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REVIEW

Bone Marrow derived Cell Therapy in Critical Limb Ischemia: A Meta-analysis of Randomized Placebo Controlled Trials

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WHAT THIS PAPER ADDS

A meta-analysis was performed of randomized placebo controlled trials on bone marrow -derived cell therapy in critical limb ischemia. This is an update of the meta-analysis by Teraa et al., published in *Annals of Surgery* in 2013. Since the publication of that article, the results of five additional placebo controlled trials involving 276 patients have been published. The 2013 meta-analysis found an advantage of cell therapy, with a divergent effect between the placebo controlled and non-placebo controlled trials. In the current analysis of only placebo controlled trials, no improvement with cell therapy was observed in amputation rates, survival, or amputation free survival.

Objective/Background: Critical limb ischemia (CLI) is the most advanced stage of peripheral artery disease (PAD), and many patients with CLI are not eligible for conventional revascularization. In the last decade, cell based therapies have been explored as an alternative treatment option for CLI. A meta-analysis was conducted of randomized placebo controlled trials investigating bone marrow (BM) derived cell therapy in patients with CLI.
Methods: The MEDLINE, Embase, and the Cochrane Controlled Trials Register databases were systematically searched, and all included studies were critically appraised by two independent reviewers. The meta-analysis was performed using a random effects model.
Results: Ten studies, totaling 499 patients, were included in this meta-analysis. No significant differences were observed in major amputation rates (relative risk [RR] 0.91; 95% confidence interval [CI] 0.65–1.27), survival (RR 1.00; 95% CI 0.95–1.06), and amputation free survival (RR 1.03; 95% CI 0.86–1.23) between the cell treated and placebo treated patients. The ankle brachial index (mean difference 0.11; 95% CI 0.07–0.16), transcutaneous oxygen measurements (mean difference 11.88; 95% CI 2.73–21.02), and pain score (mean difference −0.72; 95% CI −1.37 to −0.07) were significantly better in the treatment group than in the placebo group.
Conclusions: This meta-analysis of placebo controlled trials showed no advantage of stem cell therapy on the primary outcome measures of amputation, survival, and amputation free survival in patients with CLI. The potential benefit of more sophisticated cell based strategies should be explored in future randomized placebo controlled trials.

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INTRODUCTION

Critical limb ischemia (CLI) is at the end of the peripheral artery disease (PAD) spectrum and associated with high amputation and mortality rates and poor quality of life.[1–3] In the last decade, cell based therapies have been explored as a treatment option for patients with CLI with no option for surgical or endovascular revascularization. Since 2002, several studies have suggested beneficial effects of cell based therapies.[4–6] However, the initial pioneering studies

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were heterogeneous, small, non-controlled, and non-blinded, which made them susceptible to bias and thus prevented definite conclusions about treatment effects.

Results of larger and placebo controlled trials have become available during the last few years. The results of the studies published until 2012 were previously reviewed and summarized,[7] with the conclusion that bone marrow (BM)-derived cell therapy was a promising strategy in CLI. Importantly, the effects of the placebo controlled and non-placebo controlled trials showed divergence, with no benefit on amputation rates if only placebo controlled trials were analyzed. However, because only five placebo controlled trials were available at that time, this result could have been caused by the lack of statistical power.

Since 2012, the results of five additional randomized placebo controlled trials have been published.[8–12] These additional studies provide a significant increase in the number of patients treated with cell based therapy in well-designed placebo controlled trials and may provide stronger evidence and new guidance on the clinical applicability and effect of BM derived cell therapy in CLI. Therefore, a meta-analysis was performed that included only the randomized placebo controlled trials on BM derived cell therapy in CLI.

METHODS

Search strategy

On 15 April 2015, MEDLINE, Embase, and the Cochrane Controlled Trial Register were searched using identical search criteria to those used by Teraa et al. (see Appendix 1)[7] to identify new trials published since the initial search on 24 February 2012. Inclusion and exclusion criteria were defined before the literature search, as listed in Table 1. Studies using the contralateral limb as an internal control were included if treatment was randomized. The articles were independently screened for eligibility by two reviewers (S.P.W. and M.T.). Disagreements were resolved by consensus.

