Upper extremity ischemia treated with tissue repair cells from adult bone marrow

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Background: Unreconstructable critical ischemia with gangrene of the upper extremity is rarely due to atherosclerosis alone, and few treatment options exist. We describe a patient with gangrene of both hands as a result of unreconstructable atherosclerotic disease of both upper extremities who was successfully treated with tissue repair cells (TRCs) produced from the patient’s bone marrow.

Methods: A patient with type 1 diabetes was referred with bilateral upper extremity digital gangrene due to unreconstructable forearm and hand atherosclerosis. He was evaluated for therapeutic angiogenesis using TRCs.

Results: Following the intramuscular injection of TRCs produced from autologous bone marrow stem cells, the patient demonstrated improved arterial perfusion and a durable clinical response with healing of all amputation sites and cessation of pain.

Conclusions: The production of TRCs results in the expansion of stem and early progenitor cells, including CD90+ mesenchymal cells and endothelial progenitor cells. This is the first reported case of end-stage upper extremity ischemia treated with TRCs harvested from adult bone marrow. (J Vasc Surg 2010;52:723-9.)

Unreconstructable critical limb ischemia (CLI) due to atherosclerosis primarily occurs in the lower limbs and results in amputation in up to 40% at one year. Unreconstructable critical ischemia with gangrene of the upper extremity is infrequently due to atherosclerosis alone, with most resulting from thromboangiitis obliterans or systemic sclerosis. While no adequate approved alternative therapies exist, angiogenesis using bone marrow stem cells has been reported to improve outcomes in patients with upper extremity CLI. These patients had ischemic rest pain or finger ulceration as a result of thromboangiitis obliterans (Buerger’s disease) or collagen vascular disease as opposed to atherosclerotic disease.

There is a growing body of literature documenting clinical benefits of cell-based therapy for advanced coronary artery disease and lower extremity arterial occlusive disease. These studies generally harvest large volumes of bone marrow to obtain the necessary stem cells. We report the first case of unreconstructable atherosclerotic disease of both upper extremities treated successfully with tissue repair cells (TRCs) produced from autologous adult bone marrow stem cells.

CASE REPORT

A 63-year-old man with a 46-year history of Type 1 diabetes was referred with bilateral upper extremity digital gangrene (Fig 1). Fingertip ischemic rest pain and ischemic ulceration had begun 2 years earlier and progressed, resulting in increasing use of oral narcotic analgesics and nonhealing amputations of the distal phalanges of both index fingers and the left ring finger. Gangrene progressed proximal to the amputation sites, and ischemic rest pain developed in the right ring finger. A collagen vascular evaluation was negative, and computed tomographic arteriography (CTA) revealed unreconstructable forearm and hand atherosclerosis, showing occlusion of all forearm arteries at the wrist and occlusion of metacarpal and digital arteries. The patient was referred for evaluation for therapeutic angiogenesis.

The patient’s history included hypertension, coronary artery disease, atrial fibrillation, congestive heart failure, renal dysfunction, and lower extremity peripheral arterial disease (PAD). He had undergone right below-knee amputation and a left femoral-to-submalleolar posterior tibial artery bypass with saphenous vein followed by amputation of two left toes. He denied tobacco use and rarely used alcohol. Family history was noncontributory.

Axillary and brachial pulses were palpable bilaterally, but radial and ulnar pulses were bilaterally absent. The majority of the left index finger was gangrenous, extending to the tip from 1.5 cm distal to the web space. The right index finger had gangrene extending from the proximal interphalangeal joint distally. The right ring finger showed gangrene distal to the interphalangeal joint.

Lower extremities had palpable femoral pulses but no palpable popliteal pulses. The patient had a well-healed right below-knee amputation. The left femoral-to-posterior tibial artery bypass graft was patent. The left foot was well perfused.

Upper extremity noninvasive studies demonstrated equal brachial artery pressures and normal upper arm pulse volume recordings (PVRs). Forearm PVRs were attenuated, and the digital PVRs were nearly flat (Fig 2). Radial and ulnar artery Doppler signals were monophasic bilaterally. Bilateral arteriography demonstrated
normal arteries to the level of the brachial artery (Fig 3). The arteries of forearms and hands showed atherosclerotic occlusion with severe small vessel disease of both hands (Fig 4) without option for revascularization.

