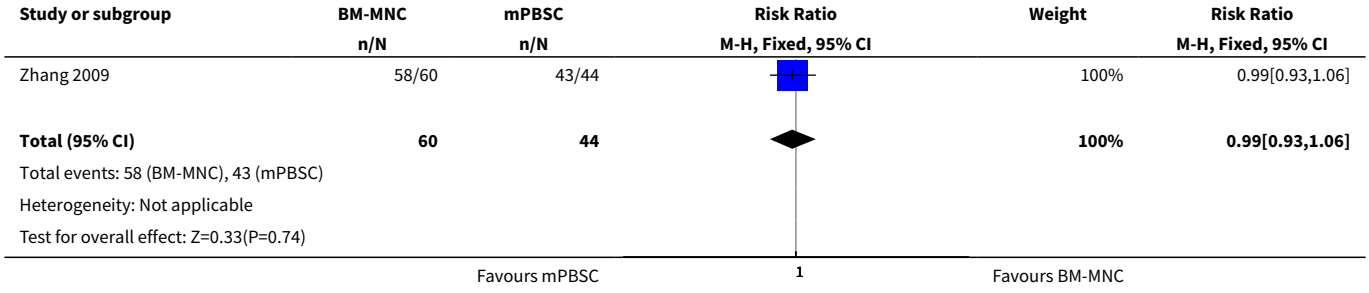
Analysis 1.2. Comparison 1 BM-MNCs vs mPBSCs, Outcome 2 Wound/ulcer healing: number of participants with healing ulcers.



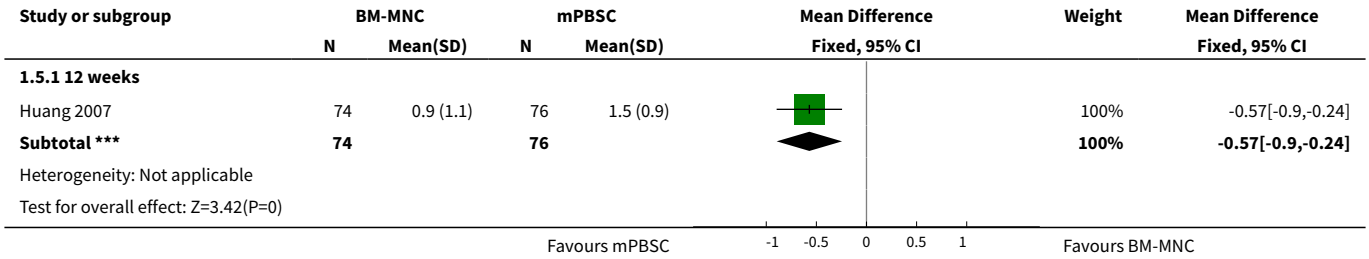
Analysis 1.3. Comparison 1 BM-MNCs vs mPBSCs, Outcome 3 Wound/ulcer healing: change in ulcer size.



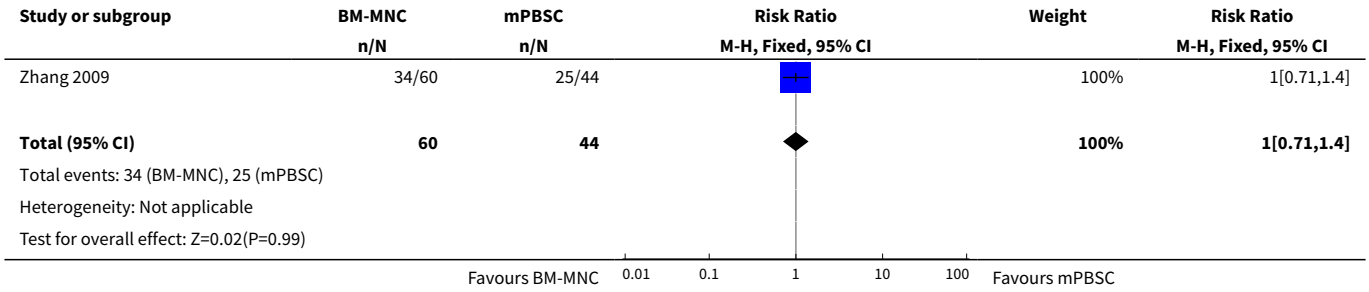
Analysis 1.4. Comparison 1 BM-MNCs vs mPBSCs, Outcome 4 Reduction in rest pain: number of participants with any reduction in rest pain score.



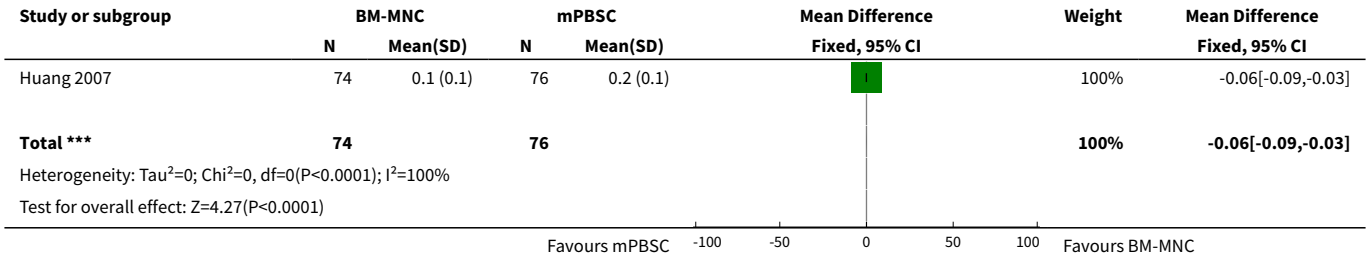
Analysis 1.5. Comparison 1 BM-MNCs vs mPBSCs, Outcome 5 Reduction in rest pain: rest pain score.



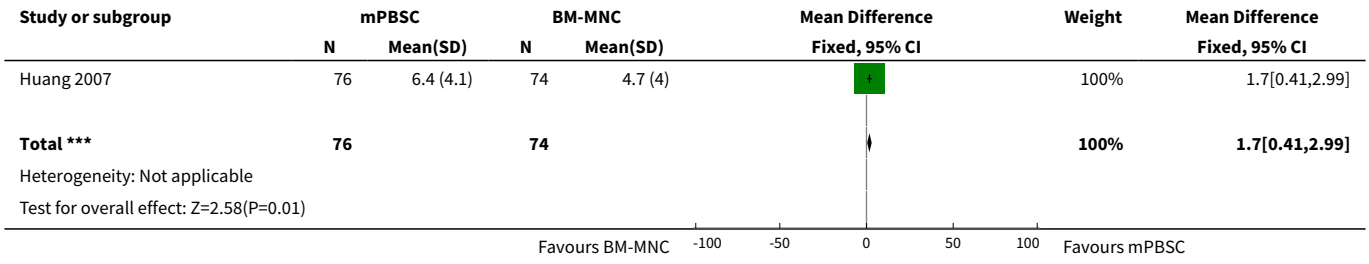
Analysis 1.6. Comparison 1 BM-MNCs vs mPBSCs, Outcome 6 Improvement in lower limb perfusion: number of participants with increased ABI.



