REVIEW ARTICLES

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Stem-cell therapies in critical limb ischemia

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Abstract

Background: Due to stimulation of muscular regeneration in ischemic extremities and increasing blood flow, stem cells are considered a promising new strategy for patients with critical limb ischemia (CLI). So, it is demonstrated that mesenchymal stem cells (MSCs), mononuclear cells derived from bone marrow, peripheral and umbilical blood, adipose tissue encourage genesis of endothelial cells (ECs) and vascular smooth muscle cells (VSMCs). By application of stem cell-based therapy, the following results are obtained: increased rate of ulcer healing, increased ankle-brachial index (ABI) and transdermal oxygen pressure ($TcPO_2$), improved revascularization, and reduced rate of amputation surgery. So, stem cell-based therapy demonstrates good clinical outcomes. However, some adverse events related to cell sampling and mobilizations are reported. In addition, because of poor cell survival in condition of ischemia their therapeutic efficacy remains low that indicates further researchers are necessary in this field.
Conclusions: Cell-based therapy is a promising approach in CLI treatment. Its promising results have been already shown in smaller studies; however, large-scale studies are entailed to ascertain their definitive role in anti-ischemic therapy.
Kew words: hypoxia, critical limb ischemia, peripheral arterial disease, transplantation, stem-cell therapy, tissue repair, angiogenesis.

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Introduction

Critical limb ischemia (CLI) represents the most severe manifestation of peripheral arterial disease (PAD). According to European Conciliation Committee, the incidence of CLI is about 500-1000 cases per 1.000.000 patients and constitutes a considerable social and economic burden. The prevalence of this disease in general population aged between 60–90 years is about 1% (0.5%–1.2%), however, there is a great difference when we compare the data from general populational studies and registers of vascular surgery. Gender differences in the prevalence of CLI varies in different studies, the Men:Women ratio is about 3:1. Even so, in older persons aged 50 or over it is equal to 1:1. It is associated with high mortality [1, 2]; about 25% of patients with this diagnostic die, and major amputations are required in 10–40% of cases when revascularization failed or in patients with "no-option" [3-5].

Due to the lack of a standard treatment guideline, CLI is still considered an orphan disease. The treatment of CLI and its symptoms includes pharmocological therapy as the first option, and invasive procedures for long-term revascularization [6]. Even the improvement of the available curative options are undoubtedly necessary, invalidity rate because of amputation in this category of patients is still high.

The main goal of the proposed treatment strategies is focused on recovery of damaged by ischemia tissues. This goal may be achieved by applications of cell-based therapies. Its beneficial effect is determined by induction vascular regeneration and angiogenesis stimulation [7]. So, cellular therapy has come into view as a new frontier in this regard.

A lot of studies have demonstrated yet that stem-cell therapy (SCT) can ensure tissue vascularization after established ischemic modification in the limbs [8-12]. Functional endothelial cells can be obtained from different cellular sources and they can be used as potential therapy in the treatment of different cardiovascular diseases. However, their therapeutical efficacy remains unclear. In this literature review different therapeutical approaces about stem cell aplication in CLI treatment are demonstrated.

In order to realize the goal of the study contemporary scientific literature containing the following key words "hypoxia", "critical limb ischemia", "peripheral arterial disease", "transplantation", "stem-cell therapy", "tissue repair", and

"angiogenesis" were selected from PubMed database. For advanced selection of literature sources, the following filters were applied: articles published in English within the time period 2002-2019. Only original research articles (preclinical, clinical and experimental studies), meta-analysis and systematized literature reviews were selected.

After the primary examination of articles' titles and exclusion the papers which did not correspond to overall goal and were not accessible for the full text review, the reference lists of relevant publications were also examined in order to find additional useful bibliography. The information was systematized and presented in the form of systematic review.

Results

According to the search strategy 872 articles on SCT and their role in CLI treatment were found. After processing 125 articles were selected as relevant. The final bibliography contains 29 articles in the area. The data about stem cells efficacy in the treatment of CLI were collected.