The Short Form (36) Health Survey (SF-36) was administered to gauge quality-of-life (QOL), and his summary of physical functioning was ranked 34/100. The patient scored “7” on a horizontal Visual Analogue Scale (VAS) for bilateral upper extremity pain.

A compassionate single-use protocol and Investigational New Drug (IND) application was approved by the United States Food and Drug Administration and the ProMedica Health System institutional review board. The patient had outpatient bone marrow aspiration (47 cc) from the posterior iliac crest under local anesthesia. The bone marrow was shipped to a centralized Aastrom Biosciences, Inc. processing facility. Cell concentration was $41 \times 10^6$ viable nucleated cells.

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Using standard protocol, red blood cells and mature granulocytes were removed using a Ficoll density gradient, resulting in $433 \times$

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**Fig 1.** Photograph of hands at presentation. Patient underwent prior amputation of right and left index fingers and left ring finger.

**Fig 2.** Photoplethysmographic waveforms of fingers of left hand at (A) initial presentation (motion artifact), (B) 1 month, (C) 3 months, and (D) 12 months after treatment. Right hand waveforms are similar.
10^6 viable mononuclear cells available for culture. Three hundred \times 10^6 viable mononuclear cells were inoculated into a closed automated cell production system for clinical-scale ex vivo culture of bone marrow derived stem and progenitor cells. The specifics of the cell culture process have been previously described. After 12 days of culture, a total of 125 \times 10^6 viable mononuclear cells (TRCs) were harvested and washed in electrolyte solution. The composition of TRCs is summarized in the Table. The CD90+ mesenchymal cell population was 49-fold increased relative to the number in the original mononuclear cell preparation. The CD90+ cell subpopulation in the TRC product has been shown to contain stem and progenitor cells and is therefore a useful surrogate marker in this respect. Importantly, TRCs have been shown to contain endothelial progenitor cells in colony formation assays and have also been shown to possess endothelial tube-formation capacity in vivo. The TRC culture process reduced the number of granulocytes, T and B lymphocytes, and red blood cells by more than 70%.

Subsequent to expansion at the processing facility, the TRC product was shipped back to the treatment center in a final product volume of 5.5 mL. This volume was expanded to 9.5 mL at the treatment center with the addition of sterile saline. TRCs were injected via 15 intramuscular injections (0.3 mL each) into the patient’s forearms (\times 2 injections), web spaces, and finger pads of both upper extremities during one treatment session (Fig 5). Injection sites were chosen to deliver the majority of the cells into ischemic tissues of the hand, as prespecified in the protocol for the single-use IND.

Two months post-injection, the patient reported diminished pain in his fingers and reduced need for analgesics. At 3 months, he developed erythema, swelling, and purulent drainage from his left and right index fingers as a result of demarcation and separation of the gangrenous tissue and infection with methicillin-resistant staphylococcus aureus. Intravenous vancomycin was begun, and he underwent amputation of the gangrenous tissue and infected bone in both index fingers. These wounds were left open. The distal phalanx of the left ring finger was amputated and closed with a palmar flap. The distal phalanx of the right ring finger was debrided. Good bleeding was observed in all wounds. He remained on vancomycin and piperacillin/tazobactam and received 14 hyperbaric oxygen treatments to assist with infection control during the week prior to discharge. His hands and proximal fingers were pink and warm. Pulsatile perfusion as assessed by PVR was markedly improved (Fig 2). All open amputation sites healed by secondary intention, and the left ring finger flap healed primarily.

Six months post-injection, analgesics were no longer required and the patient scored his worst pain as a “1” on aVAS. Laser Doppler imaging was available and performed for both hands. Fig 5 shows uniform increased perfusion at specific injection sites. Nine months following treatment, he reported no pain. All amputation sites were healed.

At his 12-month follow up (Fig 6), the patient rated “0” for pain on aVAS and was no longer taking analgesics. The SF-36 results documented marked improvement in QOL, with a 35% increase in his physical summary. Specifically, his physical functioning score increased by 15%, physical role functioning by 100%, bodily pain by 29%, general health by 21%, and vitality by 20%.