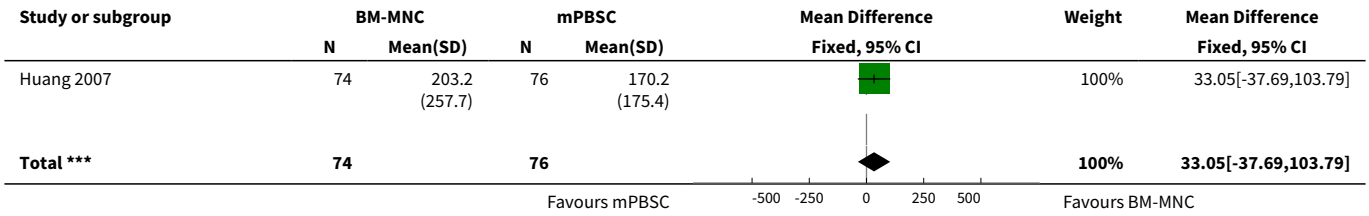
Analysis 1.7. Comparison 1 BM-MNCs vs mPBSCs, Outcome 7 Improvement in lower limb perfusion: ABI score.



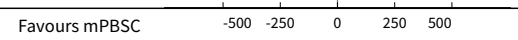
Analysis 1.8. Comparison 1 BM-MNCs vs mPBSCs, Outcome 8 Improvement in lower limb perfusion: TcO_2 reading in mmHg.



Analysis 1.9. Comparison 1 BM-MNCs vs mPBSCs, Outcome 9 Improvement in ischaemic symptoms: PFWD in metres at 12 weeks.



Study or subgroup	BM-MNC N	BM-MNC Mean(SD)	mPBSC N	mPBSC Mean(SD)	Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
Heterogeneity: Not applicable							
Test for overall effect: Z=0.92(P=0.36)							

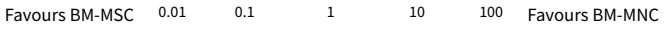


Comparison 2. BM-MNCs vs BM-MSCs

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Amputation rate	1	37	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
2 Wound/ulcer healing: number of participants with healing ulcers	1	22	Risk Ratio (M-H, Fixed, 95% CI)	2.0 [1.02, 3.92]
3 Reduction in rest pain: rest pain score	1	37	Mean Difference (IV, Fixed, 95% CI)	0.0 [-0.61, 0.61]
4 Improvement in lower limb perfusion: ABI score	1	37	Mean Difference (IV, Fixed, 95% CI)	0.05 [0.01, 0.09]
5 Improvement in lower limb perfusion: TcO_2 reading in mmHg	1	37	Mean Difference (IV, Fixed, 95% CI)	8.0 [3.46, 12.54]
6 Improvement in ischaemic symptoms: PFWT in minutes at 24 weeks	1	37	Mean Difference (IV, Fixed, 95% CI)	0.60 [-0.01, 1.21]
7 Improvement in vascularity and blood supply: number of participants with increase in numbers of collateral vessels	1	37	Risk Ratio (M-H, Fixed, 95% CI)	1.98 [1.12, 3.49]

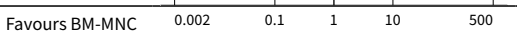
Analysis 2.1. Comparison 2 BM-MNCs vs BM-MSCs, Outcome 1 Amputation rate.

Study or subgroup	BM-MSC n/N	BM-MNC n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
Lu 2011	0/18	0/19			Not estimable
Total (95% CI)	**18**	**19**			**Not estimable**
Total events: 0 (BM-MSC), 0 (BM-MNC)					
Heterogeneity: Not applicable					
Test for overall effect: Not applicable					



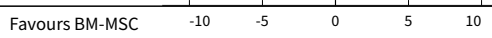
Analysis 2.2. Comparison 2 BM-MNCs vs BM-MSCs, Outcome 2 Wound/ulcer healing: number of participants with healing ulcers.

Study or subgroup	BM-MSC n/N	BM-MNC n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
Lu 2011	10/11	5/11		100%	2[1.02,3.92]
Total (95% CI)	**11**	**11**		**100%**	**2[1.02,3.92]**
Total events: 10 (BM-MSC), 5 (BM-MNC)					
Heterogeneity: Not applicable					
Test for overall effect: Z=2.02(P=0.04)					



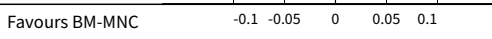
Analysis 2.3. Comparison 2 BM-MNCs vs BM-MSCs, Outcome 3 Reduction in rest pain: rest pain score.

Study or subgroup	BM-MSC N	BM-MSC Mean(SD)	BM-MNC N	BM-MNC Mean(SD)	Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
Lu 2011	18	1.4 (1)	19	1.4 (0.9)		100%	0[-0.61,0.61]
Total ***	**18**		**19**			**100%**	**0[-0.61,0.61]**
Heterogeneity: Not applicable							
Test for overall effect: Not applicable							



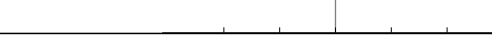
Analysis 2.4. Comparison 2 BM-MNCs vs BM-MSCs, Outcome 4 Improvement in lower limb perfusion: ABI score.