Table 1. Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Study type: RCT	Animals
Therapy: BM derived cell therapy	Children or neonates
Comparator: placebo	Review or case report ($n < 10$)
Outcome: major amputation, survival, ABI, Tco_2, pain score	No CLI or diabetic foot
	Language not English, Dutch, or German
	Gene or growth factor therapy
	Diagnostic, prognostic, or etiologic studies

Note. RCT = randomized controlled trial; BM = bone marrow; ABI = ankle brachial index; Tco_2, transcutaneous oxygen; CLI = critical limb ischemia.

Critical appraisal, data extraction, and management

Quality assessment and critical appraisal of the newly included trials was also performed by the two reviewers independently, according to a modified version of the Consolidated Standards of Reporting Trials (CONSORT) guidelines.[13] Also performed were an additional risk of bias analysis and analysis by the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system, which rates quality of evidence and grading strength of recommendations in systematic reviews.[14]

Primary outcomes were major amputation, survival, and amputation free survival (AFS). Secondary outcomes were ankle brachial index (ABI), transcutaneous oxygen (Tco_2) measurements, pain score, and ulcer healing. Data from the last follow up available were used for the analyses. Pain scores were converted to a scale ranging from 0 (no pain) to 4 (severe pain), and changes in pain score (Δ pain score) were analyzed. If raw data were unavailable, but only graphs or figures, GetData Graph Digitizer 2.25 software (S. Fedorov)[15] was used to extract the data. If SEMs were reported instead of SDs, SDs were calculated assuming that the data were distributed parametrically. If medians and interquartile ranges were reported, means and SDs were estimated using methods described previously.[16]

Statistical analysis

All statistical analyses in this meta-analysis were performed using Review Manager 5.1 software (The Cochrane Collaboration, Copenhagen, Denmark). A random effects model was applied to calculate treatment effects because statistical and methodological heterogeneity was assumed. The weighted mean difference or relative risk (RR) and the respective 95% confidence intervals (CI) were calculated to express the treatment effects. Heterogeneity between the studies included in the analyses was determined using the chi-square test. Inconsistency was quantified with the I^2 statistic, where I^2 values $< 25\%$ represent mild inconsistency, values between 25% and 50% represent moderate inconsistency, and values $> 50\%$ suggest severe heterogeneity between the studies. Statistical significance was assumed at $p < .05$.

Sensitivity analyses were performed by repeating the main computations using a fixed effects model and by repeating the main computations without the studies that used the contralateral leg as a control, because it was not ruled out that stem cells, owing to a systemic effect, could influence the results in the control leg. Sensitivity analyses were also performed for studies including > 50 patients and studies investigating intramuscular versus intra-arterial administration. Funnel plots were visually inspected for small study effects or publication bias.

RESULTS

Search results and study characteristics

The same search strategy used since the initial search in 2012 found new articles that met the search criteria after

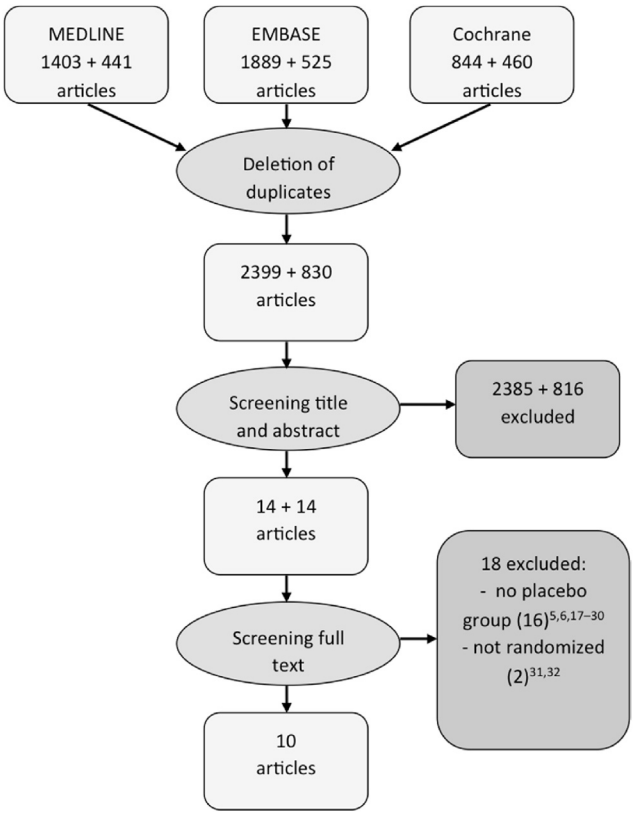

Figure 1. Flow chart of study selection (first search in 2012 plus second search in 2015).