**DISCUSSION**

Injection of TRCs into the ischemic tissue of a patient with end-stage upper extremity ischemia due to multilevel medium- and small-vessel atherosclerotic disease resulted in increased arterial perfusion, elimination of ischemic rest pain, and healing of all previously gangrenous digital amputation sites. Tissue perfusion was clinically improved, and standardized photoplethysmography demonstrated measurable increase. The patient’s clinical improvement and objectively measured increased arterial perfusion persisted beyond 1 year of follow up. The onset of improved perfusion was evident at 1 month. Ischemic rest pain was diminished at 1 month and resolved by 3 months as documented on the horizontal VAS for pain, which has been shown to be a sensitive and accurate representation of pain intensity.

Patients with advanced ischemia and gangrene face inferior outcomes with medical therapies and/or revascularization compared with patients with less severe clinical presentations, such as rest pain or digital ulceration. Critical limb ischemia patients with overt gangrene are often excluded from investigations of new therapies because of poor prognosis. In the absence of revascularization, amputation at the level of gangrenous or ischemic tissue, as performed prior to referral in this case, is futile and can lead to acceleration of necrosis and significant risk of infection.

The underlying pathophysiology of upper extremity CLI is important in terms of prognosis and response to therapy. Inflammatory occlusive diseases, such as thromboangiitis obliterans (TAO) and collagen vascular disease (CVD), are different pathologic entities than atherosclerotic occlusive disease. TAO patients respond particularly well to therapeutic angiogenesis and have a more favorable response than patients with atherosclerosis. However, patients with TAO and CVD of the upper
extremities also respond reasonably well to good medical care. McClafferty et al12 followed 44 patients with upper extremity CLI due to TAO or CVD for an average of 15.2 years. Patients with TAO appeared to have improved outcomes compared with those with CVD; however, even in CVD patients, tissue loss was modest. Often these patients have normal arterial perfusion to the wrist but have distal small-vessel disease. The patient reported herein had occlusion of both radial and ulnar arteries in addition to advanced palmar and digital occlusive disease. The multilevel nature of his occlusive disease eliminated all options for surgical or endovascular intervention and caused his severe ischemia (gangrene) with its attendant poor prognosis. The patient’s unremitting ischemic rest pain was an indicator of the additional proximal tissue at risk of necrosis.

Autologous bone marrow cells represent an attractive alternative to gene therapy for therapeutic angiogenesis in patients with CLI. Bone marrow contains a mixed population of cells such as fibroblasts, osteoblasts, myogenic cells, and endothelial cells. These can differentiate into various tissues. However, when injected into ischemic tissue, bone marrow mononuclear cells are unlikely to result in ectopic tissue formation.7,8 Tateishi et al15 investigated bone marrow-implanted limbs with immunohistochemical methods and showed an increase in capillary numbers but no evidence of either bone formation or fibrosis.

**Table.** Comparison of tissue repair cells

<table>
<thead>
<tr>
<th>Cell population</th>
<th>Cell types labeled</th>
<th>Cell count at harvest (culture inoculated cells) (\times 10^6)</th>
<th>Cell count (TRC product) (\times 10^6)</th>
<th>Fold increasea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total viable bone marrow mononuclear cells</td>
<td>All cells</td>
<td>300</td>
<td>125</td>
<td>0.4</td>
</tr>
<tr>
<td>Stem and progenitor cells</td>
<td>Mesenchymal stem and progenitor cells</td>
<td>0.8</td>
<td>39</td>
<td>49</td>
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<tr>
<td>% CD90+</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Leukocytes</td>
<td>All leukocytes</td>
<td>289</td>
<td>86</td>
<td>0.3</td>
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<tr>
<td>% CD45+</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CD14+ Auto+</td>
<td>Activated macrophages</td>
<td>0.8</td>
<td>32</td>
<td>40</td>
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<td>CD66b+</td>
<td>Granulocytes</td>
<td>183</td>
<td>20</td>
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<tr>
<td>CD3+</td>
<td>T cells</td>
<td>32</td>
<td>9.2</td>
<td>0.3</td>
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<tr>
<td>CD19+</td>
<td>B cells</td>
<td>19</td>
<td>1.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>Red blood cells and erythroid precursors</td>
<td>105</td>
<td>1.0</td>
<td>0.01</td>
</tr>
<tr>
<td>% Gly-A+</td>
<td></td>
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</table>

TRC, Tissue repair cell.

aFold increase refers to the multiplier effect of the preparation of the tissue repair cells product.