Study or subgroup	BM-MSC N	BM-MSC Mean(SD)	BM-MNC N	BM-MNC Mean(SD)	Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
Lu 2011	18	0.2 (0.1)	19	0.1 (0.1)		100%	0.05[0.01,0.09]
Total ***	**18**		**19**			**100%**	**0.05[0.01,0.09]**
Heterogeneity: Not applicable							
Test for overall effect: Z=2.53(P=0.01)							

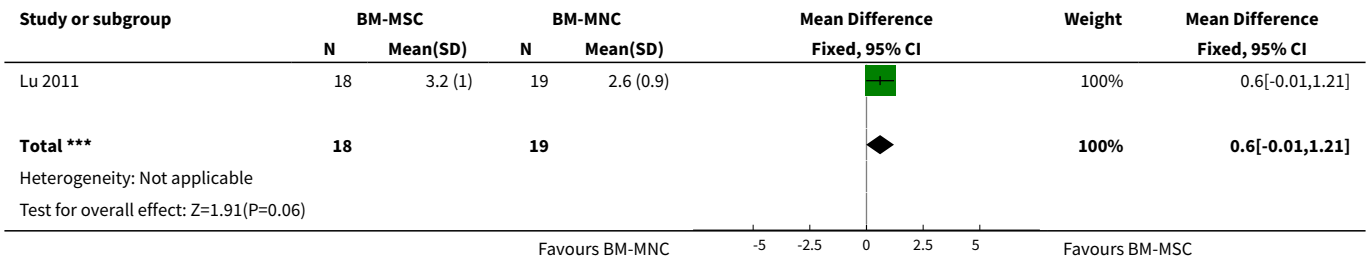


Analysis 2.5. Comparison 2 BM-MNCs vs BM-MSCs, Outcome 5 Improvement in lower limb perfusion: TcO_2 reading in mmHg.

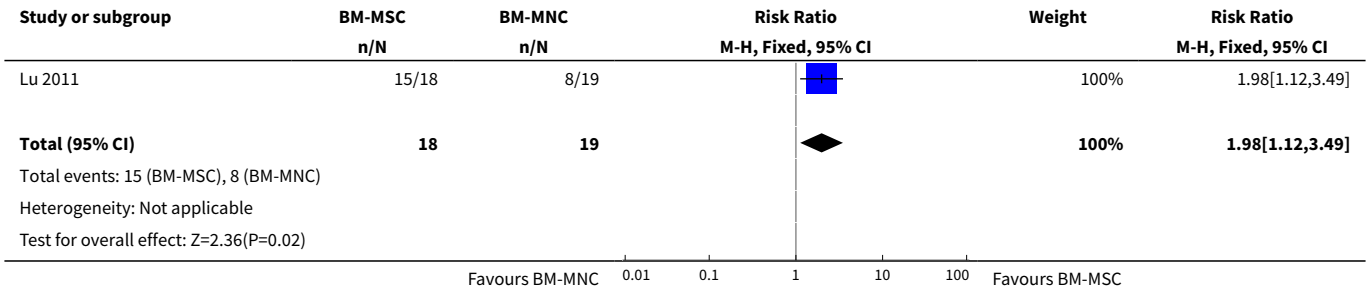
Study or subgroup	BM-MSC N	BM-MSC Mean(SD)	BM-MNC N	BM-MNC Mean(SD)	Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
Lu 2011	18	24.4 (7.6)	19	16.4 (6.4)		100%	8[3.46,12.54]
Total ***	**18**		**19**			**100%**	**8[3.46,12.54]**
Heterogeneity: Not applicable							
Test for overall effect: Z=3.45(P=0)							



Analysis 2.6. Comparison 2 BM-MNCs vs BM-MSCs, Outcome 6 Improvement in ischaemic symptoms: PFWT in minutes at 24 weeks.



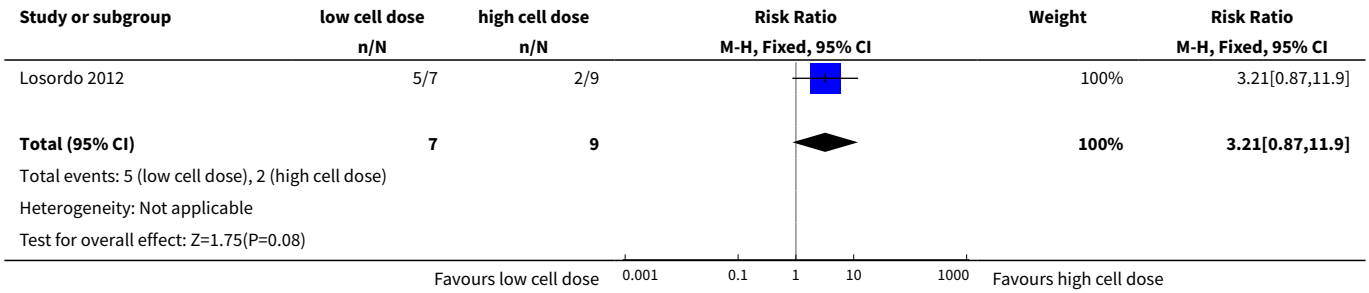
Analysis 2.7. Comparison 2 BM-MNCs vs BM-MSCs, Outcome 7 Improvement in vascularity and blood supply: number of participants with increase in numbers of collateral vessels.



Comparison 3. Low cell dose vs high cell dose

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Amputation rate	1	16	Risk Ratio (M-H, Fixed, 95% CI)	3.21 [0.87, 11.90]

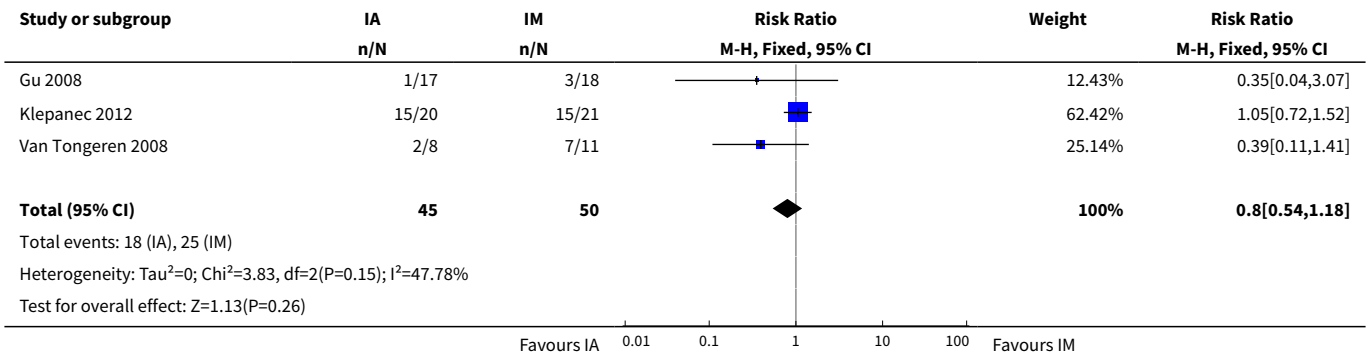
Analysis 3.1. Comparison 3 Low cell dose vs high cell dose, Outcome 1 Amputation rate.