duplications were deleted. After the titles and abstracts were screened based on the inclusion and exclusion criteria, 14 full text articles were assessed, and, ultimately, data from five articles, including 276 patients, were eligible for further analysis. These articles were added to the placebo controlled studies from the previous meta-analysis,[7] which resulted in 10 articles eligible for inclusion (Fig. 1). The reasons for the exclusion of 18 articles are summarized in Fig. 1.[5,6,17–32]

The articles were assessed for quality and risk of bias, and these results are summarized in Table 2 and Supplementary Fig. 1. There was no disagreement between the reviewers on any of the topics. Characteristics of the studies are summarized in Table 3. In addition, a GRADE evidence profile was reconstructed and is summarized in Supplementary Table 1.

The results represent data from 499 patients who were enrolled in the 10 included randomized controlled trials (RCTs). The median number of patients per study was 40.5 (range 10.0—160.0). Of the included patients, 240 were treated with cell therapy, 196 patients were treated with placebo, and 63 were treated with cell therapy in one leg and placebo in the other. The mean age and the percentage of patients with Fontaine grade IV and diabetes were similar between the treatment and control groups. The mean follow up of the studies was 7.5 months, with one study reporting 3 months or less of follow up and only three studies with a follow up of more than 6 months (Table 3).

The cell type injected and the administration route varied between the studies. Two studies used intra-arterial administration,[11,33] and the remaining studies applied the cells intramuscularly. Five of 10 studies used BM derived mononuclear cells (BMMNC),[3,4,8,11,33] one used BM derived mesenchymal stem cells (BMMSC),[9] one RCT used Ixmyelocel-T, a commercial pre-expanded cell product obtained from BM,[34] one study used $CD34^+$ cells,[12] and one used $CD133^+$ cells.[10] One study had a three-armed trial design with a BMMNC, a BMMSC, and a placebo group.[35] Patients with bilateral CLI were randomized to BMMNC or BMMSC in one leg and placebo in the other; hence, the contralateral leg served as an internal control. The original publication used the placebo treated limbs of both groups as a merged control group, without separated data for the BMMNC group and the BMMSC group. Therefore, the study groups were stratified in a BMMSC group, a BMMNC group, and the control group containing the contralateral limbs of both groups. This resulted in double -counting of the placebo treated patients, as described previously by Teraa et al.[7]

Major amputation, survival, and AFS

From nine studies reporting amputation rates, amputation occurred in 56 of 277 limbs treated with cell therapy and in 58 of 270 limbs in the control groups. Cell therapy did not significantly reduce major amputation rates overall compared with placebo (RR 0.91; 95% CI 0.65—1.27; Fig. 2A). If studies using the contralateral leg as a control

Table 2. Critical appraisal.

Study	Randomization[a]	Allocation concealment[a]	Blinding[b]	Loss to follow up[c]	Treated in assigned group[d]
Benoit et al.[3]	+	+	+	+	+
Li et al.[8]	−	−	+/−	+	+
Lu et al.[35]	+	−	+	−	+
Gupta et al.[9]	+	+	+	+	+
Losordo et al.[12]	+	−	+	+/−	+
Powell et al.[34]	+	+	+	+	+
Raval et al.[10]	−	+	+	+	−
Tateishi-Yuyama et al.[4]	+	+	+	−	+
Teraa et al.[11]	+	+	+	+	+
Walter et al.[33]	−	−	+	+	+

Note. [a] (+) = clearly defined; (−) = inadequate/not reported. [b] (+) = double blind; (+/−) = single blind; (−) = not blinded. [c] (+) < 5%; (+/−) = 5—10%; (−) > 10%. [d] (+) = All; (−) = crossover.

Table 3. Characteristics of included articles.

