Nonrevascularization-based treatments in patients with severe or critical limb ischemia

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Objective: **The aim of this systematic review was to synthesize the existing evidence about various nonrevascularization-based therapies used to treat patients with severe or critical limb ischemia (CLI) who are not candidates for surgical revascularization.**

Methods: **We systematically searched multiple databases through November 2014 for controlled randomized and nonrandomized studies comparing the effect of medical therapies (prostaglandin E_1 and angiogenic growth factors) and devices (pumps and spinal cord stimulators). We report odds ratios (ORs) and 95% confidence intervals (CIs) of the outcomes of interest pooling data across studies using the random effects model.**

Results: **We included 19 studies that enrolled 2779 patients. None of the nonrevascularization-based treatments were associated with a significant effect on mortality. Intermittent pneumatic compression (OR, 0.14; 95% CI, 0.04-0.55) and spinal cord stimulators (OR, 0.53; 95% CI, 0.36-0.79) were associated with reduced risk of amputation. A priori established subgroup analyses (combined vs single therapy; randomized vs nonrandomized) were not statistically significant.**

Conclusions: **Very low-quality evidence, mainly due to imprecision and increased risk of bias, suggests that intermittent pneumatic compression and spinal cord stimulators may reduce the risk of amputations. Evidence supporting other medical therapies is insufficient. (J Vasc Surg 2015;62:1330-9.)**

Critical limb ischemia (CLI) is characterized by at-rest pain, ulcers, or gangrene in one or both lower limbs due to pre-existing peripheral arterial occlusive disease. An estimated 5% to 10% of PAD patients aged >50 years develop CLI ≤5 years.[1] Up to 50% of CLI patients are not eligible to receive first-line treatments such as bypass surgery or endovascular interventions.[2] Various nonrevascularization modalities have been used in practice to salvage patients with CLI who are not candidates for bypass or endovascular intervention. The efficacy of these interventions is unclear and thought to be modest.[3-6] A Cochrane systematic review of spinal cord stimulation (SCS) for CLI demonstrated possible improvement in the limb salvage rate but did not investigate overall mortality.[7]

Therapy with prostanoids has also been investigated and showed some favorable results in limb salvage, but the evidence was not conclusive.[8] More recent investigations of therapies to support angiogenesis have shown some promise, but the trials were small, and the results were also inconclusive.[9,10]

Thus far, CLI remains as a condition associated with significant morbidity and mortality and a recognized public health burden with unmet needs. We conducted a systematic review and meta-analysis of comparative studies that evaluated the effect of various nonrevascularization-based interventions in patients with CLI and their effect on mortality, risk of limb loss, and wound healing.

METHODS

The methods and reporting of this systematic review complies with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[11] statement in agreement with the methodology for clinical practice guidelines for the management of arteriovenous access.[12] Following an a priori protocol developed by a committee from the Society for Vascular Surgery, we planned to synthesize the best available evidence on the available

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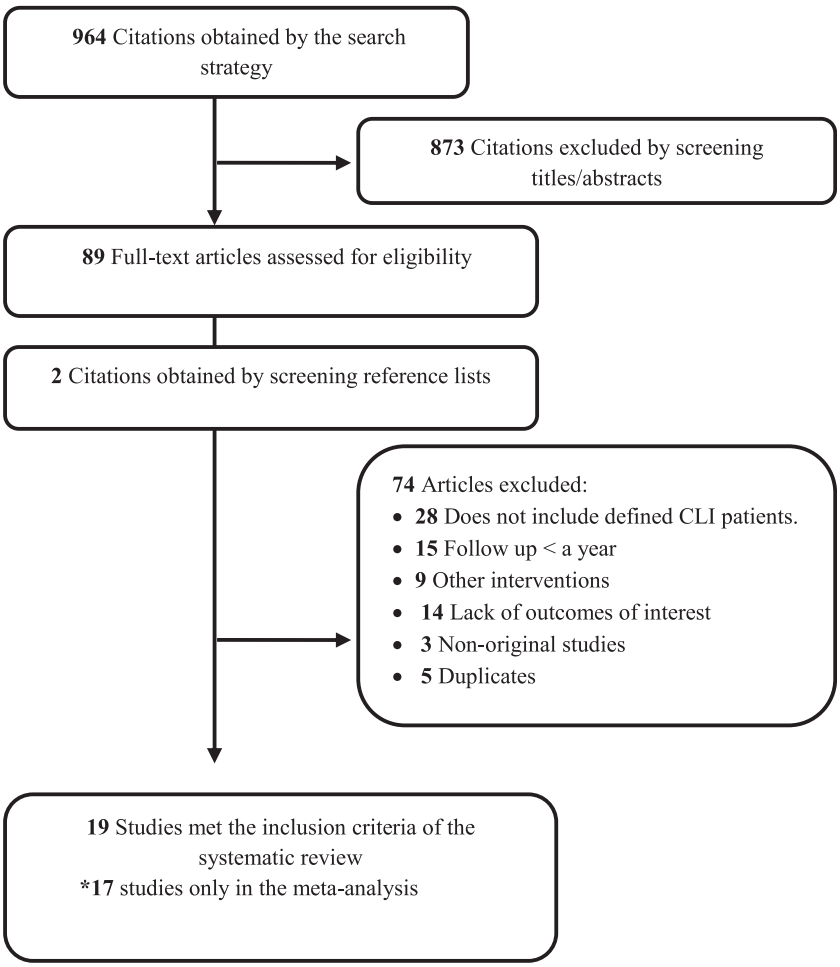

Fig. Flowchart shows the selection process of eligible studies. *CLI,* Critical limb ischemia.

nonrevascularization-based treatments and to assess the quality of evidence and magnitude of benefit in major amputations or limb loss, mortality, and wound or ulcer healing.

Study eligibility. We conducted a literature search for studies that evaluated and assessed the effect of nonrevascularization-based treatments in patients with CLI. We limited our inclusion criteria to original randomized or nonrandomized comparative studies that enrolled adult patients with CLI and had minimum of 1 year of follow-up. Studies that included any alternative treatment as a comparison group in the same study were deemed eligible. Severe or CLI-eligible patients were defined by the following criteria: rest pain, tissue loss, ulcer, or gangrene; met the Rutherford classification 4 to 6; or had an ankle pressure <70 mm Hg, toe pressure <50 mm Hg, flat peripheral vascular resistance, or transcutaneous oxygen pressure of <40 mm Hg.

Literature search. A comprehensive literature search was conducted by an expert reference librarian with input from a study investigator with expertise in systematic reviews. The search included the electronic databases MEDLINE, Embase, Cochrane Central Register of Controlled Trials, and Cochrane Database of Systematic Reviews, CINAHL, and Scopus, using various combinations of controlled vocabulary supplemented with key words. The detailed strategy is included in the Appendix (online only).

Pairs of reviewers working independently identified original studies eligible for further review by screening abstracts and titles. If an abstract was deemed relevant, the manuscript was retrieved, and the full-text version was reviewed for further assessment. Any inclusion or exclusion disagreements were discussed and reconciled by the senior investigator (M.H.M.). Previously described data sources, including citing articles and relevant systematic reviews, were searched manually for possible studies, and duplicates were excluded. We expanded the search to include all languages, with last date of inclusion of November 2014.

Data extraction. Two reviewers independently extracted data from each study on patient demographics, baseline characteristics, study design variables, sample size, interventions and comparator interventions, and outcome measures when reported. Also extracted for each study were variables related to CLI, history of chronic illnesses, and disease-specific effect size, when possible.

Table I. Baseline characteristics of the included studies

Study ID	*Study design*	*Patients, No.*	*Follow-up, months*	*Age, mean*	*Study setting*	*Intervention, No.*	*Comparison, No.*	*Inclusion criteria*
Amann,[26] 2003	Cohort	112	18	68	Multicenter	SCS (match group) (41)	2 control groups: • SCS(no-match group) (32) • Conventional conservative management (39)	• Patients had chronic, stable CLI were not suitable for vascular reconstruction according to the responsibility of a vascular surgeon.
Anghel,[27] 2011	RCT	43	15	64	Single institution	VEGF/HGF gene therapy (29)	Placebo (14)	• Rest pain for at least 1 month either or without distal ulceration or gangrene.
Belch,[19] 2011	RCT	525	12	70	Multicenter	NV1FGF (266)	Placebo (259)	• CLI with skin lesions (ischemic ulcer[s] or minor gangrene). • Objective evidence of CLI, including ankle systolic pressure <70 mm Hg, or toe systolic pressure <50 mm Hg or $TcPo_2$ <30 mm Hg.
Claeys,[20] 1999	RCT	86	12	69	Single institution	SCS + IV PGE1 (45)	PGE1 (14)	• Peripheral vascular disease with ankle systolic pressure <40 mm Hg, foot $TcPo_2$ <20 mm Hg and unrelenting rest pain despite analgesic medication. • Proof that reconstructive surgery or angioplasty was impossible by arteriography or patient condition and the presence of nonhealing foot ulcers or dry gangrene.
Dormandy,[30] 2000	RCT	624	12	68	Multicenter	PGI2 (iloprost) (427)	Placebo (207)	• Patients with trophic skin changes or rest pain due to severe arterial disease.
Idei,[29] 2011	NRT	97	36	42.1-69.2[a]	Multicenter	BM-MNC (51)	Non BM-MNC (46)	• Patients had severe rest pain and nonhealing ulcers.
Jivegard,[24] 1995	RCT	51	18	73	Multicenter	SCS (25)	Analgesic (26)	• Severe chronic (duration >2 weeks) lower limb ischemia in atherosclerotic and diabetic patients with rest pain and/or ischemic ulcerations.
Klomp,[25] 1999	RCT	120	18	73	Multicenter	SCS (60)	Best medical treatment (60)	• Have persistent pain at rest for >2 weeks or ischemic skin lesions, ankle systolic pressure <50 mm Hg. • Patients with rest pain and have diabetes and incompressible vessels, absent palpable ankle pulses.
Kavros,[31] 2008	Cohort	48	18	70	Single institution	IPC + wound care (24)	Wound care only (24)	• Patients with chronic non-healing toe or transmetatarsal amputation wounds and tissue loss of the foot attributable to chronic CLI.
Lund,[23] 1999	Cohort	70	24	73	Single institution	IV HER + oral warfarin + standard treatment (42)	Standard treatment (28)	• Patients with severe rest pain or ischemic lesions also a toe blood pressure <30 mm Hg (according to the second European Consensus Statement for CLI).
Napoli,[32] 2008	Cohort	36	18	71	Single institution	BM-MNC (intra-arterial) (18)	Placebo (18)	• History of intermittent claudication, rest pain, non-healing ischemic ulcers and were not candidates for surgical revascularization.
Nikol,[33] 2008	RCT	107	12	72	Multicenter	NV1FGF (51)	Placebo (saline injection) (56)	• Signs of healing of the trophic lesions (reduction in ulcer size or depth) were required to be absent for ≥2 weeks before the first administration of the study drug.

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Table I. Continued.

Study ID	*Study design*	*Patients, No.*	*Follow-up, months*	*Age, mean*	*Study setting*	*Intervention, No.*	*Comparison, No.*	*Inclusion criteria*
Powell,[21] 2008	RCT	106	12	71	Multicenter	HGF plasmid angiogenic gene therapy (80)	Placebo (26)	• Patients with severe, chronic symptoms and complications from inadequate limb perfusion that included ulceration, gangrene, and rest pain due to impaired peripheral blood flow. • Patients with rest pain or ischemic ulcers were enrolled into the trial if their baseline $TcPo_2$ was 40 mm Hg and/or toe pressure was 50 mm Hg or ankle pressure was 70 mm Hg.
Powell,[22] 2010	RCT	27	12	77	Multicenter	HGF plasmid angiogenic gene therapy (21)	Placebo (6)	• Subjects needed to have appropriately sized ischemic peripheral ulcer(s) or tissue loss. • Ankle systolic pressure (in the dorsalis pedis or posterior tibial arteries) of <70 mm Hg or toe systolic pressure <50 mm Hg.
Powell,[34] 2012	RCT	86	12	68	Multicenter	Cellular therapy (Ixmyelocel-T) (48)	Placebo (24)	• CLI of the lower extremities defined as persistent recurring ischemic rest pain for at least 2 weeks; and/or • Ulceration or gangrene of the foot or toe with absent palpable pedal pulses with toe systolic pressure ≤50 mm Hg or ankle systolic pressure ≤70 mm Hg.
Ubbink,[18] 1999	RCT	111	18	73	Multicenter	SCS (56)	Standard treatment (55)	• Persistent rest pain for at least 2 weeks, undergoing treatment with analgesics, or ulceration or gangrene of foot or toes. • 2-Doppler ankle systolic pressure ≤50 mm Hg or ankle/brachial pressure index ≤35%. For patients with diabetes mellitus. • Incompressible ankle arteries, absence of arterial ankle pulsations with physical examination.
Van Tongeren,[35] 2008	RCT	27	12	68	Single institution	BM-MNC (IM + intra-arterial) (8)	BM-MNC (I.M. route) (11)	• Patients suffering from CLI (ischemic rest pain or ulcers): or • In case of persistent (>12 months) profound disabling claudication and a maximum walking distance of 100 m.
Brass,[28] 2006[b]	RCT	383	6-12[a]	69.7	Multicenter	Lipo-ecraprost IV (PGE1 analog) (189)	Placebo I.V. (190)	• Patients were 40 years old and were able to provide informed consent directly or through an authorized representative. • CLI clinically defined by the presence of distal extremity pain at rest requiring use of analgesics for at least 2 weeks or the presence of peripheral ischemic ulcers or areas of gangrene. • Patients must have had hemodynamic evidence of CLI. • In the case of patients with rest pain only (Fontaine stage III), CLI diagnosis required a highest ankle systolic pressure (posterior tibial or dorsalis pedis) ≤50 mm Hg in the affected limb, toe systolic pressure ≤30 mm Hg; or • Pedal $TcPo_2$ of ≤30 mm Hg (with a limb/chest $TcPo_2$ ratio ≤0.5).