![](image.png) **Fig 4.** Bilateral arteriography showing severe occlusive disease of the distal radial ulnar and interosseus arteries bilaterally and small vessel disease of both hands.
TRCs represent an array of bone marrow mononuclear cells important in angiogenesis. The tissue production process occurs over 12 days in an automated, closed good manufacturing practice-compliant system and results in the expansion of stem and early progenitor cells, including CD90+ mesenchymal cells and endothelial progenitor cells. TRCs have been shown to have proangiogenic potential in vitro and in vivo. Red blood cells, granulocytes, and T and B lymphocytes are reduced in number relative to the starting material.

Mesenchymal cells (MSCs) and bone marrow mononuclear cells have been injected into ischemic limbs. Bone marrow mononuclear cells and MSCs differentiate into endothelial cells; however, only MSCs differentiate into vascular smooth muscle cells. MSCs secrete significantly higher amounts of proangiogenic cytokines, such as vascular endothelial growth factor, basic fibroblast growth factor, and stromal cell-derived factor-1-alpha and are likely to become apoptotic under hypoxic conditions.

The mechanism of improved perfusion following bone marrow stem cell injection can be attributed to vasculogenesis, which is a result of the endothelial progenitor cells derived from the mononuclear cells undergoing lineage-specific differentiation into new blood vessel growth. As similar attempts with single-factor approaches to angiogenesis for PAD patients have not been successful, the angiogenic functions of TRCs as well as their modulation of local inflammatory processes underscore the attractive hypothesis that a multimodal approach to the ischemic limb...
utilizing a single therapeutic with multiple mechanisms of action could be successful in variable limb ischemia circumstances.

Bartsch et al.\(^2\)\(^1\) used both intraarterial and intramuscular delivery of bone marrow mononuclear cells when they treated 13 patients with chronic PAD and intermittent claudication. Prior to injection, they induced limb ischemia with a thigh blood pressure cuff to produce a hyperemic response to maximize homing of the intraarterially delivered stem cells. Walking distance, ankle-brachial index, capillary venous oxygen saturation, and venous occlusion plethysmographic evaluation of arterial perfusion were all improved.

Cobellis et al.\(^2\)\(^2\) documented that two intraarterial infusions of bone marrow mononuclear cells into the femoral artery were well tolerated, although its necessity or efficacy was not demonstrated. Nevskaya et al.\(^2\)\(^3\) reported two patients with ischemic fingers with ulceration due to systemic sclerosis. One was treated with peripheral blood mononuclear cells and the other with bone marrow mononuclear cells. Both patients experienced ulcer healing, improved skin blood flow, and improved flow-mediated brachial artery reactivity. However, after treatment, circulating endothelial precursors were only found in the patient treated with bone marrow stem cells. This observation may be relevant to the durability of response to treatment; however, the authors failed to report long-term outcome.

Our patient is the first to be treated with bone marrow harvested TRCs for gangrene of both hands resulting from multilevel atherosclerotic disease and has demonstrated improved arterial perfusion and durable clinical response with healing and cessation of pain. The TRC method is designed to create a mixed population of cells with greatest potential efficacy for treating limb ischemia while minimizing the discomfort and risk associated with marrow harvest. Harvesting 60 cc of bone marrow is sufficient to produce TRCs and has substantial advantage over the 500 to 600 cc bone marrow volume required when bone marrow stem cells are used primarily. Sustained improved perfusion suggests that therapy with TRCs may be biologically different and more durable than gene therapy using viral or plasmid vectors to deliver intracellular pro-angiogenic proteins.\(^2\)\(^4\)

TRCs are currently under evaluation in a randomized, controlled trial of patients with CLI of the lower extremities. This case demonstrates the potential of TRC therapy for advanced CLI. The randomized controlled trial will offer a more objective look at the value of TRCs in a broader base of patients with CLI.

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REFERENCES


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