Study	n[a]	Therapy	Control	Administration route	Follow up	Mean age (y)[a]	Fontaine IV (%)[a]	Diabetes (%)
Benoit et al.[3]	34/14	BMMNC	Placebo	IM	6 mo	72.5/65.7	68/50	53/43
Li et al.[8]	29/29	BMMNC	Placebo	IM	6 mo	63.0/61.0	59/64	41/45
Lu et al.[35]	21/41 limbs	BMMNC	Placebo	IM	24 wk	63.0 ± 8.0	100/100	100/100
Lu et al.[35]	20/41 limbs	BMMSC	Placebo	IM	24 wk	65.0 ± 10.0	100/100	100/100
Gupta et al.[9]	10/10	BMMSC	Placebo	IM	6 mo	46.7/43.0	70/80	NA
Losordo et al.[12]	16/12	CD34+	Placebo	IM	12 mo	67.1/66.2	58/44	63/42
Powell et al.[34]	48/24	Ixmyelocel-T	Placebo	IM	12 mo	69.2/67.3	60/67	44/63
Raval et al.[10]	3/7	$CD133^+$	Placebo	IM	12 mo	65.0/85.0	29/33	43/33
Tateishi-Yuyama et al.[4]	22/22 limbs	BMMNC	Placebo	IM	24 wk	69.0 ± 11.0	70	65
Teraa et al.[11]	81/79	BMMNC	Placebo	IA	6 mo	69.0/65.0	63/63	36/39
Walter et al.[33]	19/21	BMMNC	Placebo	IA	3 mo	64.4/64.5	79/71	53/48

Note. NA = not available; IM = intramuscular; IA = intra-arterial; SC = subcutaneous; BMMNC = bone marrow derived mononuclear cells; BMMSC = bone marrow derived mesenchymal stem cells; ACP = angiogenic cell precursors; M-PBMC = mobilized peripheral blood mononuclear cells.

[a] Cell treated group/placebo group.

were excluded, the RR was 0.96 (95% CI 0.68—1.34). Overall survival was similar between the treatment and placebo groups (RR 1.00; 95% CI 0.95—1.06). AFS was reported or could be retrieved in eight studies and did not significantly differ between the treatment and control group, with a RR of 1.03 (95% CI 0.86—1.23; Fig. 2B).

Ulcer healing

Six studies evaluated complete ulcer healing in 253 limbs. No significant benefit of BM derived cell therapy was observed compared with control (RR 1.40; 95% CI 0.99—1.97; Fig. 2C), especially when studies using the contralateral limb as control were excluded (RR 1.09; 95% CI 0.68—1.76).

ABI and Tco_2

The ABI at the end of follow up in six studies was compared and was significantly higher in the therapeutic group than in the control group (mean difference 0.11; 95% CI 0.07—0.16 [$p < .01$]; Fig. 2D). When studies without a separate control group were excluded, the mean difference in ABI was slightly smaller (0.10; 95% CI 0.04—0.16). Four studies reported that Tco_2 was also significantly higher in the group treated with BM derived cell therapy compared with the control group, with a mean difference of 11.88 (95% CI 2.73—21.02; $p = .01$), as shown in Fig. 2E. When studies with the contralateral leg as the control were excluded, the mean difference was less pronounced (3.69; 95% CI 2.62—4.76). Importantly, the chi-square test showed considerable heterogeneity between the studies for ABI and Tco_2.

Pain score

Decreases in pain scores were significantly greater in the cell treated group than in the control group. The mean decrease in pain score was 1.3 in the treatment group and 0.6 in the placebo group. The mean difference in the decrease between the treatment and placebo groups was −0.72 (95% CI −1.37 to −0.07; $p = .03$), as shown in Fig. 2F. Analysis of only studies with a separate control population showed a mean difference of −0.44 (95% CI −1.35 to 0.46).