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Table I. Continued.

Study ID	*Study design*	*Patients, No.*	*Follow-up, months*	*Age, mean*	*Study setting*	*Intervention, No.*	*Comparison, No.*	*Inclusion criteria*
Spincemaille,[36] 2000[b]	RCT	120	24	73.1	Single	SCS + medical treatment	Medical treatment only	• In the case of patients with ulcers or gangrene (Fontaine stage IV), the ankle and toe pressure cutoffs were ≤70 mm Hg and ≤50 mm Hg, respectively. • Patients with CLI selected on the basis of the European Consensus Document on CLI; or • Patients with nonreconstructable peripheral arterial occlusive disease

BM-MNC, Bone marrow mononuclear cells; *CLI,* critical limb ischemia; *HGF,* hepatocyte growth factor; *HER,* hydroxyethylrutoside; *IM,* intramuscular; *IV,* intravenous; *IPC,* intermittent pneumatic compression; *NRT,* nonrandomized trial; *NV1FGF,* nonviral 1 fibroblast growth factor; *PGE1,* prostaglandin E_1; *PGI2,* prostacyclin; *RCT,* randomized controlled trial; *SCS,* spinal cord stimulation; *$TcPo_2$,* transcutaneous oxygen pressure; *VEGF,* vascular endothelial growth factor.

[a]Range.

[b]Studies were not included in the meta-analysis.

Risk of bias assessment. To appraise the risk of bias (study quality), we used The Newcastle-Ottawa Scale[13] for nonrandomized studies and the Cochrane Collaboration's tool in assessing the risk of bias in randomized trials.[14]

Outcomes. The outcomes of interest were mortality, major amputation, limb loss, and wound healing. Mortality was defined as postintervention ≥1 year all-cause mortality. Major amputation was defined as above-ankle ischemia-related amputation. Wound healing was reported as improved wound signs or complete closure.

Data synthesis and statistical analysis. We pooled odds ratios (ORs) of the outcomes of interest using the DerSimonian and Laird random-effect models[15] with heterogeneity estimated by the Mantel-Haenszel model. We assessed the overall heterogeneity across the included studies using the I^2 statistic and the Cochran Q test, where $I^2 > 50\%$ and a conservative P value $<.10$ suggest high heterogeneity. STATA 12.1 software (StataCorp LP, College Station, Tex) was used in all statistical analyses. We performed subgroup analysis to compare outcomes based on study design, length of follow-up, treatment combinations (single therapy vs combined), and studies before and after 1999. We also performed a subgroup analysis comparing studies that used hemodynamic criteria vs mixed criteria (ie, hemodynamic or clinical) to ascertain CLI diagnosis, based on the suggested objective criteria for evaluating catheter-based treatment of CLI.[16] Our a priori hypothesis was to stratify outcomes in accordance with the Society for Vascular Surgery Lower Extremity Threatened Limb Classification System.[17]

RESULTS

The search yielded 964 citations, of which 19 studies ultimately met the inclusion criteria but only 17, which enrolled 2779 patients, were included in the meta-analysis (Fig). Of these, 13 studies were randomized controlled trials, and four studies were nonrandomized cohort studies. Six studies[18-23] used hemodynamic criteria to diagnose patients with CLI, and 13 studies[24-35] used mixed criteria to designate their patients with a diagnosis of CLI and enroll in the study (Table I). The studied interventions varied, including SCS, intermittent pneumatic compression (IPC), prostacyclin, hydroxyethylrutoside plus warfarin, bone marrow mononuclear cell (BM-MNC), nonviral 1-fibroblast growth factor (NV1FGF), and hepatocyte growth factor (HGF) gene therapy trials. The risk of bias in the included trials was moderate. Table II summarizes the risk of bias indicators in the included studies. We were not able to assess publication bias because of the small number of the included studies per intervention.

Attempts to stratify end points using the Society for Vascular Surgery Lower Extremity Threatened Limb Classification System[17] were not possible due to insufficient reporting of variables of interest.

Meta-analysis. The results of meta-analysis are reported in Table III and shown as forest plots in Supplementary Figs 1-19 (online only). Subgroup analysis was done on study type in BM-MNC and by whether patients treat with SCS received prostaglandin E_1 (PGE1).

SCS therapy was evaluated in five studies.[18,20,24-26] Four of these studies[18,24-26] had patients who were treated with SCS only, and one study included patients who were treated additionally with PGE1.[20] SCS was associated with reduced risk of amputation (OR, 0.53; 95% confidence interval [CI], 0.36-0.79), as reported in Table III, *B.* SCS therapy was not associated with a change in mortality (OR, 0.76; 95% CI, 0.39-1.49) or ulcer healing (OR, 6.17; 95% CI, 1.87-20.30), as reported in Table III, *A* and *C*, respectively.

Three types of cellular (biological) therapies were used to target angiogenesis: BM-MNC, NV1FGF, and HGF. These were combined for each type of therapy and also pooled to give an overall estimate for angiogenic therapies. None of the angiogenesis therapies showed a statistically significant effect on mortality, amputation, or ulcer healing, as reported in Table III.

IPC use was associated with reduced risk of amputation (OR, 0.14; 95% CI, 0.04-0.55). In one nonrandomized study, hydroxyethylrutoside plus warfarin combination

Table II. Risk of bias assessment

Study ID	*Random sequence generation*	*Allocation concealment*	*Blinding*	*Attrition bias*	*Selective outcome reporting*
Randomized controlled trials					
Powell,[34] 2012	Blocks 2:1	Yes	Patients, care givers, data collectors	Low	Prespecified outcomes (efficacy and safety) were reported
Belch,[19] 2011	Stratification by diabetes status and by country, managed centrally by a central IVRS. The randomization code list, with a block size of 4	Yes, voice-activated response system	Care givers, data collectors, data analysts and outcome assessors	Low	Prespecified outcomes (efficacy and safety) were reported
Jivegard,[24] 1995	Stratification according to Pocock and Simon for gender, age (cutoff was 70 years), diabetes and ischemic ulceration	Not reported	Not reported	Low	Prespecified outcomes (efficacy) were reported
Van Tongeren,[35] 2008	Random number table	Not reported	Care givers	Low	Prespecified outcomes (efficacy) were reported; however, the study did not report any mortality outcomes (safety), which is relevant to such condition and interventions
Ubbink,[18] 1999	Independent randomization by stratification was performed for diabetes, ankle blood pressure, and the participating center	Yes, central randomization	Not reported	Low	Prespecified outcomes were reported; however, skin microcirculation status was used a surrogate outcome to qualify better ulcer healing. Also, the study did not report any mortality outcomes, which is relevant to such condition and interventions
Powell,[21] 2008	Blocks of 1:1:1:1	Not reported	Double-blind	Low	Prespecified outcomes (efficacy and quality of life assessment) were reported. Other relevant safety and efficacy outcomes, such as amputation, were not reported
Powell,[22] 2010	Randomization ratio 3:1	Not reported	Double-blind	Low	Prespecified outcomes (efficacy and safety) were reported
Anghel,[27] 2011	2:1 ratio	Not reported	Double-blind	High	Prespecified outcomes (efficacy and safety) were reported
Claeys,[20] 1999	Not reported	Not reported	Not reported	Low	Prespecified outcomes (efficacy and safety, 1-year and 2-year outcome) were reported
Klomp,[25] 1999	Random-numbers table, the list was held independently of the investigators	Not reported	Not blinded	Low	Prespecified outcomes (efficacy and quality of life assessment) were reported

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Table II. Continued.

Study ID	*Random sequence generation*	*Allocation concealment*	*Blinding*	*Attrition bias*	*Selective outcome reporting*
Nikol,[33] 2008	Permuted blocks (4:1). In this multinational study, only eight of the sites were able to enroll complete blocks, and this led to a slight imbalance in numbers between the placebo and NV1FGF groups	Yes	Patients, caregivers, data analysts, and outcome assessors	High	Prespecified outcomes (efficacy and safety) were reported
Dormandy,[30] 2000	Not reported	Not reported	Patients, caregivers, and outcome assessors	Low	Prespecified outcomes (efficacy and tolerability) were reported

	Representativeness of the exposed cohort	*Selection of the nonexposed cohort*	*Ascertainment of exposure*	*Outcome of interest was not present at start of study*	*Comparability of cohorts*
Nonrandomized cohort studies					
Napoli,[32] 2008	Somewhat representative	Drawn from the same community	No description	Yes	Study controls for most important factor
Amann,[26] 2003	Truly representative	Drawn from the same community	Secure records	Yes	No attempt to control for confounding.
Kavros,[31] 2008	Truly representative	Drawn from the same community	Secure records	Yes	Study controls for most important factor
Lund,[23] 1999	Truly representative	Drawn from the same community	Secure records	Yes	No attempt to control for confounding
Idei,[29] 2011	Truly representative	Drawn from the same community	Secure records	Yes	Study controls for most important factor

IVRS, Interactive voice response system; *NV1FGF,* nonviral 1 fibroblast growth factor.

therapy was associated with decreased mortality (OR, 0.3; 95% CI, 0.09-0.96). None of the other therapies showed statistical significance in mortality, amputation, or ulcer healing (wound improvement), as reported Table III.

NV1FGF showed a protective effect in patients who were diagnosed solely based on the hemodynamic criteria compared with the mixed criteria-diagnosed population (OR, 0.36 [95% CI, 0.14-0.92] vs OR, 1.25 [95% CI 0.83-1.88]; $P = .01$), as reported in Table III, *B.* Enrollment using the hemodynamic criteria did not show any significant difference among the other interventions, as reported in Table III. The event rates of included individual studies are summarized in Table IV.

Some studies did not report a clear distinction in their inclusion criteria to assess their eligibility in our quantitative analysis: patients received prior surgical interventions,[36] presence of disagreement of CLI-defining criteria with our protocol (or inclusion of mixed population), or the outcomes of interest were not reported clearly at or >1 year[28]; therefore, the consensus was to include these studies in the systematic review only but not in the meta-analysis.

The CIRCULASE trial[28] enrolled 190 patients with CLI who received PGE1 analog and reported that 16.2% underwent major amputations and 23.2% were ulcer-free by 6 months. At the 1-year follow-up, the trial reported a 14% all-cause mortality rate.

Spincemaille et al[36] randomized 60 patients to receiving SCS implantations, in addition to their usual medication, and reported 40% and 48% limb amputations after 1 and 2 years of follow-up, respectively.

DISCUSSION

This systematic review and meta-analysis showed that the use of IPC or SCS might be associated with a reduced risk of amputation. None of the other included nonrevascularization-based treatments were associated with a significant effect on mortality, amputation, or wound healing. One small nonrandomized study from the late 1990s suggested a potential benefit of hydroxyethylrutoside plus warfarin, but these findings have not been replicated.