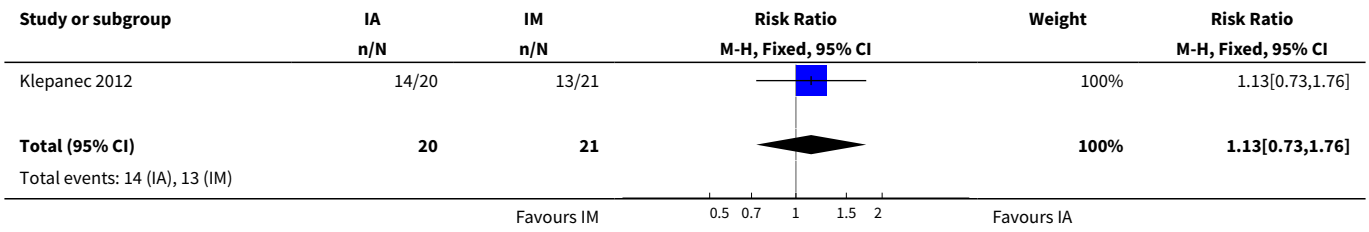
Comparison 4. Route of injection: IM injection vs IA injection

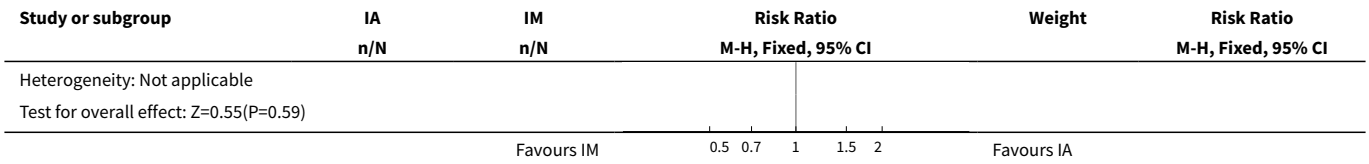
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Amputation rate	3	95	Risk Ratio (M-H, Fixed, 95% CI)	0.80 [0.54, 1.18]
2 Wound/ulcer healing: number of participants with healing ulcer	1	41	Risk Ratio (M-H, Fixed, 95% CI)	1.13 [0.73, 1.76]
3 Reduction in rest pain: number of participants with reduction in rest pain score	1	32	Risk Ratio (M-H, Fixed, 95% CI)	1.22 [0.91, 1.64]
4 Improvement in lower limb perfusion: number of participants with increased ABI	1	35	Risk Ratio (M-H, Fixed, 95% CI)	0.93 [0.43, 2.00]
5 Improvement in lower limb perfusion: ABI score	1	27	Mean Difference (IV, Fixed, 95% CI)	-0.17 [-0.37, 0.03]
6 Improvement in lower limb perfusion: number of participants with improved TcO_2 reading	2	62	Risk Ratio (M-H, Fixed, 95% CI)	1.22 [0.86, 1.72]
7 Improvement in vascularity and blood supply: number of participants with increase in numbers of collateral vessels	1	15	Risk Ratio (M-H, Fixed, 95% CI)	0.91 [0.40, 2.11]

Analysis 4.1. Comparison 4 Route of injection: IM injection vs IA injection, Outcome 1 Amputation rate.

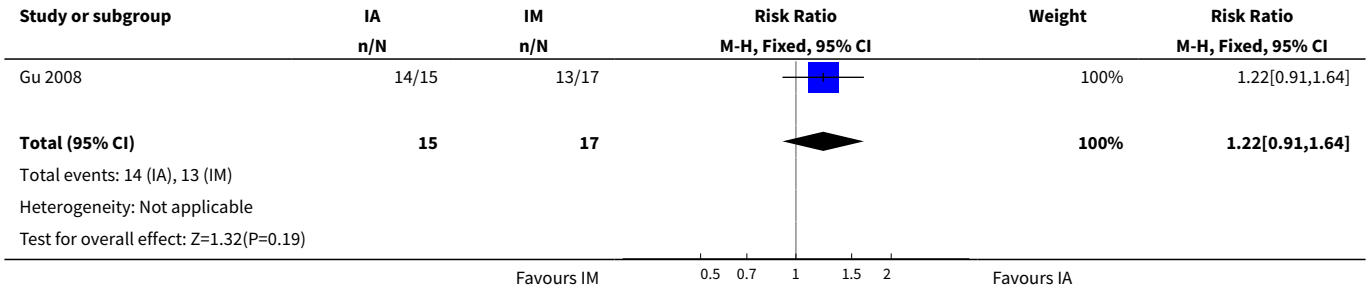


Analysis 4.2. Comparison 4 Route of injection: IM injection vs IA injection, Outcome 2 Wound/ulcer healing: number of participants with healing ulcer.

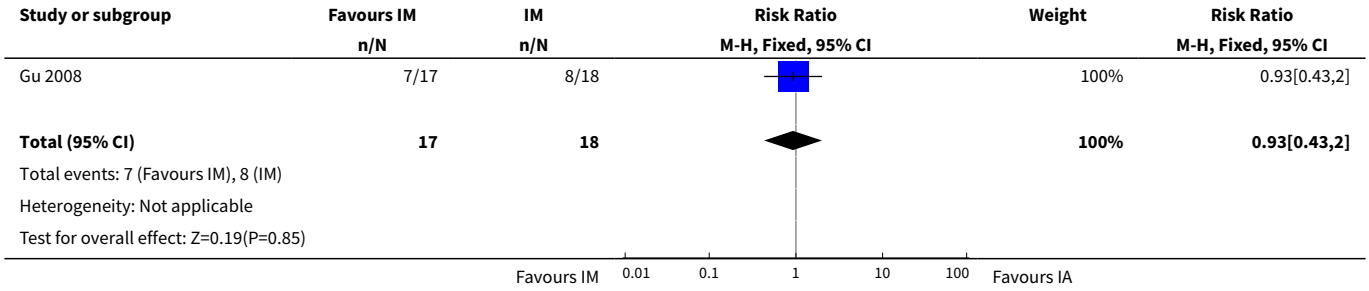




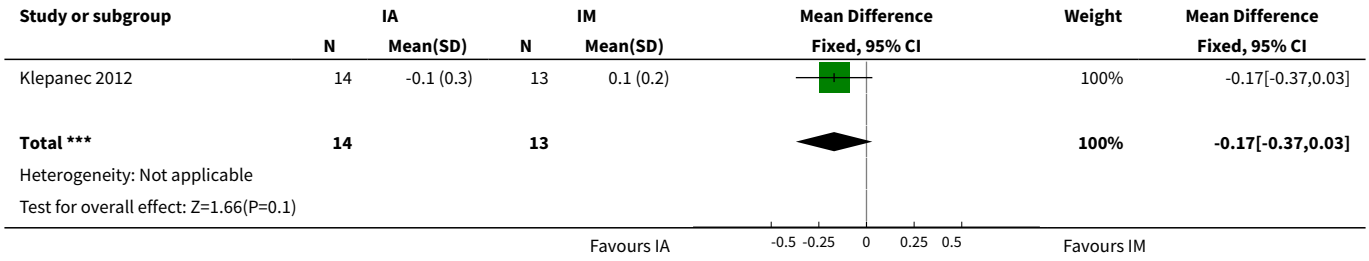
Analysis 4.3. Comparison 4 Route of injection: IM injection vs IA injection, Outcome 3 Reduction in rest pain: number of participants with reduction in rest pain score.