Safety issues

Results from the included articles showed that BM derived cell therapy appeared to be relatively safe. Observed adverse effects were mostly mild and transient. The most frequently reported adverse effects were pain and tenderness at the BM aspiration site. Other reported treatment related adverse events and effects were a transient and well tolerated hematocrit decrease in the treatment group compared with the control group,[3] two patients each with groin hematoma,[11,33] or malignancies,[3,33] and one patient each with stent thrombosis,[33] pseudoaneurysm,[33] moderate hypotension,[12] or worsening of CLI after the injection.[12]

Sensitivity analyses

The sensitivity analyses based on fixed effects models did not substantially change the observed effects, and only the difference in ulcer healing became statistically significant (random effects model: RR 1.40; 95% CI 0.99—1.97; fixed effects model: RR 1.49; 95% CI 1.15—1.93). Sensitivity analysis based on studies including > 50 patients did not substantially change the observed effects, except for ulcer healing (all studies included: RR 1.40; 95% CI 0.99—1.79; studies including > 50 patients: RR 1.71; 95% CI 1.32—2.21). When trials using intramuscular or intra-arterial administration were compared, a smaller effect size for intra-arterial administration was observed; however, the differences were not significant. This may have been owing to the small number of trials (two studies) investigating the intra-arterial route.[11,33] The funnel plots were not indicative of small study effects or publication bias, based on visual inspections.