IPC use was associated with statistically significant improvements in ulcer healing and amputation, but given that these results were derived from single small nonrandomized studies, replication of such results is needed, and the effect needs to be verified in larger randomized controlled trials (quality of evidence, very low, C). SCS did show improvements in amputation rates; however, these estimates lacked precision. In addition, several angiogenic

Table III. A, Meta-analysis results of mortality

Intervention	*Subgroup*	*OR (95% CI)*	I^2, *%*	P *(Heterogeneity)*	P *(Interaction)*
SCS	All studies	0.76 (0.39-1.49)	0.0	.79	
	Without PGE1[a]	0.84 (0.33-2.11)	0.0	.53	.78
	With PGE1[a]	0.69 (0.26-1.83)	0.0	–	
	Mixed-criteria CLI diagnosis	0.84 (0.33-2.11)	0.0	.53	.78
	Hemodynamic criteria-only CLI diagnosis	0.76 (0.39-1.49)	0.0	–	
BM-MNC	Mixed-criteria CLI diagnosis	0.54 (0.21-1.38)	–	.70	
	Only RCT	1.00 (0.09-11.61)	–	–	–
NV1FGF	All studies	0.81 (0.32-2.07)	64.3	.09	
	Mixed-criteria CLI diagnosis	0.44 (0.15-1.27)	–	–	.09
	Hemodynamic criteria-only CLI diagnosis	1.18 (0.74-1.88)	–	–	
HGF	All studies	1.27 (0.33-4.93)	0.0	.95	
	Mixed-criteria CLI diagnosis	0.96 (0.08-11.61)	–	–	.76
	Hemodynamic criteria-only CLI diagnosis	1.52 (0.27-8.53)	0	.763	
IPC	Mixed-criteria CLI diagnosis	0.60 (0.15-2.47)	–	–	–
HER + warfarin	Mixed-criteria CLI diagnosis	0.30 (0.09-0.96)	–	–	–
Prostacyclin	Mixed-criteria CLI diagnosis	1.11 (0.67-1.83)	–	–	–

BM-MNC, Bone marrow mononuclear cells; *CI,* confidence interval; *CLI,* critical limb ischemia; *HER,* hydroxyethylrutoside; *IPC,* intermittent pneumatic compression; *NV1FGF,* nonviral 1 fibroblast growth factor; *OR,* odds ratio; *PGE1,* prostaglandin E_1; *RCT,* randomized controlled trial.
[a]Studies using hemodynamic criteria.

Table III. B, Meta-analysis results of major amputation

Intervention	*Subgroup*	*OR (95% CI)*	I^2, *%*	P *(Heterogeneity)*	P *(Interaction)*
SCS	All studies	0.53 (0.36-0.79)	0.0	.54	
	With PGE1	0.76 (0.25-2.32)	–	–	.59
	Without PGE1	0.52 (0.31-0.87)	8.7	.33	
BM-MNC	Hemodynamic criteria-based CLI diagnosis	0.13 (0.01-1.16)	86.5	.001	
	RCTs only	0.50 (0.14-1.84)	30.4	.23	–
NV1FGF	All studies	0.72 (0.22-2.42)	82.3	.017	
	Mixed-criteria CLI diagnosis	0.36 (0.14-0.92)	–	–	.01
	Hemodynamic criteria-based CLI diagnosis	1.25 (0.83-1.88)	–	–	
HGF	All studies	0.32 (0.08-1.35)	32.8	.22	–
	Mixed-criteria CLI diagnosis	0.18 (0.04-0.76)	–	–	.23
	Hemodynamic criteria-based CLI diagnosis	0.80 (0.11-5.59)	–	–	
IPC	Mixed-criteria CLI diagnosis	0.14 (0.04-0.55)	–	–	–
HER + warfarin	Hemodynamic criteria-based CLI diagnosis	0.79 (0.33-1.89)	–	–	–
Prostacyclin	Mixed-criteria CLI diagnosis	0.85 (0.55-1.30)	–	–	–

BM-MNC, Bone marrow mononuclear cells; *CI,* confidence interval; *CLI,* critical limb ischemia; *HER,* hydroxyethylrutoside; *IPC,* intermittent pneumatic compression; *NV1FGF,* nonviral 1 fibroblast growth factor; *OR,* odds ratio; *PGE1,* prostaglandin E_1; *RCT,* randomized controlled trial; *SCS,* spinal cord stimulator.

therapies showed trends toward improvement, but the trials varied significantly in the type, dose, and delivery of the treatment. When all angiogenic therapies were combined, there was significant improvement in limb loss, but none of the individual therapies demonstrated a statistically significant effect (quality of evidence, very low, C).

Patients with CLI are often elderly, present with multiple comorbidities, and experience significant pain, nonhealing wounds, impaired mobility, and reduced quality of life. Although revascularization is the first-line therapy for many patients, many others are poor candidates due to physiologic or anatomic reasons. As a result, there is considerable interest in alternative noninvasive forms of treatment that can ameliorate the debilitating effects of CLI.

At present, medical therapy is considered largely palliative. Regenerative approaches (eg, cell and gene therapies) are largely unproven. Previous evidence suggested the positive effect of SCS, generally agreeing with our results. Therapeutic angiogenesis has been sought as a promising therapy to improve ankle-brachial index, rest pain, and transcutaneous oxygen pressure, but the evidence from small trials has been limited mainly due to design issues or early stop.[37] There is great interest from physicians and industry in developing alternative treatment approaches, with significant research in recent years, prompting the need for this systematic review.

Limitations of this study include the relatively low quality of the evidence mainly due to imprecision (ie, small sample size and wide CIs) and the risk of bias. There was a significant amount of heterogeneity among studies due to differing design and inclusion criteria as well as the variation in the anatomic site and severity of ischemia.

The strengths of this systematic review include the expansive and comprehensive literature search, the inclusion of all publication languages, selecting studies in

Table III. C, Meta-analysis results of ulcer healing

Intervention	*OR (95% CI)*	I^2	P *(Heterogeneity)*
SCS + PGE1	6.17[a] (1.87-20.30)	–	–
HGF	6.72[a] (0.33-136.21)	–	–
HER + warfarin	0.79[a] (0.33-1.89)	–	–
IPC	7.00[b] (1.82-26.89)	–	–
Prostacyclin	1.44[b] (0.86-2.42)	–	–

CI, Confidence interval; *HER,* hydroxyethylrutoside; *HGF,* hepatocyte growth factor; *IPC,* intermittent pneumatic compression; *OR,* odds ratio; *PGE1,* prostaglandin E_1; *SCS,* spinal cord stimulator.
[a]Studies using hemodynamic criteria.
[b]Studies using mixed criteria (clinical presentation with or without hemodynamic diagnosis).

Table IV. Summary of event rates of included studies

		Limb loss		
Study ID	*Mortality, %*	*Patients with limb loss, %*	*Limbs lost, %*	*Ulcer healing, %*
Jivegard,[24] 1995	31	45	–	–
Ubbink,[18] 1999	–	35	–	–
Klomp,[25] 1999	6	45	–	–
Claeys,[20] 1999	26	17	–	21
Lund,[23] 1999	23	–	66	–
Dormandy,[30] 2000	13	27	–	13
Amann,[26] 2003	–	35	–	–
Kavros,[31] 2008	21	63	–	–
Napoli,[32] 2008	5	33	–	–
Powell,[21] 2008	6	–	–	–
Nikol,[33] 2008	18	25	–	–
Van Tongreen,[35] 2008	–	33	–	–
Powell,[22] 2010	19	30	–	26
Idei,[29] 2011	16	59	47	–
Anghel,[27] 2011	7	44	–	–
Blech,[19] 2011	16	23	–	–
Powell,[34] 2012	3	19	–	–

duplicate, and adherence to the TransAtlantic Inter-Society Consensus for the Management of Peripheral Arterial Disease definition of CLI.

CONCLUSIONS

Overall low-quality evidence suggests that IPC and SCS may reduce the risk of amputation. Evidence supporting other medical therapies is insufficient. Given the quality of the evidence, further research is needed to define optimal nonsurgical approaches to CLI treatment.

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AUTHOR CONTRIBUTIONS

Conception and design: MC, MM
Analysis and interpretation: AAD, MS, ZW, MC, MM
Data collection: AAD, MS, NA, CU, ME
Writing the article: AAD, MS, NA, ZW, MC, MM
Critical revision of the article: AAD, MS, NA, CU, ZW, ME, MC, MM
Final approval of the article: AAD, MS, NA, CU, ZW, ME, MC, MM
Statistical analysis: AAD, MS
Obtained funding: AAD, MS, MM
Overall responsibility: MM
AAD and MS equally contributed to this study as first authors.

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Additional material for this article may be found online at www.jvascsurg.org.

APPENDIX (online only).

Search strategy

Ovid. Database(s): Embase 1988 to 2012 Week 46, Ovid MEDLINE in-process and other nonindexed citations and Ovid MEDLINE 1946 to present, Evidence-Based Medicine Reviews—Cochrane Central Register of Controlled Trials November 2012, Evidence-Based Medicine Reviews—Cochrane Database of Systematic Reviews 2005 to October 2012

No.	*Searches*	*Results*
1	exp Ischemia/	455,614
2	exp peripheral occlusive artery disease/	87,563
3	exp Peripheral Vascular Diseases/	950,368
4	exp Atherosclerosis/	143,727
5	exp arteriosclerosis/	272,300
6	exp intermittent claudication/	13,290
7	exp Arterial Occlusive Diseases/	264,275
8	(ischemia or ischaemia or ischemic or ischaemic or "circulation disorder*" or "circulation failure*" or "circulation disturbance*" or "circulatory disorder*" or "circulatory failure*" or "circulatory disturbance*" or ((arter* or vascul* or vein or veno or peripheral*) adj2 (steno* or lesio* or block* or occlus* or obliterat* or insufficiency or obstruct*)) or "peripheral arterial disease*" or "peripheral artery disease*" or "peripheral occlusive disease*" or "peripheral angiopath*" or PVD or PAOD or atherosclero* or atherogenesis or atheroma* or "peripheral vascular disease*" or (intermitten* adj claudicat*) or arteriosclor* or CLI).mp. [mp = ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, nm, ps, rs, ui, tx, ct]	1,164,776
9	or/1-8	1,688,305
10	exp Leg/	156,979
11	exp Leg Ulcer/	24,967
12	exp lower extremity/	227,598
13	(leg or legs or foot or feet or toe or toes or knee* or ankle* or thigh* or calf or "calfs lower limb*" or "lower extremit*" or buttock* or hip or hips).mp. [mp = ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, nm, ps, rs, ui, tx, ct]	970,420
14	or/10-13	976,948
15	9 and 14	110,077
16	exp Prostaglandins/	183,122
17	exp Alprostadil/	17,209
18	exp Epoprostenol/	27,584
19	exp Iloprost/	6984
20	exp prostaglandin E1/	17,209
21	exp prostacyclin/	27,584
22	exp beraprost/	1238
23	(Prostaglandin* or prostanoid* or "AS-013" or iloprost or ventavis or liprostin or alprostadil or taprostene or beraprost* or "TTC-909" or clinprost or misoprostol or cicaprost or cisaprost or Epoprostenol or ciprostene or prostavasin or lipoecraprost or "lipo-ecraprost" or prostacyclin or PGE* or PGI*).mp. [mp = ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, nm, ps, rs, ui, tx, ct]	347,934
24	or/16-23	356,675
25	exp Electric Stimulation Therapy/ or exp Electric Stimulation/	336,941
26	exp spinal cord stimulation/	2992
27	((spinal adj3 stimulat*) or (dors* adj column* adj3 stimulat*) or (epidur* adj spin* adj3 stimulat*) or (epidur* adj electric* adj stimulat*)).mp. [mp = ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, nm, ps, rs, ui, tx, ct]	8508
28	or/25-27	339,049
29	((compression or mechanical*) adj2 (device* or pump* or therap* or treatment*)).mp. [mp = ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, nm, ps, rs, ui, tx, ct]	24,119
30	exp Transplantation, Autologous/	56,169
31	exp bone marrow transplantation/	86,023
32	exp Leukocytes, Mononuclear/	968,596
33	(autotransplant* or "autologous transplantat*" or "auto transplant*" or "Bone Marrow Transplant*" or "bone marrow graft*" or "bone marrow transfusion*" or mononuclear or (autologous adj3 cell*)).mp. [mp = ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, nm, ps, rs, ui, tx, ct]	330,583
34	or/29-33	1,210,237
35	exp Gene Therapy/	93,398
36	exp cell transplantation/	146,595

(*Continued on next page*)

Continued.