Study 1: Eriko Tateishi-Yuyama et al. 2002 [13]. **Study characteristics:** Prospective clinic randomized study (n=47). **Results:** The efficacy and safety of autologous implantation of bone marrow-mononuclear cells (BM-MNCs) in patients with ischaemic limbs because of peripheral arterial disease were evaluated. All the patients enrolled in this study were assigned in two groups: group A (n=25) and group B (n=22). Patients with unilateral limb ischemia from the first group were treated by injection of BM-MNCs into the gastrocnemius of the ischemic limb and saline solution into the opposite limb. Patients from group B presented bilateral leg ischemia. They also were treated by transplantation of (BM-MNCs) in one leg. However, injection of peripheral blood-mononuclear cells was used as control. The safety and feasibility of the treatment were evaluated according to the following criteria: ABI and rest pain. Follow-up period consisted of 24 weeks with an intermediate evaluation at 4 weeks. Significant improvement of ABI, transcutaneous oxygen pressure ($TcPO_2$), rest pain, and pain-free walking time were recorded in legs injected with BM-MNCs. So, the beneficial effect of BM-MNCs transplantation in achievement of therapeutic angiogenesis was clinically demonstrated. It is determined by the cells ability to supply endothelial progenitor cells and to secrete various angiogenic factors or cytokines.

Study 2: Takashi Iwase et al. 2005 [14]. **Study characteristics:** Animal study. **Results:** In this study efficacy of stem cells transplantation of mesenchymal stem cells (MSCs) and BM-MNCs in CLI was evaluated; also, their therapeutic potential was compared. For this purpose, a rat model of hindlimb ischemia was developed. Analysis of 3 weeks follow-up period was done and the following criteria were studied: laser Doppler perfusion index, blood perfusion, and capillary density. The data analysis demonstrated a greater improvement of ischemia in MSC-group.

Study 3: Ivana Rosova et al. 2007 [12]. **Study characteristics:** Animal study. **Results:** The MSCs properties and their impact in injured tissue regeneration were studied. It was demonstrated that intra-arterial administration of BM-MSCs in 24 hours may induce revascularization enhancement in ischemic limb. Also, the study data analysis clearly suggested that preculturing MSC under hypoxic condition improves their regenerative potential.

Study 4: Nihan Ranjan Dash et al. 2009 [10]. **Study characteristics:** Randomized experimental study (n=24). **Results:** The efficacy and feasibility of autologous BM-MSCs in combination with standard wound dressing regimen in the treatment of lower extremities chronic non-healing ulcers were evaluated. Researches demonstrated quicker regeneration of trophic ulcers with improvement of pain-free walking distance in the experimental group with no significant alteration in the biochemical parameters.

Study 5: Gabriel P Lasala et al. 2010 [15]. **Study characteristics:** Phase I clinical trial. **Results:** The researchers demonstrated the efficacy of autologous BM-MNSc and MSCs infusion in ischemia treatment. After a ten months follow-up the improvements of life quality, walking time and ABI were achieved. In addition, increased perfusion in ischemic limbs was confirmed by angiographic and 99mTc-TF perfusion scintigraphy scores.

Study 6: J Hoffmann et al. 2010 [11]. **Study characteristics:** Animal study. **Results:** The effectiveness of BM-MSCs in the treatment of occlusive arterial diseases was studied. In addition, the cells properties in hypoxic conditions and normoxia were examined. It was demonstrated that due to better survival in hypoxic conditions and improved production of vascular endothelial growth factor (VEGF) BM-MSCs transplantation leads to an increase in vessel density when comparing to other groups.