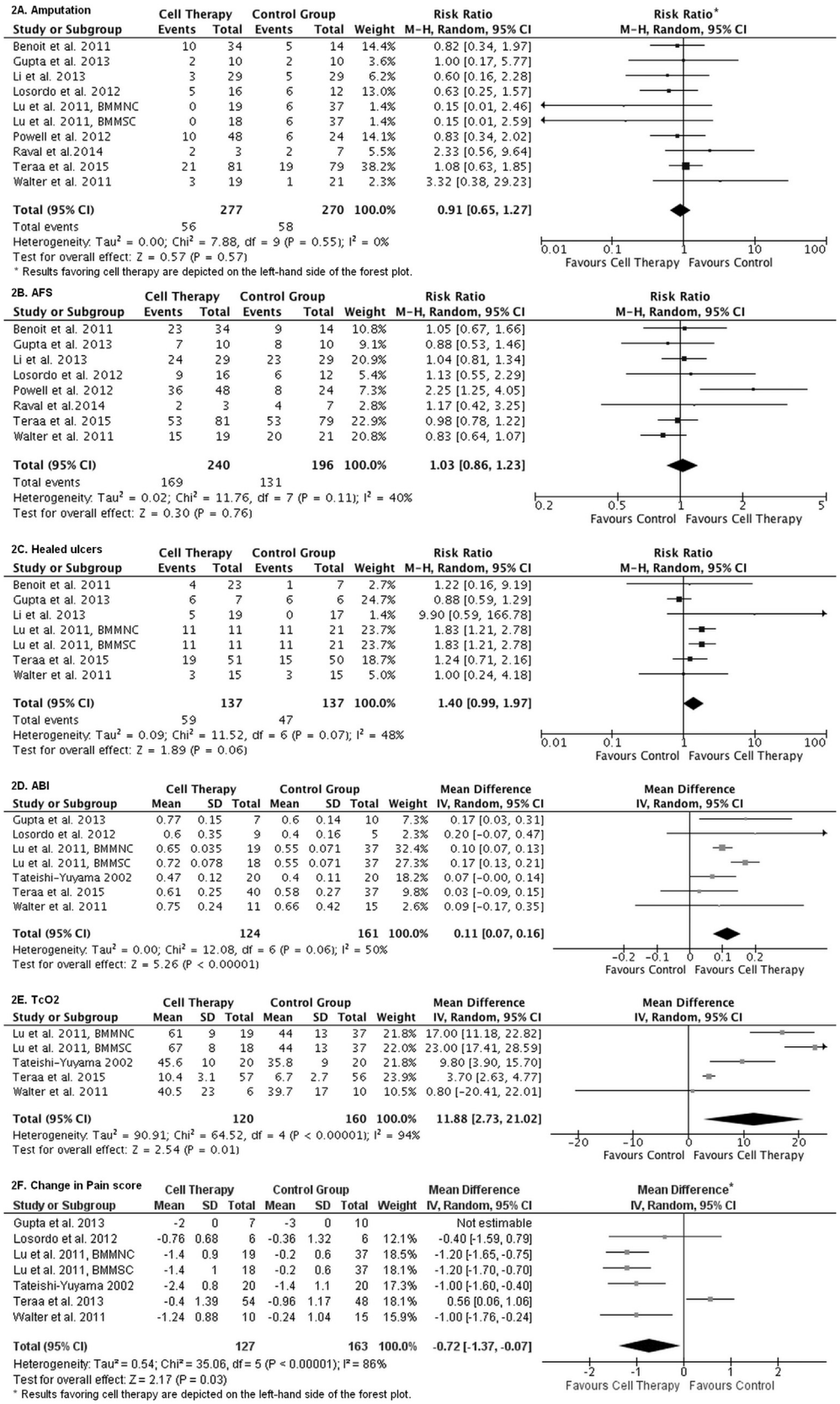

Figure 2. Meta-analysis of endpoints in placebo controlled bone marrow (BM) derived cell therapy trials. *Note*. CI = confidence interval; BMMNC = BM derived mononuclear cells; BMMSC = BM derived mesenchymal stem cells; AFS = amputation free survival; ABI = ankle brachial index; TcO_2 = transcutaneous oxygen.

DISCUSSION

The present meta-analysis of 10 randomized placebo controlled trials investigating BM derived cell therapy in 499 patients with CLI showed no differences in amputation rates, survival, or AFS between cell treated and placebo treated patients. Although ABI, Tco_2, and pain scores appeared to be better in the groups treated with BM derived cells, the percentage of healed ulcers was not significantly different between the groups. Notably, BM derived cell therapy seemed to be a relatively safe treatment option because the adverse events were mostly mild and transient.

Previous meta-analyses of trials of cell therapy that were and were not placebo controlled showed promising amputation rates, survival, and AFS. In particular, the first non-placebo controlled and relatively small studies showed advantages of cell therapy, but the larger studies and placebo controlled trials showed less convincing results on hard end points such as amputation, survival, and AFS. The present analysis included five additional placebo controlled trials comprising 276 additional patients and did not show significant effects of BM derived cell therapy on the primary outcome measures. Because improvement occurred in the treatment arm and in the placebo arm in several trials,[3,9,11,12,33] an adequate double blinded design is of great importance.

ABI and Tco_2 seemed better in the cell therapy group; however, analyses showed large heterogeneity based on chi-square tests, and therefore these results are less reliable. Moreover, the value of an increased ABI or Tco_2 level is questionable if amputation rates, survival, and ulcer healing do not improve.

The current meta-analysis mainly included studies that investigated the intramuscular administration of BM derived cells,[3,4,8–10,12,34,35] and most investigators used BMMNC.[3,4,8,11,33,35] The limited numbers of trials and patients precluded decent subgroup analyses for the different administration routes or cell types.

That circulating BM derived progenitor cells are dysfunctional and that levels are lower in patients with CLI than in healthy controls because of prolonged pro-inflammatory stimuli has been suggested.[36] This might explain the absence of treatment effect seen in this meta-analysis. However, Gremmels et al. suggested that this disease mediated cell dysfunction in patients with CLI is reversed in BMMSCs,[37] making them possibly more effective in neovascularization therapy for CLI. In addition, BMMSCs might provide additional benefit when used in an allogeneic approach, for example off the shelf availability and circumvention of BM aspiration procedures in the frail patient with CLI.[38]

Although a clear difference between trials that used intra-arterial and intramuscular administration was not observed, evidence shows that BM derived cells mainly act via paracrine pathways. One advantage of intramuscular administration could be the creation of "local depots" of stem cells with increased local paracrine activity and local release of arteriogenic cytokines.[18,39] This suggests that intramuscular administration might be better and should be explored in future clinical trials.

This meta-analysis has some limitations. The number of published trials is relatively small, and the included studies had a small sample size. Most of the studies were pilot studies[3,4,8–10,12,34,35] and did not include a sample size calculation or were designed for safety analysis. Hence, most studies were not powered to prove therapeutic efficacy.

Only four of the 10 studies randomized $>$ 50 patients, and just one included $>$ 100 patients, which could lead to small study bias. The GRADE analysis of the included studies generally showed low study quality for all outcomes (see Supplementary Material).

Follow up time was generally limited, and none of the included trials had a follow up $>$ 12 months. The interval between the start of inclusion and publication of the trial result was relatively long for most trials, which can cause heterogeneity in the included patient population, for example because of changes in secondary prevention. There is no generally accepted unequivocal definition of the "no option" patient with CLI. Patient ineligibility for revascularization is often determined in multidisciplinary consensus meetings; therefore, differences among and within trials can arise in which patients are included in a trial, which could influence outcomes in different studies.