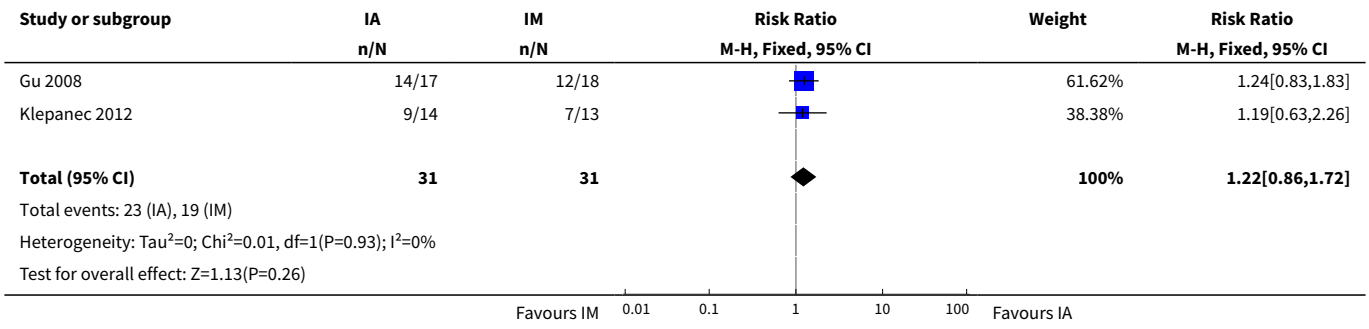
Analysis 4.4. Comparison 4 Route of injection: IM injection vs IA injection, Outcome 4 Improvement in lower limb perfusion: number of participants with increased ABI.



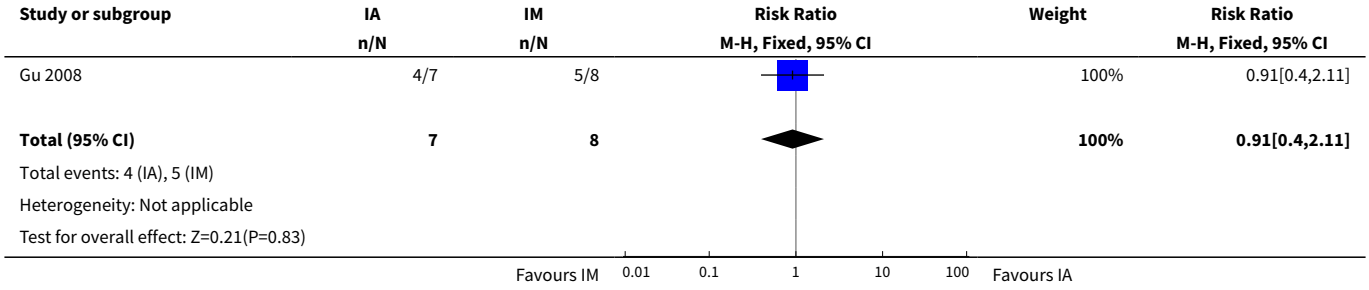
Analysis 4.5. Comparison 4 Route of injection: IM injection vs IA injection, Outcome 5 Improvement in lower limb perfusion: ABI score.



Analysis 4.6. Comparison 4 Route of injection: IM injection vs IA injection, Outcome 6 Improvement in lower limb perfusion: number of participants with improved TcO_2 reading.



Analysis 4.7. Comparison 4 Route of injection: IM injection vs IA injection, Outcome 7 Improvement in vascularity and blood supply: number of participants with increase in numbers of collateral vessels.



APPENDICES

Appendix 1. CENTRAL search strategy, 17 February 2017

Search run on Fri Feb 17 2017		
#1	MESH DESCRIPTOR Arteriosclerosis	868
#2	MESH DESCRIPTOR Arteriolosclerosis EXPLODE ALL TREES	0
#3	MESH DESCRIPTOR Arteriosclerosis Obliterans	71
#4	MESH DESCRIPTOR Atherosclerosis	619
#5	MESH DESCRIPTOR Arterial Occlusive Diseases	724
#6	MESH DESCRIPTOR Intermittent Claudication	712
#7	MESH DESCRIPTOR Ischemia	789

(Continued)

#8	MESH DESCRIPTOR Peripheral Vascular Diseases EXPLODE ALL TREES	2201
#9	(atherosclero* or arteriosclero* or PVD or PAOD or PAD):TI,AB,KY	9119
#10	((arter* or vascular or vein* or veno* or peripher*) near3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)):TI,AB,KY	7966
#11	(peripheral near3 dis*):TI,AB,KY	3371
#12	(claudic* or IC):TI,AB,KY	3063
#13	(isch* or CLI):TI,AB,KY	23713
#14	arteriopathic:TI,AB,KY	7
#15	dysvascular*:TI,AB,KY	10
#16	(leg near3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)):TI,AB,KY	95
#17	(limb near3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)):TI,AB,KY	145
#18	((lower near3 extrem*) near3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)):TI,AB,KY	77
#19	((iliac or femoral or popliteal or femoro* or fempop* or crural) near3(occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)):TI,AB,KY	1008
#20	MESH DESCRIPTOR Leg EXPLODE ALL TREES WITH QUALIFIERS BS	1107
#21	MESH DESCRIPTOR Iliac Artery	144
#22	MESH DESCRIPTOR Popliteal Artery	278
#23	MESH DESCRIPTOR Femoral Artery	810
#24	MESH DESCRIPTOR Tibial Arteries	33
#25	(((femor* or iliac or popliteal or fempop* or crural or poplite* or infrapopliteal or inguinal or femdist* or inguinal or infrainquinal or tibial) near3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*))):TI,AB,KY	1157
#26	#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	43742
#27	MESH DESCRIPTOR Stem Cell Transplantation EXPLODE ALL TREES	1531
#28	MESH DESCRIPTOR Hematopoietic Stem Cell Mobilization EXPLODE ALL TREES	259
#29	MESH DESCRIPTOR Stem Cells EXPLODE ALL TREES	690