No.	*Searches*	*Results*
37	("gene therap*" or "dna therap*" or "gene treatment*" or "dna treatment*" or "cell transplant*").mp. [mp = ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, nm, ps, rs, ui, tx, ct]	269,431
38	35 or 36 or 37	278,663
39	24 or 28 or 34 or 38	208,5803
40	exp Survival Analysis/ or exp Survival/ or exp Survival Rate/	759,187
41	exp Wound Healing/	167,811
42	exp amputation/	38,966
43	(survival or (heal* adj3 (wound* or injur*)) or (limb* adj3 (loss or lose or losing)) or amputat*).mp. [mp = ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, nm, ps, rs, ui, tx, ct]	1,769,690
44	or/40-43	1,845,348
45	15 and 39 and 44	2147
46	exp controlled study/	3,976,677
47	exp randomized controlled trial/	657,875
48	((control$ or randomized) adj2 (study or studies or trial or trials)).mp. [mp = ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, nm, ps, rs, ui, tx, ct]	5,094,428
49	meta analysis/	104,932
50	meta-analys$.mp.	168,861
51	exp "systematic review"/	54,671
52	(systematic* adj review$).mp.	12,8401
53	exp cohort studies/	1,460,154
54	exp longitudinal study/	947,405
55	exp retrospective study/	728,211
56	exp prospective study/	604,682
57	exp comparative study/	2,348,824
58	exp clinical trial/	1,603,933
59	exp cross-sectional study/	234,939
60	crossover procedure/	35,484
61	exp cross-over studies/	89,269
62	((clinical or comparative or cohort or longitudinal or retrospective or prospective or concurrent or "cross-sectional" or crossover or "cross-over") adj (study or studies or survey or surveys or analysis or analyses or trial or trials)).mp.	6,627,315
63	("crossover procedure" or "cross-over procedure"). mp. [mp = ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, nm, ps, rs, ui, tx, ct]	39,324
64	or/46-63	10,177,464
65	45 and 64	1336
66	from 45 keep 1247-1970	724
67	limit 66 to (clinical trial, all or clinical trial, phase I or clinical trial, phase ii or clinical trial, phase III or clinical trial, phase IV or clinical trial or comparative study or guideline or meta analysis or multicenter study or practice guideline or randomized controlled trial or twin study or validation studies) [Limit not valid in Embase, CCTR, CDSR; records were retained]	259
68	65 or 67	1337
69	limit 68 to (book or book series or editorial or erratum or letter or note or addresses or autobiography or bibliography or biography or comment or dictionary or directory or interactive tutorial or interview or lectures or legal cases or legislation or news or newspaper article or overall or patient education handout or periodical index or portraits or published erratum or video-audio media or webcasts) [Limit not valid in Embase, Ovid MEDLINE, Ovid MEDLINE in-process, CCTR, CDSR; records were retained]	98
70	68 not 69	1239
71	from 45 keep 1971-2147	177
72	70 or 71	1341
73	remove duplicates from 72	987

CCTR, Cochrane Controlled Trials Register; *CDSR,* Cochrane Database of Systematic Reviews.

Scopus. TITLE-ABS-KEY (ischemia or ischaemia or ischemic or ischaemic or "circulation disorder*" or "circulation failure*" or "circulation disturbance*" or "circulatory disorder*" or "circulatory failure*" or "circulatory disturbance*" or (arter* W/2 steno*) or (arter* W/2 lesio*) or (arter* W/2 block*) or (arter* W/2 occlus*) or (arter* W/2 obliterat*) or (arter* W/2 insufficiency) or (arter* W/2 obstruct*) or (vascul* W/2 steno*) or (vascul* W/2 lesio*) or (vascul* W/2 block*) or (vascul* W/2 occlus*) or (vascul* W/2 obliterat*) or (vascul* W/2 insufficiency) or (vascul* W/2 obstruct*) or (vein W/2 steno*) or (vein W/2 lesio*) or (vein W/2 block*) or (vein W/2 occlus*) or (vein W/2 obliterat*) or (vein W/2 insufficiency) or (vein W/2 obstruct*) or (veno W/2 steno*) or (veno W/2 lesio*) or (veno W/2 block*) or (veno W/2 occlus*) or (veno W/2 obliterat*) or (veno W/2 insufficiency) or (veno W/2 obstruct*) or (peripheral* W/2 steno*) or (peripheral* W/2 lesio*) or (peripheral* W/2 block*) or (peripheral* W/2 occlus*) or (peripheral* W/2 obliterat*) or (peripheral* W/2

insufficiency) or (peripheral* W/2 obstruct*) or "peripheral arterial disease*" or "peripheral artery disease*" or "peripheral occlusive disease*" or "peripheral angiopath*" or PVD or PAOD or atherosclero* or atherogenesis or atheroma* or "peripheral vascular disease*" or (intermitten* W/1 claudicat*) or arteriosclor* or CLI)

TITLE-ABS-KEY (leg or legs or foot or feet or toe or toes or knee* or ankle* or thigh* or calf or calfs "lower limb*" or "lower extremit*" or buttock* or hip or hips)

TITLE-ABS-KEY (Prostaglandin* or prostanoid* or "AS-013" or iloprost or ventavis or liprostin or alprostadil or taprostene or beraprost* or "TTC-909" or clinprost or misoprostol or cicaprost or cisaprost or Epoprostenol or ciprostene or prostavasin or lipoecraprost or "lipo-ecraprost" or prostacyclin or PGE* or PGI* or (spinal W/3 stimulat*) or (dors* W/1 column* W/3 stimulat*) or (epidur* W/1 spin* W/3 stimulat*) or (epidur* W/1 electric* W/1 stimulat*) or (compression W/2 device*) or (compression W/2 pump*) or (compression W/2 therap*) or (compression W/2 treatment*) or (mechanical* W/2 device*) or (mechanical* W/2 pump*) or (mechanical* W/2 therap*) or (mechanical* W/2 treatment*) or autotransplant* or "autologous transplantat*" or "auto transplant*" or "Bone Marrow Transplant*" or "bone marrow graft*" or "bone marrow transfusion*" or mononuclear or (autologous W/3 cell*) or "gene therap*" or "dna therap*" or "gene treatment*" or "dna treatment*" or "cell transplant*")

TITLE-ABS-KEY (survival or (heal* W/3 wound*) or (heal* W/3 injur*) or (limb* W/3 loss) or (limb* W/3 lose) or (limb* W/3 losing) or amputat*)

TITLE-ABS-KEY ((meta W/1 analys*) or (systematic* W/2 review*) or (control* W/2 stud*) or (control* W/2 trial*) or (randomized W/2 stud*) or (randomized W/2 trial*) or "comparative stud*" or "comparative survey*" or "comparative analys*" or "cohort stud*" or "cohort survey*" or "cohort analys*" or "longitudinal stud*" or "longitudinalsurvey*" or "longitudinal analys*" or "retrospective stud*" or "retrospective survey*" or "retrospective analys*"or "prospective stud*" or "prospective survey*" or "prospective analys*" or "concurrent stud*" or "concurrentsurvey*" or "concurrent analys*" or "clinical stud*" or "clinical trial*" or "cross-sectional stud*" or "cross-sectional analys*" or "cross-over stud*" or "cross-over analys*" or "cross-over procedure" or "crossover stud*" or"crossover analys*" or "crossover procedure")

1 and 2 and 3 and 4 and 5

PMID(0*) or PMID(1*) or PMID(2*) or PMID(3*) or PMID(4*) or PMID(5*) or PMID(6*) or PMID(7*) or PMID(8*) or PMID(9*)

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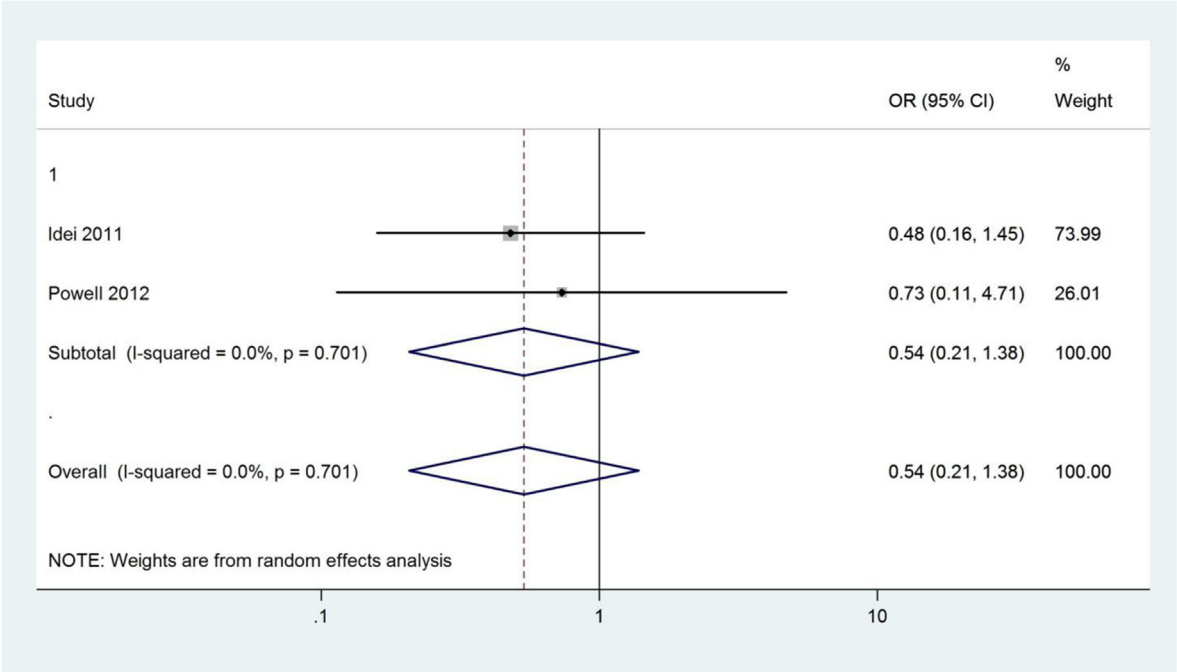
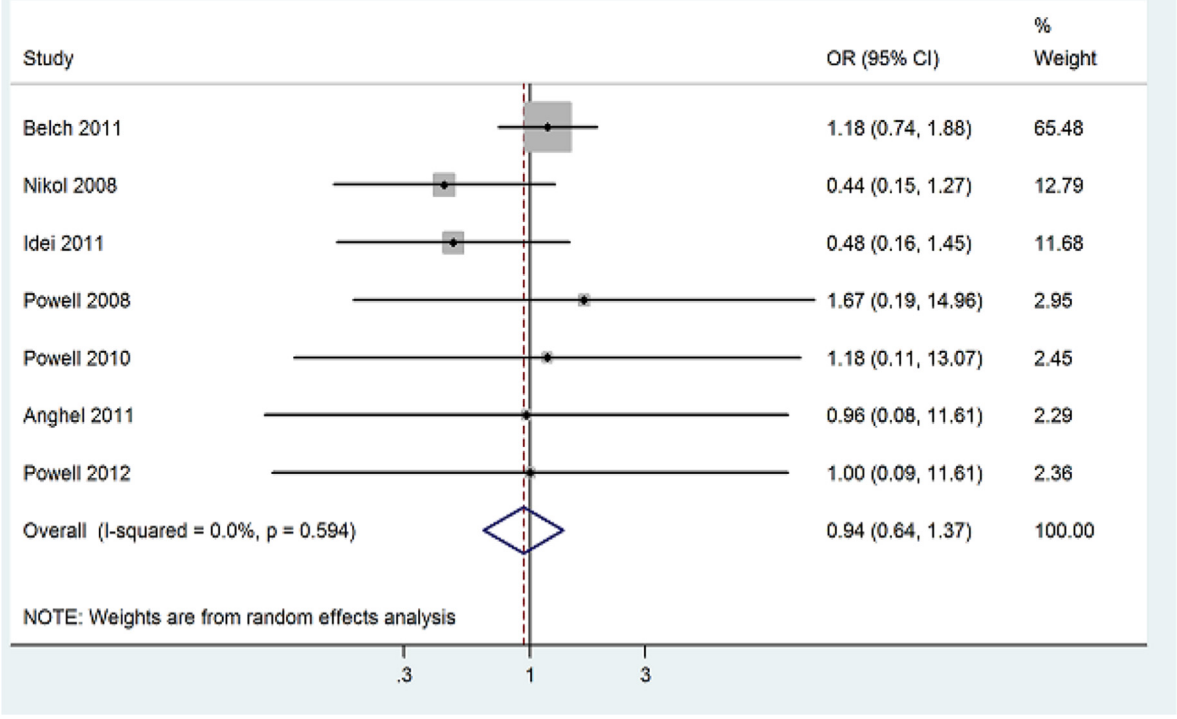
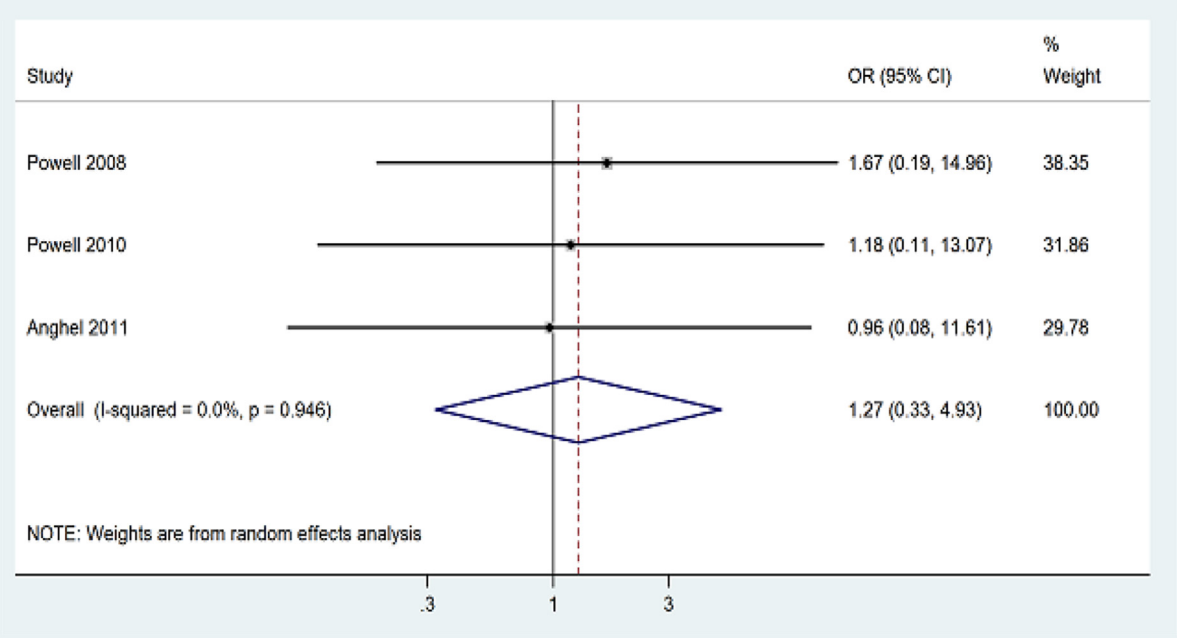