In conclusion, this meta-analysis of 10 placebo controlled trials of BM derived cell therapy in 499 patients with CLI showed no advantage of cell therapy on the primary outcome measures of amputation, survival, and AFS. This meta-analysis underlines the need for future well designed double blinded and placebo controlled randomized trials that are adequately powered, to investigate specific BM derived cell therapeutic strategies. From the available evidence it is believed that future cell therapy in CLI should focus on specific cellular therapies. It has recently been seen that the neovascularization capacity of mesenchymal stem cells is not compromised in patients with CLI, suggesting that autologous mesenchymal stem cells may be suitable for cellular therapy in patients with CLI.[16] A joint international effort would be advisable to assure that a future trial would be adequately powered and finished within an acceptable timeframe.

CONFLICT OF INTEREST

None.

FUNDING

None.

APPENDIX 1. SEARCH SYNTAXES.

Syntax "Medline"

("Peripheral arterial disease"[TIAB] OR PAD[TIAB] OR "peripheral arterial occlusive disease"[TIAB] OR PAOD[TIAB]

OR "Critical limb ischemia"[TIAB] OR "Critical limb ischaemia"[TIAB] OR CLI[TIAB] OR "Severe limb ischemia"[TIAB] OR "Severe limb ischaemia"[TIAB] OR "arteriosclerosis obliterans"[TIAB] OR "thromboangitis obliterans"[TIAB] OR Buerger[TIAB] OR "Fontaine 3"[TIAB] OR "Fontaine 4"[TIAB] OR "Fontaine III"[TIAB] OR "Fontaine IV"[TIAB] OR "Rutherford 4"[TIAB] OR "Rutherford 5"[TIAB] OR "Rutherford 6"[TIAB] OR Amputation[TIAB] OR "Ischemic ulcer*"[TIAB] OR "Ischaemic ulcer*"[TIAB] OR gangrene[TIAB] OR necrosis [TIAB] OR "diabetic foot"[TIAB] OR "diabetic ulcer"[TIAB]) AND (BM[TIAB] OR "Bone marrow" [TIAB] OR BM-MNC [TIAB] OR BMMNC[TIAB] OR BMMC[TIAB] OR "Bone marrow mononuclear cell*"[TIAB] OR "bone marrow derived mononuclear cell"[TIAB] OR PB-MNC[TIAB] OR PBMNC[TIAB] OR PB-MC[TIAB] OR PBMC[TIAB] OR "Peripheral blood mononuclear cell*"[TIAB] OR "peripheral blood derived mononuclear cell*"[TIAB] OR "Stem cell*"[TIAB] OR "Progenitor cell*"[TIAB] OR cell[TIAB] OR cellular [TIAB] OR "Cell based" [TIAB] OR "Cell based" [TIAB] OR "Neovascular*"[TIAB] OR angiogen*[TIAB] OR "Mesenchymal stem cell*"[TIAB] OR "Mesenchymal stromal cell*"[TIAB] OR MSC[TIAB]) AND (therapy[TIAB] OR therapies[TIAB] OR therapeutic*[TIAB] OR intervention[TIAB] OR infusion[TIAB] OR administration[TIAB] OR injection[TIAB] OR application[TIAB] OR treatment[TIAB] OR transplantation[TIAB]) AND (RCT[TIAB] OR randomis*[TIAB] OR randomiz*[TIAB] OR trial[TIAB] OR "placebo controlled"[TIAB] OR placebo controlled[TIAB] OR placebo [TIAB] OR "clinical trial"[TIAB] OR "prospective study"[TIAB] OR "double-blind"[TIAB] OR "double blind"[TIAB] OR blinded[TIAB])

Syntax "Embase"