(Continued)

#30	MESH DESCRIPTOR Bone Marrow Cells EXPLODE ALL TREES	1484
#31	((mononuclear or endothelial or mesenchymal) near3 cell*):TI,AB,KY	5253
#32	((stem or progenitor or precursor or therap*) near3 cell*):TI,AB,KY	12700
#33	((embryo* or fetal or foetal or umbilical or marrow or cord) near5 cel-l*):TI,AB,KY	2023
#34	(BM-MNC* or PB-MNC* or M-PBNNC* or AT-MSC*):TI,AB,KY	42
#35	MESH DESCRIPTOR Transplantation, Autologous	1297
#36	#27 OR #28 OR #29 OR #30 OR #31 OR #32 OR #33 OR #34 OR #35	18669
#37	#26 AND #36	1264

Appendix 2. Trials databases searches, 17 February 2017

Clinicaltrials.gov 69 studies found for: (bone marrow OR stem cells) AND (critical limb ischemia)

WHO Clinical trials register 17 records for 17 trials found for: (ischemia or ischaemia) and cells in Title and Cells in intervention

ISRCTN Register 4 results for: (ischemia or ischaemia) and cells

Appendix 3. Authors' PubMed search strategy, February 2017

#1	Search **(Peripheral arterial disease[Title/Abstract] OR Peripheral vascular disease[Title/Abstract] OR chronic limb ischemia[Title/Abstract] OR critical limb ischemia[Title/Abstract] OR CLI[Title/Abstract] OR PAD[Title/Abstract] OR limb ischaemia[Title/Abstract])**	35028
#2	Search **(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])**	9809
#3	Search **Peripheral Vascular Diseases [MeSH Terms]**	48342
#4	Search **Peripheral Arterial Diseases [MeSH Terms]**	4259
#5	Search **#1 OR #2 OR #3 OR #4**	85418
#6	Search **(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])**	117626
#7	Search**Leukocytes, Mononuclear [MeSH Terms]**	526581
#8	Search **Mesenchymal Stromal Cells[MeSH Terms]**	24202
#9	Search**bone marrow transplantation[MeSH Terms]**	42775
#10	Search **stem cells[MeSH Terms]**	167737

(Continued)

#11	Search **#6 OR #7 OR #8 OR #9 OR #10**	747219
#12	Search **#5 AND #11**	1488
#13	Search **randomized controlled trial [pt]**	428782
#14	Search **controlled clinical trial [pt]**	515129
#15	Search **randomized [tiab]**	400989
#16	Search **placebo [tiab]**	182281
#17	Search **drug therapy [sh]**	1902784
#18	Search **randomly [tiab]**	266458
#19	Search **trial [ti]**	162684
#20	Search **#13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19**	2588730
#21	Search **(animals [mh] NOT humans [mh])**	4299695
#22	Search **#20 NOT #21**	2322098
#23	Search #**12 AND #22**	234

Appendix 4. Database searches, 16 May 2018

Source	Search strategy	Hits retrieved
1. VASCULAR REGISTER IN CRSW	#1 Stem Cell OR Autologous cells OR Bone Marrow Cells AND INREGISTER #2 2017 or 2018 AND INREGISTER #3 #1 AND #2	1
2. CENTRAL via CRSO	#1 MESH DESCRIPTOR Arteriosclerosis 927 #2 MESH DESCRIPTOR Arteriolosclerosis EXPLODE ALL TREES 0 #3 MESH DESCRIPTOR Arteriosclerosis Obliterans 76 #4 MESH DESCRIPTOR Atherosclerosis 963 #5 MESH DESCRIPTOR Arterial Occlusive Diseases 804 #6 MESH DESCRIPTOR Intermittent Claudication 805 #7 MESH DESCRIPTOR Ischemia 1354 #8 MESH DESCRIPTOR Peripheral Vascular Diseases EXPLODE ALL TREES 2660 #9 (atherosclero* or arteriosclero* or PVD or PAOD or PAD):TI,AB,KY 11705 #10 ((arter* or vascular or vein* or veno* or peripher*) near3 (occlus* or re-occlus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)):TI,AB,KY 10331	520

(Continued)

#11 ((peripheral near3 dis*)):TI,AB,KY 4618

#12 (claudic* or IC):TI,AB,KY 3969

#13 (isch* or CLI):TI,AB,KY 30894

#14 arteriopathic:TI,AB,KY 7

#15 dysvascular*:TI,AB,KY 17

#16 (leg near3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)):TI,AB,KY 124

#17 (limb near3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)):TI,AB,KY 210

#18 ((lower near3 extrem*) near3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)):TI,AB,KY 106

#19 ((iliac or femoral or popliteal or femoro* or fempop* or crural) near3(occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)):TI,AB,KY 1481

#20 MESH DESCRIPTOR leg EXPLODE ALL TREES 2784

#21 MESH DESCRIPTOR Iliac Artery 158

#22 MESH DESCRIPTOR Popliteal Artery 300

#23 MESH DESCRIPTOR Femoral Artery 894

#24 MESH DESCRIPTOR Tibial Arteries 36

#25 ((((femor* or iliac or popliteal or fempop* or crural or poplite* or infrapopliteal or inguinal or femdist* or inguinal or infrainquinal or tibial) near3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)))):TI,AB,KY 1661

#26 #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 57950

#27 MESH DESCRIPTOR Stem Cell Transplantation EXPLODE ALL TREES 1753

#28 MESH DESCRIPTOR Hematopoietic Stem Cell Mobilization EXPLODE ALL TREES 278

#29 MESH DESCRIPTOR Stem Cells EXPLODE ALL TREES 735

#30 MESH DESCRIPTOR Bone Marrow Cells EXPLODE ALL TREES 1589

#31 (((mononuclear or endothelial or mesenchymal) near3 cell*)):TI,AB,KY 7061

#32 (((stem or progenitor or precursor or therap*) near3 cell*)):TI,AB,KY 17942

#33 (((embryo* or fetal or foetal or umbilical or marrow or cord) near5 cell*)):TI,AB,KY 3057

#34 ((BM-MNC* or PB-MNC* or M-PBNNC* or AT-MSC*)):TI,AB,KY 65

#35 MESH DESCRIPTOR Transplantation, Autologous 1432

#36 #27 OR #28 OR #29 OR #30 OR #31 OR #32 OR #33 OR #34 OR #35 25398

(Continued)