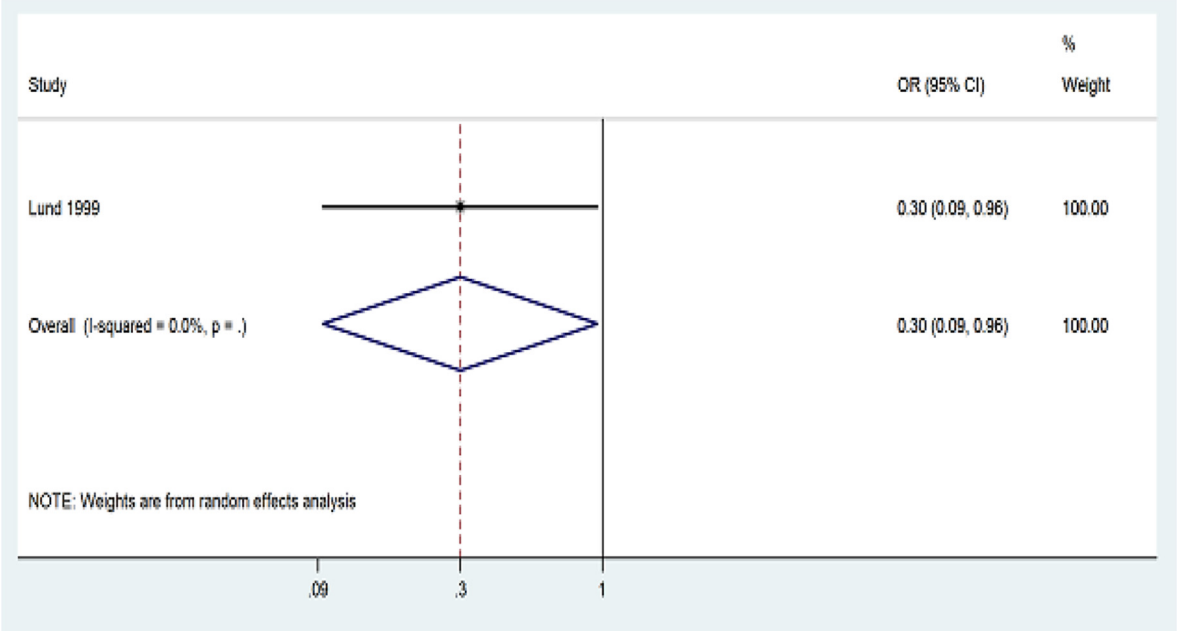
Supplementary Fig 1 (online only). Meta-analysis of mortality in patients after receiving bone marrow mononuclear cells (BM-MNC). *CI,* Confidence interval; *OR*, Odds ratio. The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *dashed vertical line* indicates the summary measure, with the associated *diamond* indicating the weighted mean difference and the lateral tips of the diamond indicating the associated CIs. The *horizontal lines* represent the 95% CIs.



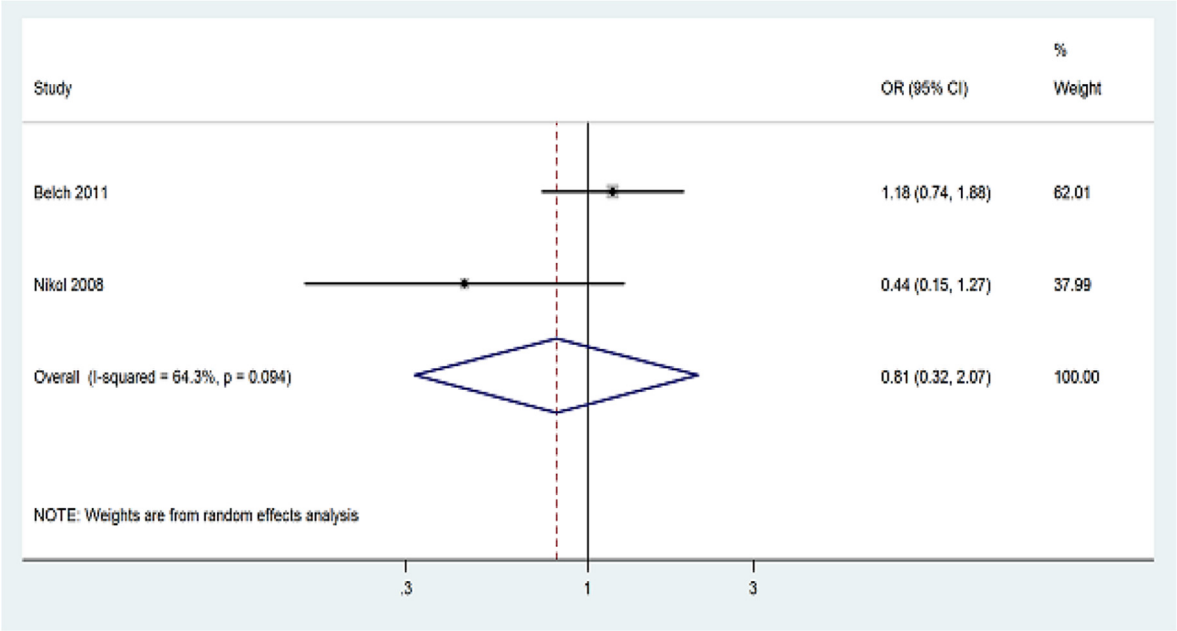
Supplementary Fig 2 (online only). Meta-analysis of mortality in patients after receiving bone marrow mononuclear cells (BM-MNC) plus nonviral 1 fibroblast growth factor (NV1FGF) plus hepatocyte growth factor (HGF) (combined effect of biological treatments). *CI,* Confidence interval; *OR*, odds ratio. The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *dashed vertical line* indicates the summary measure, with the associated *diamond* indicating the weighted mean difference and the lateral tips of the diamond indicating the associated CIs. The *horizontal lines* represent the 95% CIs.



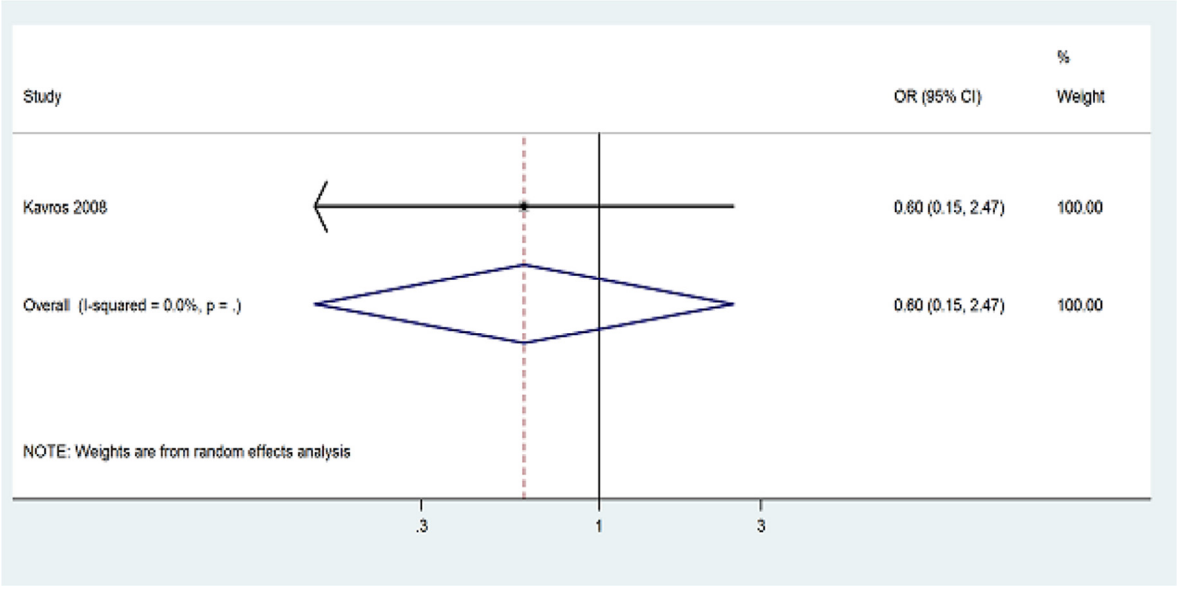
Supplementary Fig 3 (online only). Meta-analysis of mortality in patients after receiving hepatocyte growth factor (HGF). *CI*, Confidence interval; *OR*, odds ratio. The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *dashed vertical line* indicates the summary measure, with the associated *diamond* indicating the weighted mean difference and the lateral tips of the diamond indicating the associated CIs. The *horizontal lines* represent the 95% CIs.



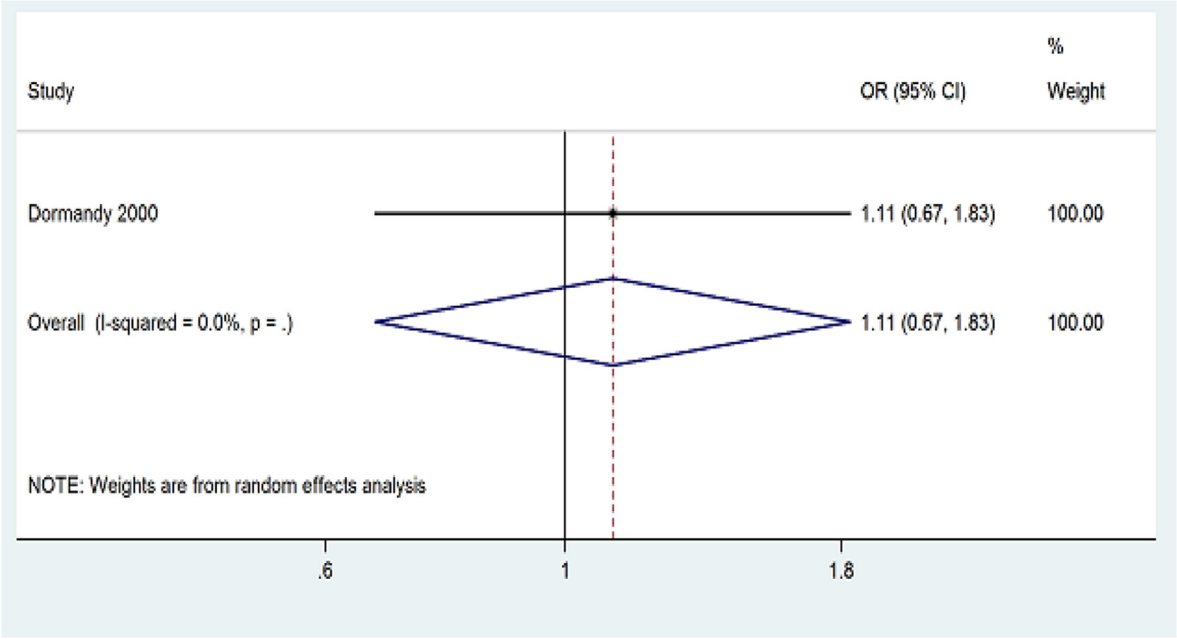
Supplementary Fig 4 (online only). Meta-analysis of mortality outcome in patients after receiving hydroxyethylrutoside plus warfarin. *CI*, Confidence interval; *OR*, odds ratio. The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *dashed vertical line* indicates the summary measure, with the associated *diamond* indicating the weighted mean difference and the lateral tips of the diamond indicating the associated CIs. The *horizontal lines* represent the 95% CIs.