("Peripheral arterial disease":ti,ab OR PAD:ti,ab OR "peripheral arterial occlusive disease":ti,ab OR PAOD:ti,ab OR "Critical limb ischemia":ti,ab OR "Critical limb ischaemia":ti,ab OR CLI:ti,ab OR "Severe limb ischemia":ti,ab OR "Severe limb ischaemia":ti,ab OR "arteriosclerosis obliterans":ti,ab OR "thromboangitis obliterans":ti,ab OR Buerger:ti,ab OR "Fontaine 3":ti,ab OR "Fontaine 4":ti,ab OR "Fontaine III":ti,ab OR "Fontaine IV":ti,ab OR "Rutherford 4":ti,ab OR "Rutherford 5":ti,ab OR "Rutherford 6":ti,ab OR Amputation:ti,ab OR "Ischemic (ulcer OR ulcers)":ti,ab OR "Ischaemic (ulcer OR ulcers)":ti,ab OR gangrene:ti,ab OR necrosis:ti,ab OR "diabetic foot":ti,ab OR "diabetic (ulcer OR ulcers)":ti,ab) AND (BM:ti,ab OR "Bone marrow":ti,ab OR BM-MNC:ti,ab OR BMMNC:ti,ab OR BMMC:ti,ab OR "Bone marrow mononuclear (cell OR cells)":ti,ab OR "bone marrow derived mononuclear cell":ti,ab OR PB-MNC:ti,ab OR PBMNC:ti,ab OR PB-MC:ti,ab OR PBMC:ti,ab OR "Peripheral blood mononuclear (cell OR cells)":ti,ab OR "peripheral blood derived mononuclear (cell OR cells)":ti,ab OR "Stem (cell OR cells)":ti,ab OR "Progenitor (cell OR cells)":ti,ab OR cell:ti,ab OR cellular:ti,ab OR "Cell based":ti,ab OR "Cell based":ti,ab OR Neovascular*:ti,ab OR angiogen*:ti,ab OR "Mesenchymal stem (cell OR cells)":ti,ab OR "Mesenchymal stromal (cell OR cells)":ti,ab OR MSC:ti,ab) AND (therapy:ti,ab OR therapies:ti,ab OR therapeutic*:ti,ab OR intervention:ti,ab OR infusion:ti,ab OR administration:ti,ab OR injection:ti,ab OR application:ti,ab OR treatment:ti,ab OR transplantation:ti,ab) AND (RCT:ti,ab OR randomis*:ti,ab OR randomiz*:ti,ab OR trial:ti,ab OR "placebo controlled":ti,ab OR placebo controlled:ti,ab OR placebo:ti,ab OR "clinical trial":ti,ab OR "prospective study":ti,ab OR "double-blind":ti,ab OR "double blind":ti,ab OR blinded:ti,ab)

Syntax "Cochrane"

("Peripheral arterial disease" OR PAD OR "peripheral arterial occlusive disease" OR PAOD OR "Critical limb ischemia" OR "Critical limb ischaemia" OR CLI OR "Severe limb ischemia" OR "Severe limb ischaemia" OR "arteriosclerosis obliterans" OR "thromboangitis obliterans" OR Buerger OR "Fontaine 3" OR "Fontaine 4" OR "Fontaine III" OR "Fontaine IV" OR "Rutherford 4" OR "Rutherford 5" OR "Rutherford 6" OR Amputation OR "Ischemic ulcer*" OR "Ischaemic ulcer*" OR gangrene OR necrosis OR "diabetic foot" OR "diabetic ulcer*") AND (BM OR "Bone marrow" OR BM-MNC OR BMMNC OR BMMC OR "Bone marrow mononuclear cell*" OR "bone marrow derived mononuclear cell" OR PB-MNC OR PBMNC OR PB-MC OR PBMC OR "Peripheral blood mononuclear cell*" OR "peripheral blood derived mononuclear cell*" OR "Stem cell*" OR "Progenitor cell*" OR cell OR cellular OR "Cell based" OR "Cell based" OR "Neovascular*" OR angiogen* OR "Mesenchymal stem cell*" OR "Mesenchymal stromal cell*" OR MSC) AND (therapy OR therapies OR therapeutic* OR intervention OR infusion OR administration OR injection OR application OR treatment OR transplantation) AND (RCT OR randomis* OR randomiz* OR trial OR "placebo controlled" OR placebo controlled OR placebo OR "clinical trial" OR "prospective study" OR "double-blind" OR "double blind" OR blinded)

APPENDIX A. SUPPLEMENTARY MATERIAL

Supplementary material related to this article can be found at http://dx.doi.org/10.1016/j.ejvs.2015.08.018

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