	#37 #26 AND #36 1796 #38 01/01/2017 TO 16/05/2018:CD 257803 #39 #37 AND #38 520	
3. Clinicaltrials.gov	Peripheral arterial disease OR Peripheral Vascular Diseases OR lower limb ischaemia | Stem Cell OR Autologous cells OR Bone Marrow Cells | Start date on or after 01/01/2017 | Last update posted on or before 03/05/2019	8
4. ICTRP Search Portal	Peripheral arterial disease OR Peripheral Vascular Diseases OR lower limb ischaemia | Stem Cell OR Autologous cells OR Bone Marrow Cells | 01/01/2017 to 03/05/2019	0
5. MEDLINE	1. ARTERIOSCLEROSIS/ 2. exp ARTERIOLOSCLEROSIS/ 3. Arteriosclerosis Obliterans/ 4. ATHEROSCLEROSIS/ 5. Arterial Occlusive Diseases/ 6. Intermittent Claudication/ 7. ISCHEMIA/ 8. Peripheral Vascular Diseases/ 9. (atherosclero* or arteriosclero* or PVD or PAOD or PAD).ti,ab. 10. ((arter* or vascular or vein* or veno* or peripher*) adj3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab. 11. (peripheral adj3 dis*).ti,ab. 12. (claudic* or IC).ti,ab. 13. (isch* or CLI).ti,ab. 14. arteriopathic.ti,ab. 15. dysvascular*.ti,ab. 16. (leg adj3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab. 17. (limb adj3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab. 18. (lower adj3 extrem* adj3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab. 19. ((iliac or femoral or popliteal or femoro* or fempop* or crural) adj3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab. 20. exp LEG/bs [Blood Supply] 21. Iliac Artery/ 22. Popliteal Artery/	526

(Continued)

	23. Femoral Artery/ 24. Tibial Arteries/ 25. ((femor* or iliac or popliteal or fempop* or crural or poplite* or infrapopliteal or inguinal or femdist* or inguinal or infrainquinal or tibial) adj3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab. 26. 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 27. exp Stem Cell Transplantation/ 28. exp Bone Marrow Cells/ 29. ((mononuclear or endothelial or mesenchymal) adj3 cell*).ti,ab. 30. ((stem or progenitor or precursor or therap*) adj3 cell*).ti,ab. 31. ((embryo* or fetal or foetal or umbilical or marrow or cord) adj5 cell*).ti,ab. 32. (BM-MNC* or PB-MNC* or M-PBNNC* or AT-MSC*).ti,ab. 33. Transplantation, Autologous/ 34. exp Hematopoietic Stem Cell Mobilization/ 35. exp Stem Cells/ 36. or/27-35 37. 26 and 36 38. randomized controlled trial.pt. 39. controlled clinical trial.pt. 40. randomized.ab. 41. placebo.ab. 42. drug therapy.fs. 43. randomly.ab. 44. trial.ab. 45. groups.ab. 46. or/38-45 47. exp animals/ not humans.sh. 48. 46 not 47 49. 37 and 48 50. (2017* or 2018*).ed. 51. 49 and 50	
6. EMBASE	1 arteriosclerosis/ 14659 2 exp arteriolosclerosis/ 486 3 peripheral occlusive artery disease/ 21901	2878

(Continued)

4 atherosclerosis/ 113655

5 peripheral occlusive artery disease/ 21901

6 intermittent claudication/ 6103

7 ischemia/ 59992

8 exp peripheral vascular disease/ 1280555

9 (atherosclero* or arteriosclero* or PVD or PAOD or PAD).ti,ab. 192387

10 ((arter* or vascular or vein* or veno* or peripher*) adj3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab. 144769

11 (peripheral adj3 dis*).ti,ab. 43541

12 (claudic* or IC).ti,ab. 52623

13 (isch* or CLI).ti,ab. 399372

14 arteriopathic.ti,ab. 82

15 dysvascular*.ti,ab. 177

16 (leg adj3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab. 684

17 (limb adj3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab. 2162

18 (lower adj3 extrem* adj3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab. 1461

19 ((iliac or femoral or popliteal or femoro* or fempop* or crural) adj3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab. 9348

20 iliac artery/ 9843

21 popliteal artery/ 5196

22 femoral artery/ 20875

23 tibial artery/ 2263

24 ((femor* or iliac or popliteal or fempop* or crural or poplite* or infrapopliteal or inguinal or femdist* or inguinal or infrainquinal or tibial) adj3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab. 10857

25 or/1-24 1556237

26 exp stem cell transplantation/ 128994

27 exp stem cell/ 300361

28 exp bone marrow cell/ 85006

29 ((mononuclear or endothelial or mesenchymal) adj3 cell*).ti,ab. 317089

30 ((stem or progenitor or precursor or therap*) adj3 cell*).ti,ab. 415578

(Continued)

	31 ((embryo* or fetal or foetal or umbilical or marrow or cord) adj5 cell*).ti,ab. 232042 32 (BM-MNC* or PB-MNC* or M-PBNNC* or AT-MSC*).ti,ab. 994 33 autotransplantation/ 15144 34 or/26-33 860956 35 25 and 34 82480 36 randomized controlled trial/ 454282 37 controlled clinical trial/ 415959 38 random$.ti,ab. 1164643 39 randomization/ 69778 40 intermethod comparison/ 224992 41 placebo.ti,ab. 222171 42 (compare or compared or comparison).ti. 334278 43 ((evaluated or evaluate or evaluating or assessed or assess) and (compare or compared or comparing or comparison)).ab. 1614974 44 (open adj label).ti,ab. 62586 45 ((double or single or doubly or singly) adj (blind or blinded or blindly)).ti,ab. 157598 46 double blind procedure/ 123039 47 parallel group$1.ti,ab. 19496 48 (crossover or cross over).ti,ab. 71898 49 ((assign$ 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. 248541 50 (assigned or allocated).ti,ab. 289586 51 (controlled adj7 (study or design or trial)).ti,ab. 261038 52 (volunteer or volunteers).ti,ab. 171731 53 trial.ti. 213834 54 or/36-53 3469338 55 35 and 54 15884 56 (2017* or 2018*).em. 3418276 57 55 and 56 2878 58 from 57 keep 2001-2878 878	
7. CINAHL	S47 S45 AND S46 53 S46 EM 2017 OR EM 2018 350,115 S45 S32 AND S44 628	53

(Continued)

S44 S33 OR S34 OR S35 OR S36 OR S37 OR S38 OR S39 OR S40 OR S41 OR S42 OR S43 338,202

S43 MH "Random Assignment" 37,958

S42 MH "Single-Blind Studies" or MH "Double-Blind Studies" or MH "Triple-Blind Studies" 32,619

