Autologous bone marrow mononuclear cell implantation therapy is an effective limb salvage strategy for patients with severe peripheral arterial disease

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Objective: **This study was conducted to determine if intramuscular and intra-arterial stem cell injections delay or prevent major limb amputations, improve ankle-brachial index measurements, relieve rest pain, and improve ulcer healing.**
Methods: **A prospective case series with interventions occurring between December 2007 and September 2012 and a 3-month minimum follow-up was conducted at an urban tertiary care referral hospital. Patients with severe limb-threatening peripheral arterial disease, without other options for revascularization, were eligible for enrollment. Dual intramuscular and intra-arterial injection of bone marrow mononuclear cells harvested from the iliac crest was performed. Major limb amputation at 3 months was the primary outcome measure. Secondary outcome measures included ankle-brachial index measurements, rest pain, and ulceration healing. Kaplan-Meier survivorship was performed to ascertain overall survivorship of the procedure.**
Results: **No complications related to the procedure were reported. Of 49 patients (56 limbs) enrolled, two patients (two limbs) died, but had not undergone major amputation, and five limbs (8.9%) underwent major amputation within the first 3 months. Three-month follow-up evaluations were conducted on the remaining 49 limbs (42 patients). Median postprocedure revised Rutherford and Fontaine classifications were significantly lower compared with median baseline classifications. After 3 months, seven patients (nine limbs) died but had not undergone major amputation, and seven limbs (14.3%) underwent major amputation. At a mean follow-up of 18.2 months, the remaining 33 limbs (29 patients) had not undergone a major amputation. Freedom from major adverse limb events (MALE) was 91.1% (95% confidence interval, 79.9-96.2) at 3 months and 75.6% (95% confidence interval, 59.4-86.1) at 12 months.**
Conclusions: **This procedure was designed to improve limb perfusion in an effort to salvage limbs in patients for whom amputation was the only viable treatment option. The results of this analysis indicate that it is an effective strategy for limb salvage for patients with severe peripheral arterial disease. (J Vasc Surg 2015;62:673-80.)**

Although revascularization via percutaneous intervention or surgery is the preferred therapeutic option for most patients with critical limb ischemia,[1] approximately one-third of these patients are not ideal candidates because of concomitant disease or unfavorable anatomy.[2] Eventually, many patients with severe to end-stage peripheral arterial occlusive disease (PAD) may resort to limb amputation, which is associated with an array of negative consequences, including significant postoperative morbidity, physical disability, emotional toll, and financial impact.[3-5] The median financial cost of managing a patient after amputation is almost twice that of a successful limb salvage.[5]

Therapeutic angiogenesis using bone marrow mononuclear cells (BM-MNCs) has shown favorable results in patients with critical limb ischemia who are not eligible for other revascularization attempts.[6,7] The goal of this therapy is to inject stem cells extracted from BM that are able to differentiate into hematopoietic and mesenchymal cells and to secrete growth factors to promote neoangiogenesis and endothelialization in ischemic tissue.[8-12] The conclusion from a meta-analysis of 37 trials involving intramuscular autologous BM-MNC implantation was that the procedure is feasible, relatively safe, and a potentially effective therapeutic strategy for PAD patients for whom traditional revascularization is not possible.[13] The authors of another meta-analysis of seven trials (six used intramuscular injection and one used intra-arterial injection) suggested that such therapy confers a beneficial effect in patients with severe PAD.[14] However, insufficient

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Trial Registration: Use of Autologous Bone-Marrow for Mononuclear Cell Implantation Therapy as a Limb Salvage Procedure in Patients with Moderate to Severe Peripheral Arterial Disease, clinicaltrials.gov Identifier: NCT00919516, http://www.clinicaltrials.gov.
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evidence was found after a systematic review to support intramuscular injection, mainly due to the small numbers of participants and the lack of trials using the technique in general.[15]

In December 2007, we began a prospective interventional study to assess a dual intramuscular and intra-arterial autologous BM-MNC implantation technique developed by the senior author (R.F.) to treat patients with severe limb-threatening PAD.[16] The aim of this procedure was to improve tissue perfusion to salvage limbs in such patients for whom amputation had been recommended. The study objective was to determine if intramuscular and intra-arterial stem cell injections delay or prevent major limb amputations, improve ankle-brachial index (ABI) measurements, relieve rest pain, and improve ulcer healing. Enrollment was completed in September 2012, and the final analysis is presented in this report.