Study 7: R Kolvenbach et al. 2010 [16]. **Study characteristics:** Prospective, clinic randomized study (n=8). **Results:** The study included 8 patients with CLI, in all cases surgical treatment was indicated. Adjunctive intraoperative SCT with BM-derived stem cells was performed in combination with intervention. For cells' processing a point-of-care system was used. A discreet increasing of ABI was determined in five patients, and high recovery rates were obtained. However, two major amputations and one minor amputation were needed postoperatively.

Study 8: Debin Lu et al. 2011 [17]. **Study characteristics:** Randomized, double-blind, controlled study (n=20). **Results:** The therapeutic effects of intramuscular administration of BM-MSCs and BM-MNCs in patients with diabetic CLI and foot ulcers (Fontaine class IV) were studied. The follow-up period was 24 months. The researchers demonstrated better clinical results in MSCs-treated group (complete ulcer healing, improved limb perfusion, pain-free walking time, ABI, $TcPO_2$, and magnetic resonance angiography). Of note, neither cell type resulted in any adverse effects.

Study 9: Dirk H Walter et al. 2011 [18]. **Study characteristics:** Multicenter, phase II, double-blind, randomized study (n=40). **Results:** Feasibility and benefits of intraarterial injection of BM-MNC in patients with CLI were evaluated. The increase of ABI was not observed in the experimental group; however, cell therapy was associated with

accelerated ulcer healing and reduced rest pain.

Study 10: Naomi Idei et al. 2011 [19]. **Study characteristics:** Prospective, clinic randomized study (n=51). **Results:** The long-term results of BM-MSCs administration in patient with CLI were evaluated (the study included 51 patients: 25 with PAD and 26 with Buerger disease). In both groups, ABI and $TcPO_2$ significantly increased after 1 month. In addition, the COX model revealed that BM-MNC implantation correlated with prevention of major amputation. However, in patients with PAD, ABI and TcPO2 gradually decreased during 3-year follow-up and returned to baseline levels.

Study 11: Aaron Liew et al. 2012 [9]. **Study characteristics:** Literature review. **Results:** The authors provided an overview of the potential role of MSC-based therapies for CLI, put into discussion the proposed mechanism of stem cells' actions in the improvement of ischemic tissue regeneration (such as paracrine, immunomodulatory, and myogenic/endothelial differentiation effects) and analyzed certain factors: cell dose, timing, and appropriate route of administration – critical to the success. Also, data obtained from preclinical studies and current early-phase human trial were discussed. Animal researches demonstrated that by administration of MSCs and modified MSCs derived from various sources (bone marrow, umbilical cord blood, fetal membrane, and adipose tissue) significant improvement in mouse/rat models of ischemia can be obtained. In almost all cases intramuscular route was chosen. The effect is determined by enhancement of blood perfusion and capillary density. Clinical human studies also confirmed the angiogenic effect of MSC therapy. Finally, the authors highlighted the main directions in the field development.

Study 12: Gabriel P Lasala et al. 2012 [20]. **Study characteristics:** Phase II clinical trial (n=26). **Results:** The efficacy of a combined cell product (mesenchymal stem cells in conjunction with endothelial progenitor cells) given intramuscularly (gastrocnemius infusion) was evaluated and compared with a placebo product. Improvements in walking time, ABI, and life quality without any adverse effects were established only in the cell-treated limb with no modifications in the contralateral leg, where the placebo was introduced. Also, scintigraphic examinations (technetium-99m-tetrofosmin scintigraphy) were realized. It demonstrated increased perfusion exclusively in the cell-treated limbs.

Study 13: Han Cheol Lee et al. 2012 [21]. **Study characteristics:** Prospective, clinic randomized study (n=15). **Results:** The stem-cell therapy effectiveness in ischemia treatment was examined. It was demonstrated that intramuscular injections of autologous MSCs derived from adipose tissue in patients with Buerger's disease and diabetic foot are feasible and safe, clinical improvement being recorded in 66.7% of cases. During the folow-up period minor amputations were required only in five cases. Walking time, collateral blood vessels formation, ulcers recovery and clinical symptoms recovery were improved; but, ABI was unchanged.