S41 MH "Crossover Design" 11,154

S40 MH "Factorial Design" 916

S39 MH "Placebos" 8,339

S38 MH "Clinical Trials" 93,234

S37 TX "multi-centre study" OR "multi-center study" OR "multicentre study" OR "multicenter study" OR "multi-site study" 4,438

S36 TX crossover OR "cross-over" 14,482

S35 TX random* 217,617

S34 TX trial* 248,860

S33 TX "latin square" 142

S32 S23 AND S31 2,772

S31 S24 OR S25 OR S26 OR S27 OR S28 OR S29 OR S30 43,836

S30 (MH "Bone Marrow Transplantation, Autologous") 147

S29 TX BM-MNC* or PB-MNC* or M-PBNNC* or AT-MSC* 6,548

S28 TX ((embryo* or fetal or foetal or umbilical or marrow or cord) n5 cell*) 5,617

S27 TX ((stem or progenitor or precursor or therap*) n3 cell*) 27,759

S26 TX ((mononuclear or endothelial or mesenchymal) n3 cell*) 8,446

S25 (MH "Stem Cells+") 10,218

S24 (MH "Hematopoietic Stem Cell Transplantation") 4,000

S23 S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21 OR S22 (85,427

S22 (((femor* or iliac or popliteal or fempop* or crural or poplite* or infrapopliteal or inguinal or femdist* or inguinal or infrainquinal or tibial) n3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*))) 1,080

S21 (MH "Tibial Arteries") 145

S20 (MH "Femoral Artery") 1,201

S19 (MH "Popliteal Artery") 360

S18 (MH "Iliac Artery") 458

S17 TX ((iliac or femoral or popliteal or femoro* or fempop* or crural) n3(occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)) 941

(Continued)

	S16 TX ((lower n3 extrem*) n3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)) 121 S15 TX (limb n3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)) 273 S14 TX (leg n3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)) 123 S13 TX dysvascular* 172 S12 TX arteriopathic 10 S11 TX isch* or CLI 39,225 S10 TX claudic* or IC 5,793 S9 TX peripheral ADJ3 dis* 0 S8 TX ((arter* or vascular or vein* or veno* or peripher*) n3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)) 12,600 S7 TX atherosclero* or arteriosclero* or PVD or PAOD or PAD 26,253 S6 (MH "Peripheral Vascular Diseases+") 10,351 S5 (MH "Ischemia") 3,352 S4 (MH "Intermittent Claudication") 851 S3 (MH "Arterial Occlusive Diseases") 1,603 S2 (MH "Atherosclerosis") 3,297 S1 (MH "Arteriosclerosis") 4,827	
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(Continued)

14 (limb adj3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab. 32

15 (lower adj3 extrem* adj3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab. 25

16 ((iliac or femoral or popliteal or femoro* or fempop* or crural) adj3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab. 54

17 ((femor* or iliac or popliteal or fempop* or crural or poplite* or infrapopliteal or inguinal or femdist* or inguinal or infrainquinal or tibial) adj3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab. 109

18 or/1-17 4307

19 exp stem cell transplantation/ 54

20 ((mononuclear or endothelial or mesenchymal) adj3 cell*).ti,ab. 554

21 ((stem or progenitor or precursor or therap*) adj3 cell*).ti,ab. 360

22 ((embryo* or fetal or foetal or umbilical or marrow or cord) adj5 cell*).ti,ab. 295

23 (BM-MNC* or PB-MNC* or M-PBNNC* or AT-MSC*).ti,ab. 87

24 or/19-23 1074

25 18 and 24 104

26 exp CLINICAL TRIALS/ 3738

27 RANDOM ALLOCATION/ 314

28 DOUBLE BLIND METHOD/ 653

29 Clinical trial.pt. 1210

30 (clinic* adj trial*).tw. 5364

31 ((singl* or doubl* or trebl* or tripl*) adj (blind* or mask*)).tw. 2816

32 PLACEBOS/ 585

33 placebo*.tw. 3094

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35 PROSPECTIVE STUDIES/ 1072

36 or/26-35 22400

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38 ("2017" or "2018").yr. 1412

39 37 and 38 0

CONTRIBUTIONS OF AUTHORS

SFAW: designed the project and review, searched the PubMed database, undertook data extraction and analysis, performed statistical analysis, and wrote the review.
NAI: searched the PubMed database, undertook data extraction and analysis, assessed risk of bias in the included studies, and prepared tables and figures.
WFWJ: reviewed the results and edited the manuscript.
NAM: contributed to extracting and interpreting the data and assessed risk of bias in the included studies.
MKAAH: searched the databases.
HH: reviewed the findings of the analysis.
NML: contributed to designing the review, analysing data, performing statistical analyses, assessing risk of bias in the included studies, and reviewing and editing the manuscript.

DECLARATIONS OF INTEREST

SFAW: none known.
NAI: none known.
WFWJ: none known.
NAM: none known.
MKAAH: none known.
HH: none known.
NML: none known.

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DIFFERENCES BETWEEN PROTOCOL AND REVIEW

1. Under types of outcome measures, we have amended the primary outcomes to include amputation rate and deleted amputation-free survival because only one RCT reported on amputation-free survival (Losordo 2012), and most included studies used amputation rate and not amputation-free survival as the primary clinical endpoint. The 'number of newly formed collaterals in the lower limbs as analysed by angiography' has been added to the list of secondary outcomes.
2. We have revised the section under Unit of analysis issues to incorporate statements on some studies that reported their results using limbs rather than patients as the unit of analysis.
3. Unit of analysis issues - We have inserted sensitivity analyses for relevant outcomes to address the issues related to discrepancies in the unit of analysis used by studies for the same comparison group.
4. To reflect the importance of outcomes, we have made wound/ulcer healing a primary outcome and reduction in rest pain a secondary outcome.

INDEX TERMS

Medical Subject Headings (MeSH)

Amputation, Surgical [statistics & numerical data]; Bone Marrow Cells [cytology]; Bone Marrow Transplantation [*methods]; Cause of Death; Injections, Intra-Arterial; Injections, Intramuscular; Ischemia [*therapy]; Leg [*blood supply]; Leg Ulcer [therapy]; Mesenchymal Stem Cell Transplantation [*methods]; Peripheral Blood Stem Cell Transplantation [*methods]; Peripheral Blood Stem Cells [cytology]; Randomized Controlled Trials as Topic

MeSH check words

Humans