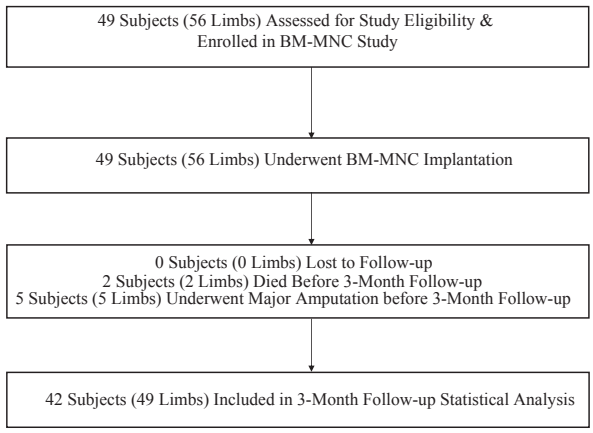

Fig 1. Flowchart depicts patient accounting. *BM-MNC,* Bone marrow mononuclear cell.

METHODS

This prospective, interventional case series was designed to assess outcomes of autologous BM-MNC implantation in patients with severe limb-threatening PAD who did not have other options for revascularization. The protocol and informed consent were approved by an Institutional Review Board. A standardized technique was followed for all procedures.[16,17] Forty-nine patients (56 limbs) gave written informed consent and were enrolled in the study between December 2007 and September 2012. A flowchart depicting patient accounting is provided in Fig 1.

Patients aged ≥18 years were eligible to participate in the study if the following criteria were met: (1) diagnosis of severe limb-threatening PAD, defined as an ABI measurement of ≤0.4 or the presence of a nonhealing ischemic ulcer(s), and (2) angiographic identification of stenosis or occlusion of two of the following lower extremity arteries: anterior tibial, posterior tibial, and peroneal. Additional stenosis or occlusion could be present proximally to these vessels. Major limb amputation had been recommended to these patients due to limb-threatening ischemia or unresolved osteomyelitis, ulcerations, or gangrene. Patients with anatomic limitations identified on angiogram that precluded a recommendation of traditional endovascular or open bypass treatments were eligible for inclusion. Excluded from the study were patients aged <18 years and those eligible to undergo traditional endovascular or open bypass for the treatment of PAD, pregnant women, prisoners, patients with developmental challenges, and those unable to consent for participation independently. Eligibility for study participation was determined by each surgeon and approved by the senior author.

At enrollment, all patients were undergoing maximum medical therapy for PAD according to the managing vascular surgeon's discretion and were receiving wound care management, when applicable. While patients were under monitored anesthesia care and local anesthesia, a three-hole BM needle (DePuy Inc, Warsaw, Ind) with a syringe containing heparin (1000 U/mL) was used to obtain autologous BM aspirate from the anterior superior iliac spine. For patients with heparin-induced thrombocytopenia, only saline was used in the syringe. To concentrate the BM cells, the aspirate (50 to 60 mL) was placed in a centrifuge and spun at 2400 rpm for 12 minutes.

The cells were reinjected immediately into the patient, both intravascularly and intramuscularly, using injection sites determined by the location of the stenosis or occlusion, or both, on a prior angiogram. For intramuscular injections, the aspirate (2 mL) was injected intramuscularly into six different lower leg sites in a standardized fashion to approximate the disease surface area. Injections specific to the calf started 8 cm above the medial and lateral malleolus in the midline of the calf and continued proximally in 8-cm increments. For intra-arterial injections, aspirate (12 mL) was injected intravascularly in a 4F catheter in 2-mL increments, flushing between injections. Artery selection was determined by occlusion location: the popliteal artery was used for tibial occlusions, and the common femoral artery was used for superficial femoral artery occlusions.

Study design. Patients were evaluated before BM-MNC implantation and then at 2 weeks and 3 months after the procedure. Routine angiography was performed before the procedure to identify the specific location of stenosis or occlusion. At the 2-week postprocedure evaluation, limbs only were assessed for possible complications related to the procedure; no study data were collected. Rest pain and ulcer status, if applicable, were evaluated before the procedure and at 3 months afterward. Revised Rutherford criteria[18] were used to classify the extent of acute ischemia, and the Fontaine classification[19] was used to categorize PAD. ABIs of the dorsalis pedis artery and posterior tibial artery were measured, when possible.