Study 14: Pawan K Gupta et al. 2013 [22]. **Study characteristics:** Prospective, double blind randomized placebo controlled multi-center study (n=12). **Results:** In the study the patients with CLI (PAD) as per Rutherford classification in category II-4, III-5, or III-6 with infra-inguinal arterial occlusive disease were included, in all of them revascularization treatment being impossible or failed. Intramuscular route of administration (gastrocnemius of the ischemic limb) was used for cells delivery. The efficacy and safety of intramuscular administration of allogeneic BM-MSCs in patients with CLI were determined (improvement in the rest pain score, ABI, ankle pressure). The regenerative effects were determined by stimulation of angiogenesis and inducing immunomodulatory environment *in situ*. In addition, adverse effect incidence in experimental group was lower than in placebo one.

Study 15: Hendrik Gremmels et al. 2013 [23]. **Study characteristics:** Study review, namely Gupta and colleagues report [14]. **Results:** The authors mentioned the importance of Gupta et al. study, as a welcome addition in the field of investigating STC in PAD. It was practically demonstrated that SCT is a promising avenue in the treatment of patients with very few other options. However, the mechanism of MSC-mediated improvements is still unclear; it indicates that additional investigations are necessary.

Study 16: Jae Choon Ryu et al. 2013 [24]. **Study characteristics:** Animal study. **Results:** The main study goal was to test hypothesis that treatment of limb ischemia with multipotential adult progenitor cells (MAPCs) promotes recovery of blood flow. The limb ischemia in mice was induced by ligation of iliac artery. MAPCs were injected intramuscularly on day 1. Optical imaging demonstrated cells' survival for 1 week. Contrast-enhanced ultrasound showed a more complete blood flow recovery in the experimental group. Fluorescent microangiography demonstrated more complete distribution of flow to microvascular units in the MAPC-treated mice. So, MAPCs efficacy in promoting flow recovery in ischemic tissue was demonstrated.

Study 17: Wing-Hon Lai et al. 2013 [8]. **Study characteristics:** Prospective, preclinical randomized, experimental study (n=15). **Results:** The researchers studied the therapeutic efficacy of endothelial-like cells (EC) in the treatment of cardiovascular diseases. Functional EC were derived from BM-MNCs (BM-EC), human embryonic stem cells (hESC-EC), and human induced pluripotent stem cells (hiPSC-E). *In vitro* (tube formation, migration and cytokine expression profiles) and *in vivo* testings (attenuation of hind-limb ischemia in mice) were performed. It was demonstrated that hESC-EC and hiPSC-EC are useful in the treatment of tissue ischemia.

Study 17: Juan Jose Parcero et al. 2014 [25]. **Study characteristics**: Case report. **Results:** The angiogenic properties of autologous adipose-derived stromal cell (ASC) were studied, as well as their safety and feasibility of clinical application. The patient, a 80-year-old female from the USA enrolled in the study, received radiation therapy due to the presence of a squamous cell carcinoma and as a result developed a non-healing lesion. Because the traditional treatment was non-effective, the patient received cellular therapy. Approximately $35.1*10^6$ autologous cells were ad-

ministered intravenously, and another 81.2*10^6 cells were implanted directly at the edges of lesions and throughout the ulcers. Complete healing, closure, and disappearance of ulcers with symptoms reduction were achieved. So, it was demonstrated that ASC can help improve or eliminate non-healing lesions and seems to be a treatment alternative in advanced ischemic disorders.

Study 18: Rita Compagna et al. 2015 [6]. **Study characteristics:** Literature review. **Results:** The aim of the study was to perform a systematic analysis of the most recent scientific literature on the application of STC in the treatment CLI of different etiologies. In the study 1031 eligible full text articles on stem cells biology, physiology, and differentiation into vascular cells were included. In addition, the data about actual indications for SCT, methodology of stem cells sampling, optimal route of administration (intramuscular *vs* intra-arterial), and the clinical and adverse effects was reported.