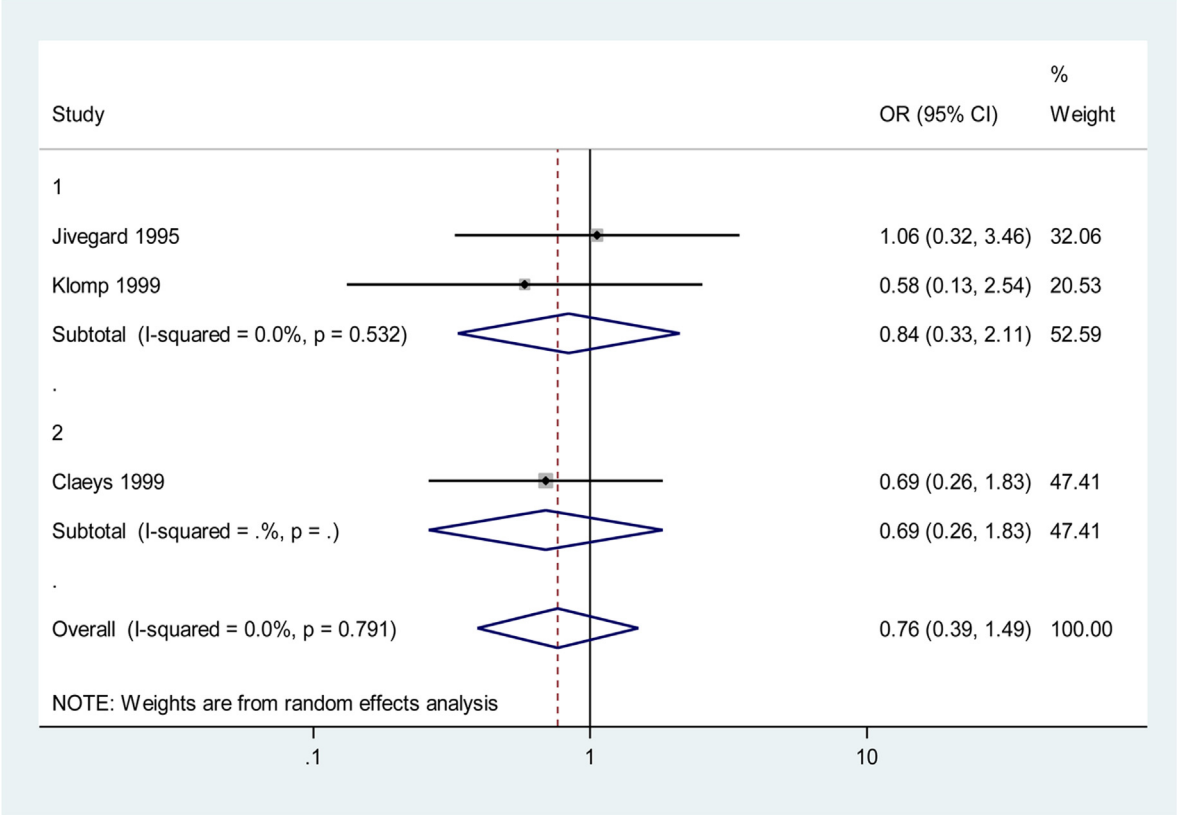
Supplementary Fig 5 (online only). Meta-analysis of mortality in patients after receiving nonviral 1 fibroblast growth factor (NV1FGF). *CI*, Confidence interval; *OR*, odds ratio. The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *dashed vertical line* indicates the summary measure, with the associated *diamond* indicating the weighted mean difference and the lateral tips of the diamond indicating the associated CIs. The *horizontal lines* represent the 95% CIs.



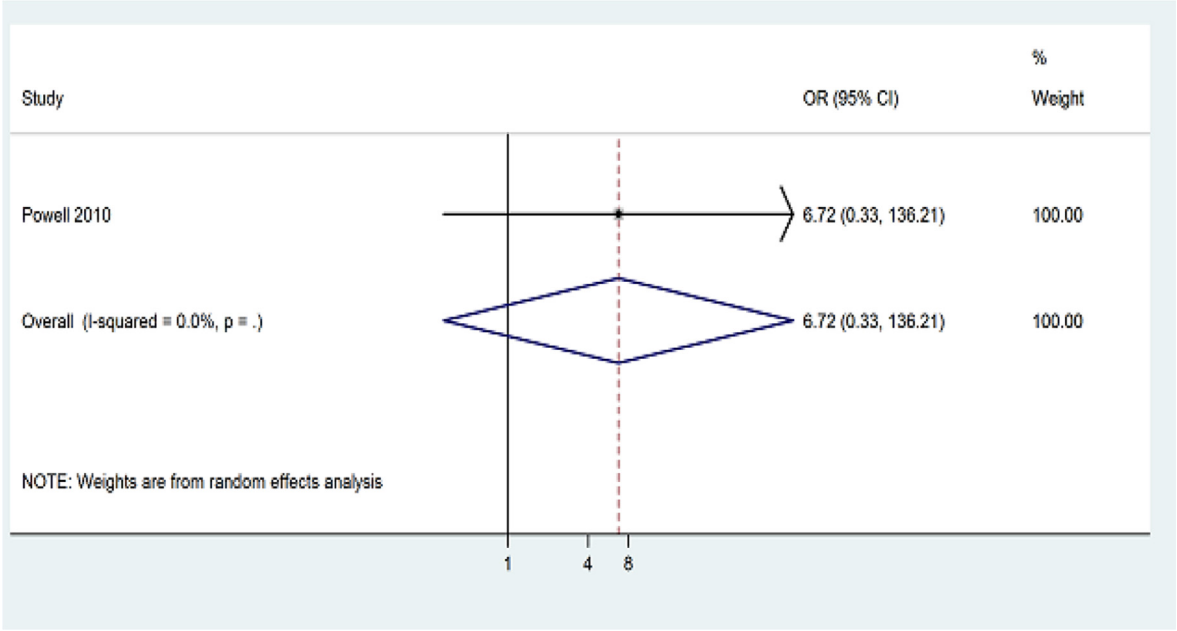
Supplementary Fig 6 (online only). Meta-analysis of mortality outcome in patients after receiving intermittent pneumatic compression (IPC). *CI*, Confidence interval; *OR*, odds ratio. The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *dashed vertical line* indicates the summary measure, with the associated *diamond* indicating the weighted mean difference and the lateral tips of the diamond indicating the associated CIs. The *horizontal lines* represent the 95% CIs.



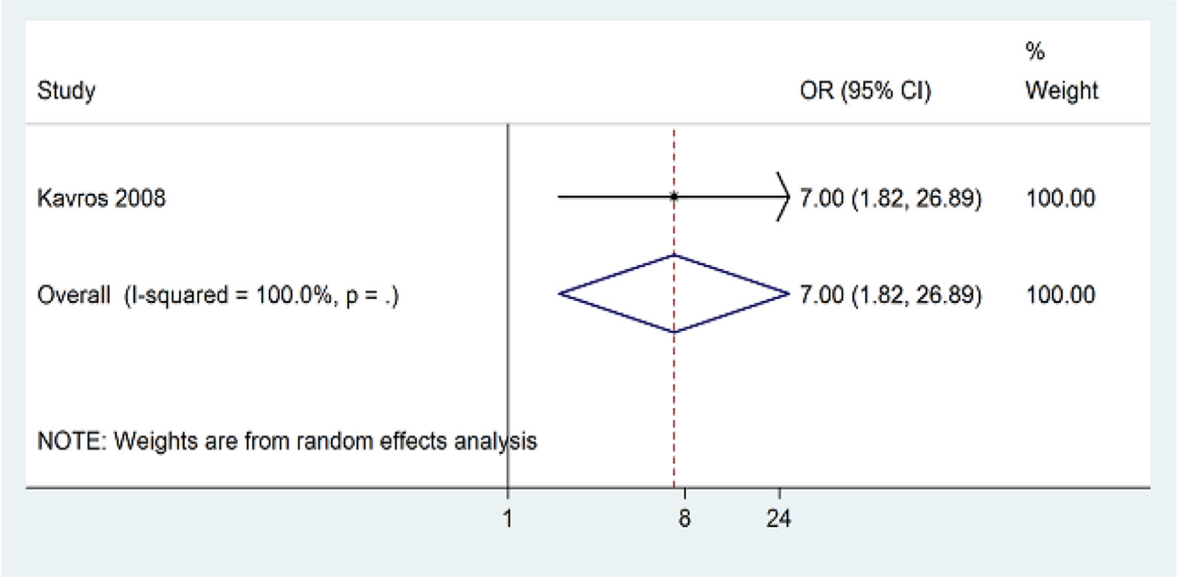
Supplementary Fig 7 (online only). Meta-analysis of mortality outcome in patients after receiving prostacyclin. *CI*, Confidence interval; *OR*, odds ratio. The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *dashed vertical line* indicates the summary measure, with the associated *diamond* indicating the weighted mean difference and the lateral tips of the diamond indicating the associated CIs. The *horizontal lines* represent the 95% CIs.



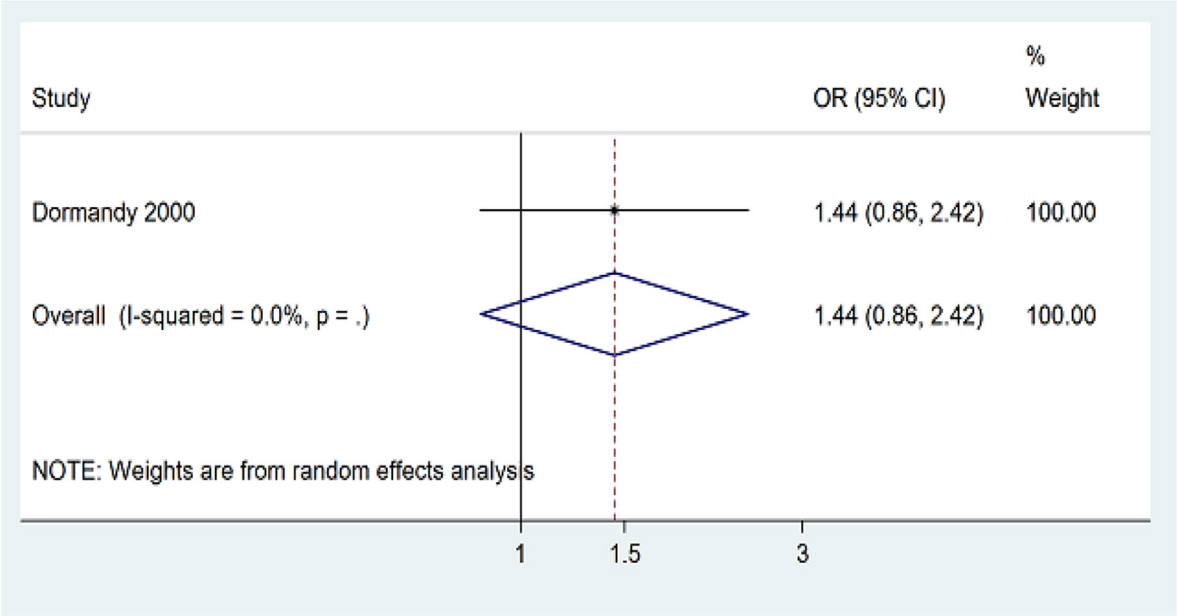
Supplementary Fig 8 (online only). Meta-analysis of mortality in patients after receiving spinal cord stimulator (SCS) implant (1 = without prostaglandin E_1 [PGE1], 2 = with PGE1). *CI*, Confidence interval; *OR*, odds ratio. The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *dashed vertical line* indicates the summary measure, with the associated *diamond* indicating the weighted mean difference and the lateral tips of the diamond indicating the associated CIs. The *horizontal lines* represent the 95% CIs.



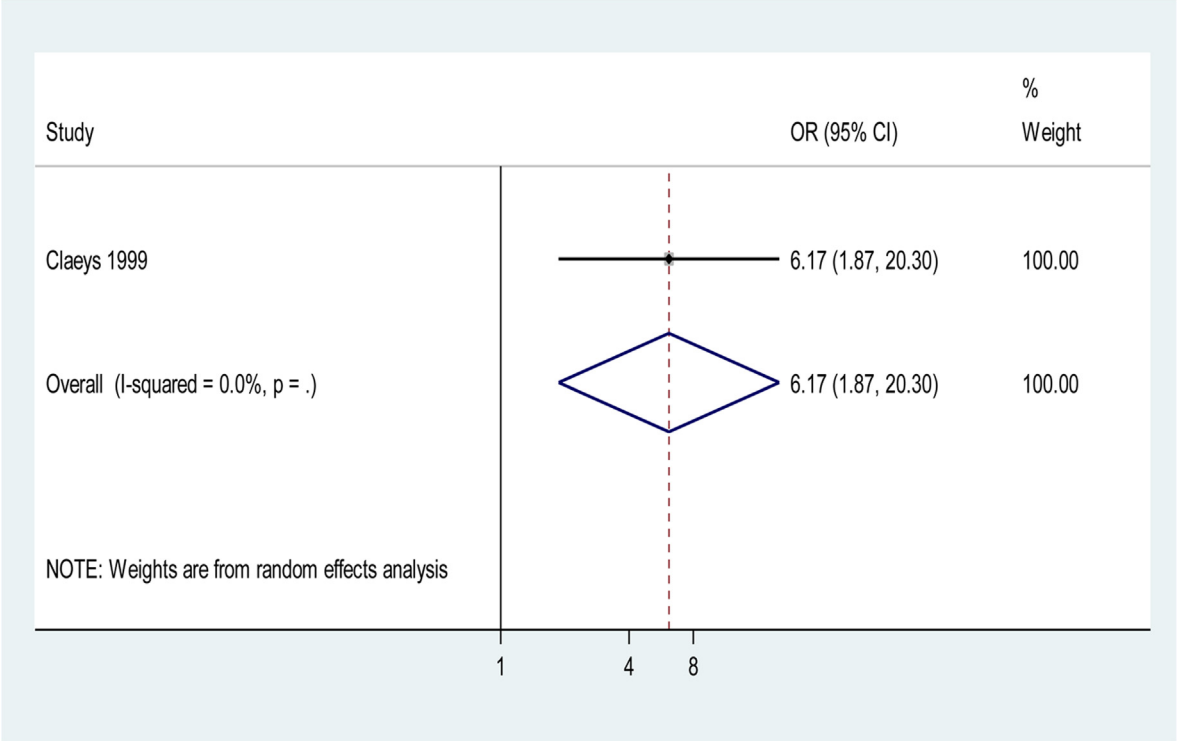
Supplementary Fig 9 (online only). Meta-analysis of patients with improved healing after receiving hepatocyte growth factor (HGF). *CI*, Confidence interval; *OR*, odds ratio. The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *dashed vertical line* indicates the summary measure, with the associated *diamond* indicating the weighted mean difference and the lateral tips of the diamond indicating the associated CIs. The *horizontal lines* represent the 95% CIs.



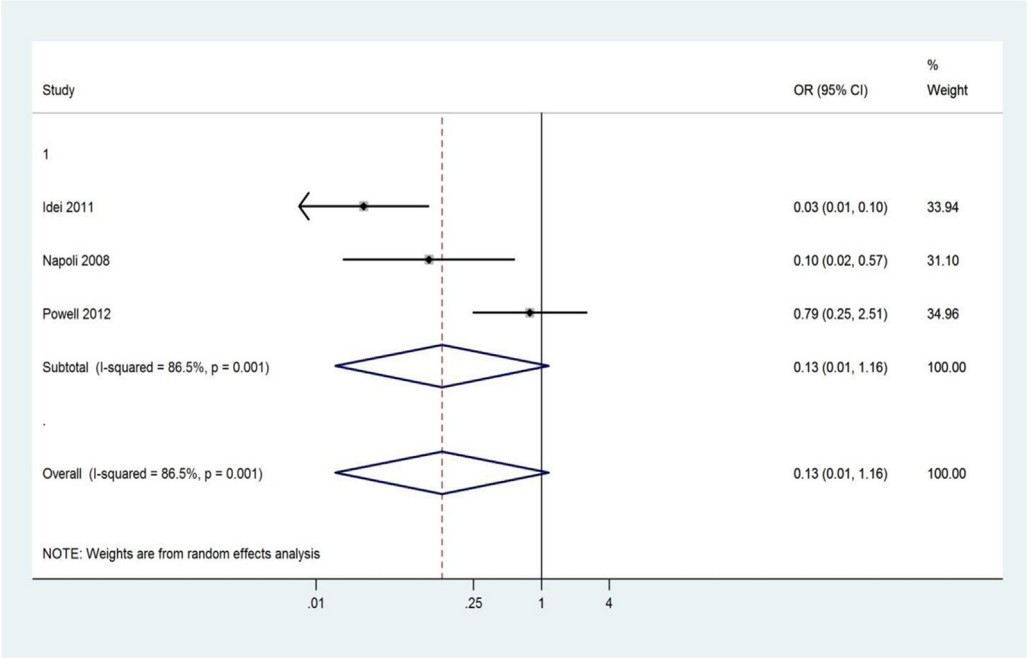
Supplementary Fig 10 (online only). Meta-analysis of patients with improved healing after receiving intermittent pneumatic compression (IPC). *CI*, Confidence interval; *OR*, odds ratio. The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *dashed vertical line* indicates the summary measure, with the associated *diamond* indicating the weighted mean difference and the lateral tips of the diamond indicating the associated CIs. The *horizontal lines* represent the 95% CIs.



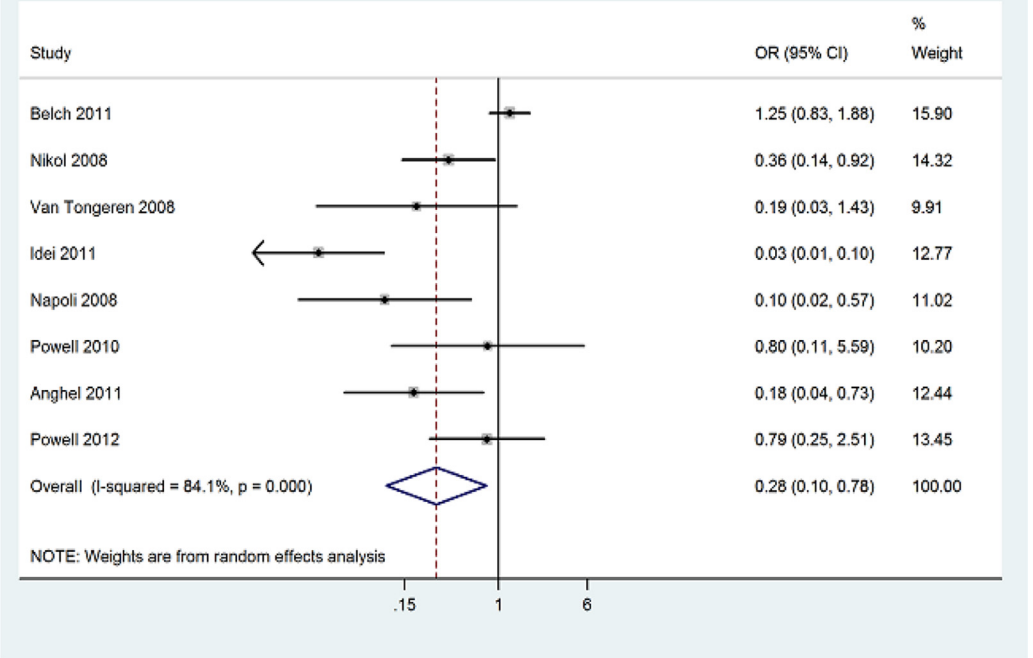
Supplementary Fig 11 (online only). Meta-analysis of patients with improved healing after receiving prostacyclin. *CI,* Confidence interval; *OR,* odds ratio. The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *dashed vertical line* indicates the summary measure, with the associated *diamond* indicating the weighted mean difference and the lateral tips of the diamond indicating the associated CIs. The *horizontal lines* represent the 95% CIs.



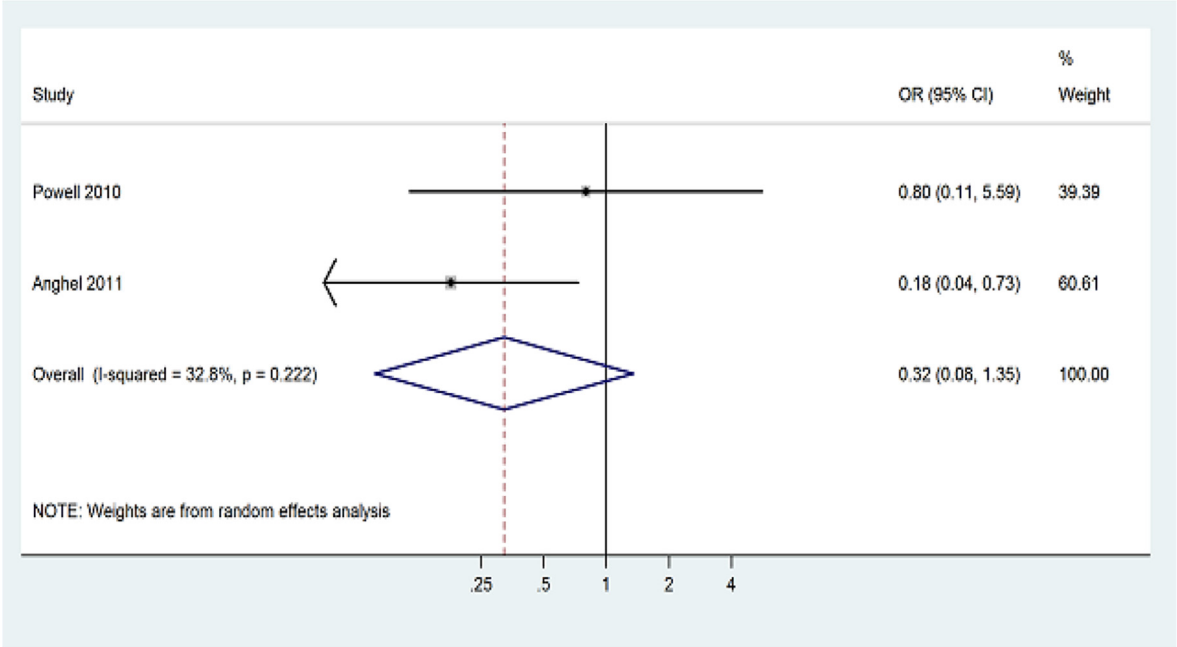
Supplementary Fig 12 (online only). Meta-analysis of patients with improved healing after receiving spinal cord stimulation (SCS) implant. *CI,* Confidence interval; *OR,* odds ratio. The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *dashed vertical line* indicates the summary measure, with the associated *diamond* indicating the weighted mean difference and the lateral tips of the diamond indicating the associated CIs. The *horizontal lines* represent the 95% CIs.