Study 19: Yanyi Xu et al. 2015 [7]. **Study characteristics:** Animal study. **Results:** The researchers demonstrated that stem cell survival can be increased even under the low oxygen and nutrient environment. The effect may be obtained by introducing a prosurvival environment into the delivery system.

Study 20: Venkatesh Ponemone et al. 2017 [26]. **Study characteristics:** Prospective, clinic randomized study (n=17). **Results:** The safety and therapeutic effectiveness of autologous bone marrow cell concentrate in revascularization of CLI patients were described. The study included 17 patients. The cellular concentrate was prepared utilizing an intraoperative point-of-care device and injected intramuscularly. Significant improvements in ABI, TcPO2, mean rest pain and intermittent claudication pain scores, wound/ulcer healing, and 6-minute walking distance were observed. Adverse effects were reported just in seven (41.2%) patients, three (17.6%) patients underwent major limb amputation (above the ankle), two (11.8%) patients underwent minor amputation (digit/s), and two unrelated deaths. So, cells' injection was found to be safe, easy, and inexpensive procedure for definite ameliorating of limb ischemia.

Study 21: Arun Sharma et al. 2019 [27]. **Study characteristics:** Literature review. **Results:** The authors presented a comprehensive review of the contemporary scientific literature about therapeutic angiogenesis with stem cells. The benefic aspects of SCT, such as improvement in ABI, $TcPO_2$, reduction of pain, and reduced rates of limb amputation were described. Challenges and limitations of the SCT (anti-ischemic mechanism, ideal cell type, therapeutic dosage, and optimal route of administration) and future study directions were put into discussion.

Study 22: Stempeutics Research Pvt. Ltd. (Bangalore, India) [28]. **Study characteristics:** Phase ½ clinical trial. **Results:** The study was performed from commercial perspective. The efficacy of off-the-shelf allogeneic BM-MSCs administered intramuscularly into the patients with CLI was estimated. No adverse reaction or rejection were found. In addition, improvement in ABI and a reduction in the number of ulcers were reported.

Study 23: Pluristem Therapeutics Inc. (Haifa, Israel) [29]. **Study characteristics:** Two phase 1 trials (n=27). **Results:** The effectiveness of allogeneic placenta-derived MSCs injected intramuscular was demonstrated. Within six-month follow-up, major amputation was necessary only in one case. Significant improvement in blood flow and quality of life was found. In addition, the total pain score reduced.

Discussion

By systematization of collected data, we determined that the cellular grafts are efficient in the treatment of CLI of different etiologies. For this purpose different cell types may be used, such as BM-MSCs, BM-MNCs, mesenchymal cells derived from adipose tissue, stem cells derived from umbilical cord blood, and their combinations. The appropriate routs of cells administration are intramuscular and intra-arterial [5, 7, 17]. For appreciation of SCT efficacy the following criteria are proposed [5-21]: ABI, $TcPO_2$, ankle pressure, capillary density, pain-free walking distance, rest pain, perfusion index, magnetic resonance angiography, wound/ulcer recovery, adverse effects, amputation rate, and life quality.

Conclusions

SCT is evolving as a promising newer tool in the management of severe peripheral vascular diseases; its application in practice may significantly improve the ischemia treatment with good clinical outcomes and reduced amputation rate. Initial animal and clinical studies are supportive of its safety and feasibility. However, the acceptance of this mode of therapy as a standard of care is still a matter of debate and supplementary studies are necessary for effectiveness evaluation and establishing its definite survival benefit.

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Authors' contribution
SV conceptualized the project and performed data collection; TM drafted the manuscript; AC interpreted the data and contributed to the final version of the manuscript; VN took the lead in writing the manuscript. All the authors revised the manuscript critically and approved the final version of the manuscript.

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Ethics approval and consent to participate
No approval was required for this study.

Conflict of Interests
No competing interests were disclosed.