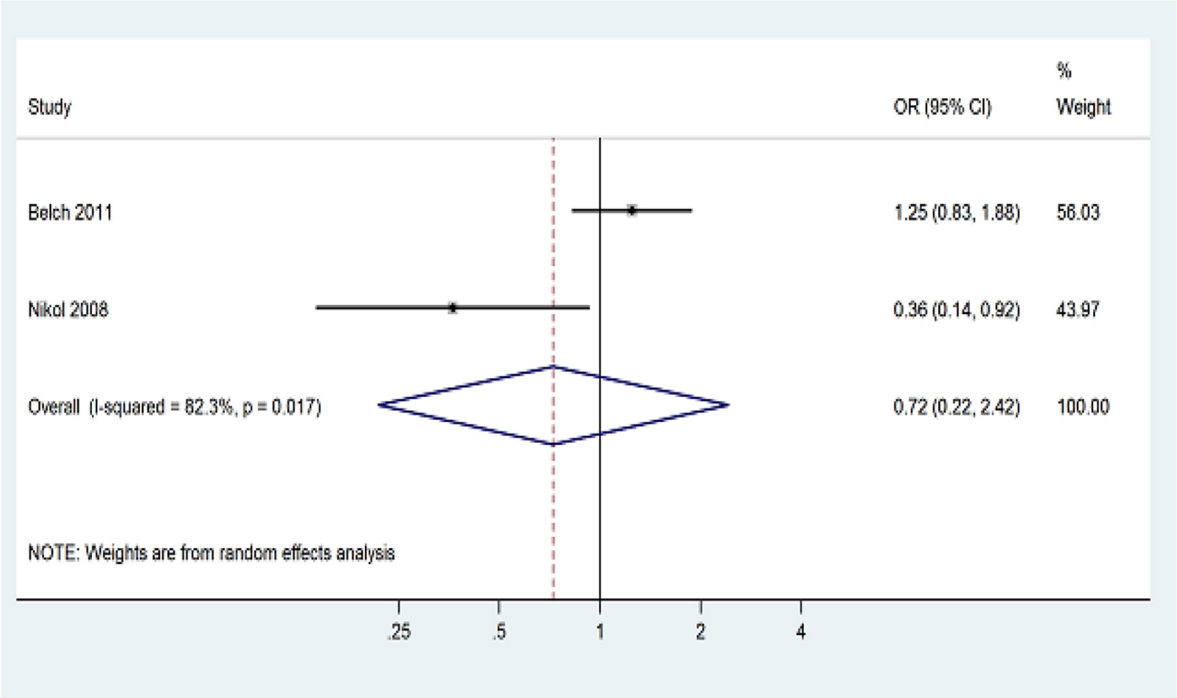
Supplementary Fig 13 (online only). Meta-analysis of patients with limb loss outcome after receiving bone marrow mononuclear cells (BM-MNC). *CI,* Confidence interval; *OR,* odds ratio. The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *dashed vertical line* indicates the summary measure, with the associated *diamond* indicating the weighted mean difference and the lateral tips of the diamond indicating the associated CIs. The *horizontal lines* represent the 95% CIs. *1* = Studies reporting patients with limb loss outcome after receiving BM-MNC.



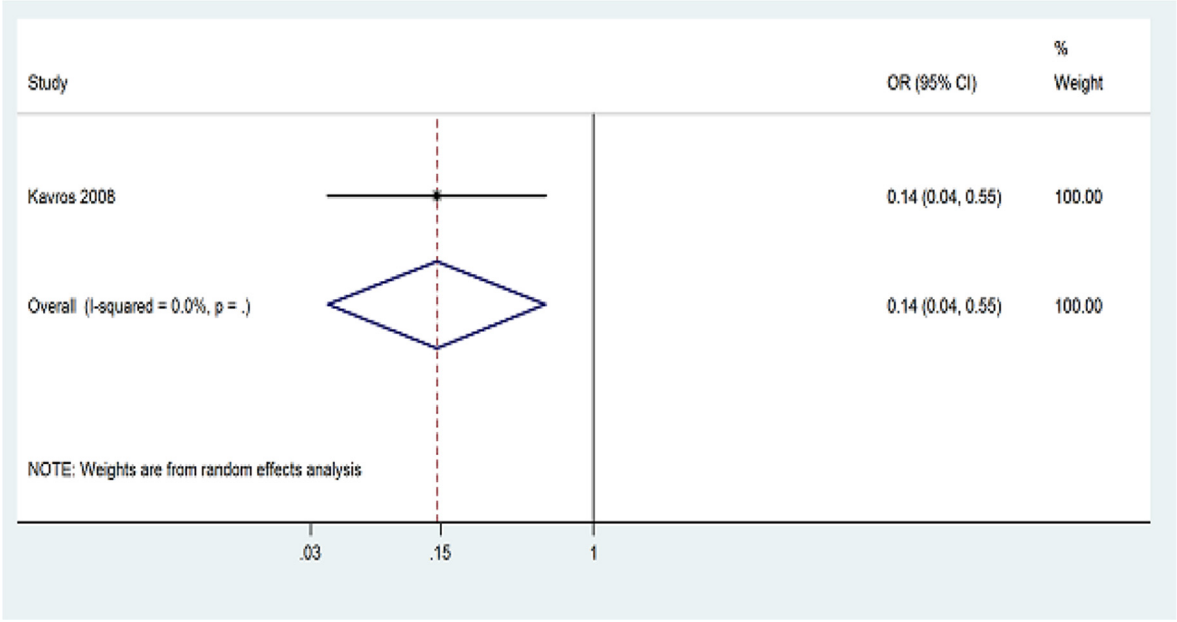
Supplementary Fig 14 (online only). Meta-analysis of patients with limb loss in patients after receiving bone marrow mononuclear cells (BM-MNC), hepatocyte growth factor (HGF), and nonviral 1 fibroblast growth factor (NV1FGF) (combined effect of biological treatments). *CI,* Confidence interval; *OR,* odds ratio. The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *dashed vertical line* indicates the summary measure, with the associated *diamond* indicating the weighted mean difference and the lateral tips of the diamond indicating the associated CIs. The *horizontal lines* represent the 95% CIs.



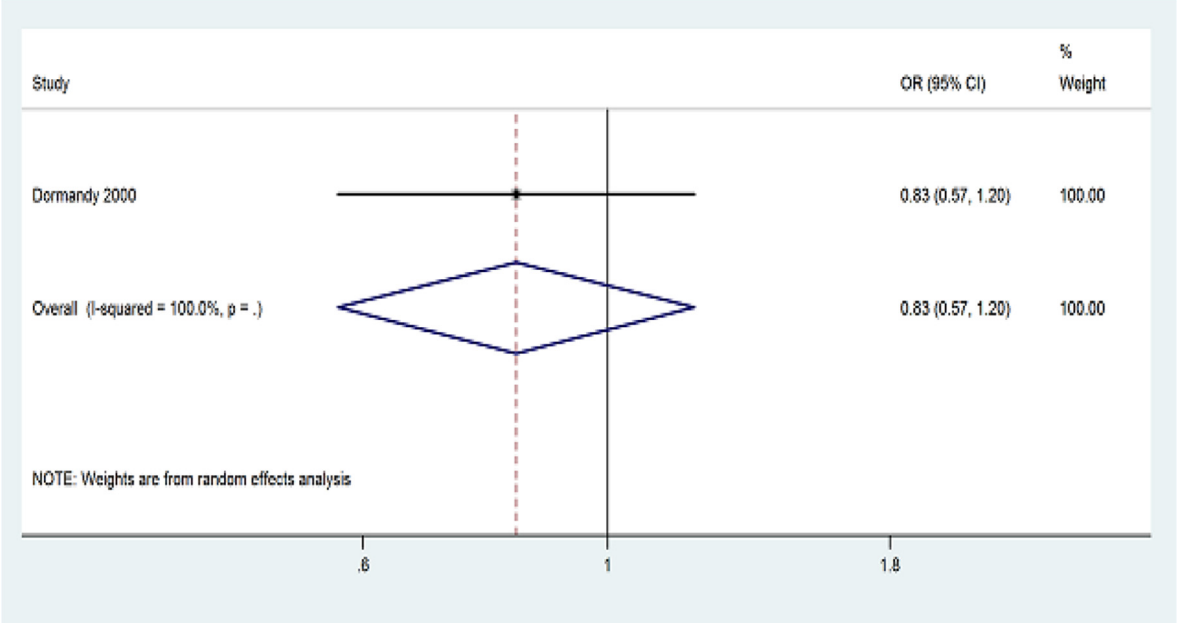
Supplementary Fig 15 (online only). Meta-analysis of patients with limb loss outcome after receiving hepatocyte growth factor (HGF). *CI,* Confidence interval; *OR,* odds ratio. The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *dashed vertical line* indicates the summary measure, with the associated *diamond* indicating the weighted mean difference and the lateral tips of the diamond indicating the associated CIs. The *horizontal lines* represent the 95% CIs.



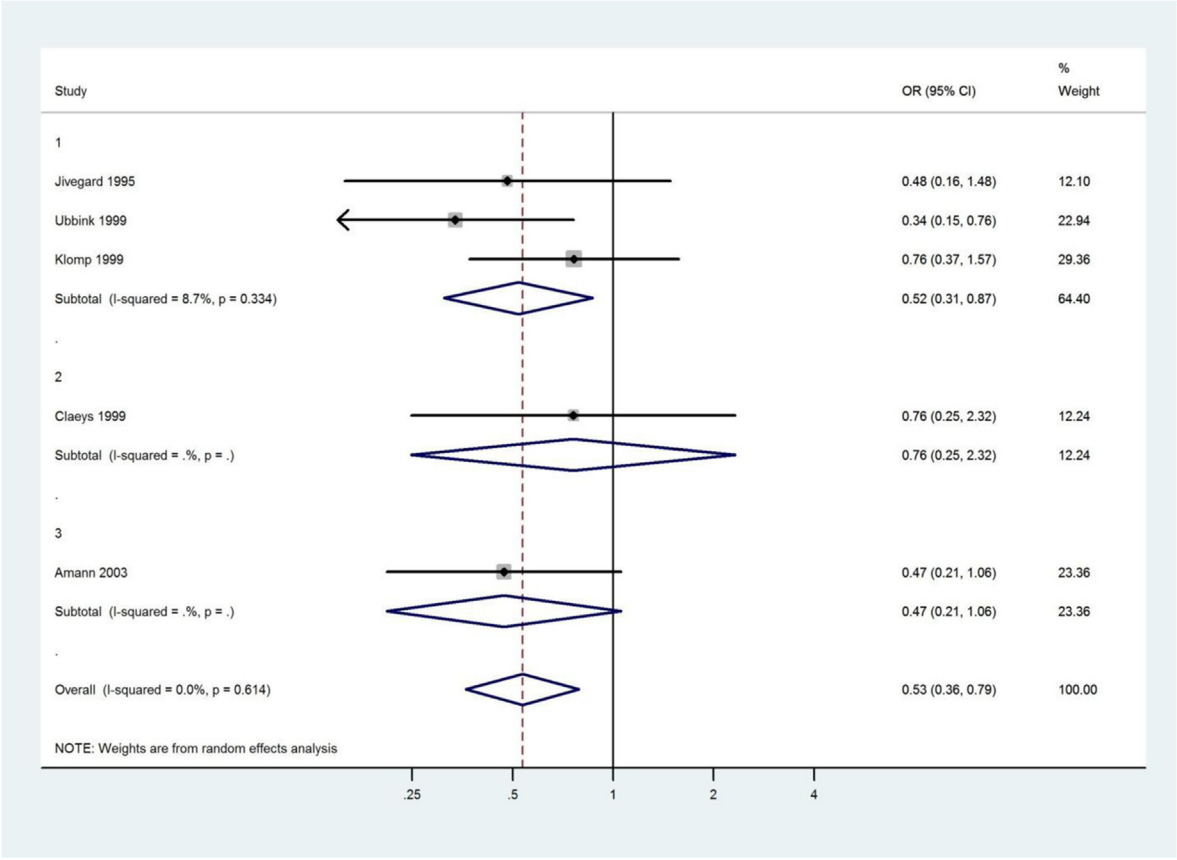
Supplementary Fig 16 (online only). Meta-analysis of patients with limb loss outcome after receiving nonviral 1 fibroblast growth factor (NV1FGF). *CI,* Confidence interval; *OR,* odds ratio. The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *dashed vertical line* indicates the summary measure, with the associated *diamond* indicating the weighted mean difference and the lateral tips of the diamond indicating the associated CIs. The *horizontal lines* represent the 95% CIs.



Supplementary Fig 17 (online only). Meta-analysis of patients with limb loss outcome after receiving intermittent pneumatic compression (IPC). *CI*, Confidence interval; *OR*, odds ratio. The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *dashed vertical line* indicates the summary measure, with the associated *diamond* indicating the weighted mean difference and the lateral tips of the diamond indicating the associated CIs. The *horizontal lines* represent the 95% CIs.



Supplementary Fig 18 (online only). Meta-analysis of patients with limb loss outcome after receiving prostacyclin. *CI*, Confidence interval; *OR*, odds ratio. The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *dashed vertical line* indicates the summary measure, with the associated *diamond* indicating the weighted mean difference and the lateral tips of the diamond indicating the associated CIs. The *horizontal lines* represent the 95% CIs.



Supplementary Fig 19 (online only). Meta-analysis of patients with limb loss outcome after receiving spinal cord stimulator (SCS) implant (*1* = without prostaglandin E_1 [PGE1], *2* = with PGE1, *3* = with adjunctive medical treatment). *CI*, Confidence interval; *OR*, odds ratio. The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *dashed vertical line* indicates the summary measure, with the associated *diamond* indicating the weighted mean difference and the lateral tips of the diamond indicating the associated CIs. The *horizontal lines* represent the 95% CIs.