Bone Marrow Stem Cell Therapy (SCT) for Peripheral Arterial Disease (PAD): an Initial Experience

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Abstract

Introduction: 30% of patients with critical limb ischaemia (CLI) are not suitable for conventional treatment. Use of stem cell therapy (SCT) is relatively new. This study shares an initial experience using SCT in 4 patients.

Methods: Approval from Institutional Medical Board Ethics Committee was obtained prior to commencement and informed consent was sought. Patients included had extensive CLI history that was no longer amenable to standard treatment. Bone marrow aspiration from the iliac crest was carried out under regional anaesthesia. This was later centrifuged and injected intramuscularly and adjacent to affected vessels. Wound surveillance was then performed.

Results: SCT was well tolerated in all 4 patients and 2 had favourable results. None developed related complications. Patients 1 and 2 showed improvement of rest pain, claudication symptoms and healing of ulcers. Angiogenesis and neovascularization can be seen in follow up angiography for patient 1. Wound healing was not noted in the other 2 patients, with both requiring amputations eventually.

Discussion: The experience while early has been invaluable. The varied response suggests that factors determining treatment success remained unknown. Likewise, most other trials have consisted of small uncontrolled patient series, with few randomized studies. Haemodialysis, diabetes mellitus and coronary arterial disease factors seemed to negatively affect angiogenesis. Severity of rest pain and number of repeated interventions, in particular bypass procedures, may negatively intervene with neo-capillary formation. SCT may eventually provide hope to patients and physicians. More research can help determine a specific group of patients that will benefit most.

Keywords: Neuropathy; Stem cells; Therapeutic angiogenesis; Endothelial progenitor

Introduction

Critical limb ischaemia (CLI) is the most severe form of peripheral arterial disease where there is inadequate blood flow to maintain metabolic requirements of the tissues at rest [1]. Treatment is often challenging, with surgical or endovascular revascularisation considered to be the gold standard. Up to 30% of patients however, are not suitable for treatment and require limb amputation, while 25% suffer mortality from the disease [2]. This is due to the high operative risks conferred by concomitant chronic co-morbidities such as ischemic heart disease, chronic renal impairment, as well as the often adverse extent of vasculopathy with diffuse involvement of multiple levels and distal stenosis. Locally, 8.9% of the population between 18 and 69 years are diabetic, and the risk of CLI is increased 4 fold in these patients [3]. CLI in diabetics preferentially involve infra-popliteal distal calf vessels with diffuse, multi-level distal stenosis compounding the difficulties faced during revascularization. Patients require repeated procedures due to a combination of premature advanced atherosclerosis, peripheral neuropathy, impaired cellular immunity and impaired wound healing. Up to 45% of all amputees are diabetic, and a diabetic patient with CLI is 10 times more likely to require an amputation despite new technology and innovation in revascularization [4].

Bone Marrow Stem Cell Therapy (SCT) in clinical practice

Embryonic and adult stem cells have the capacity to renew and generate differentiated cells. Embryonic stem cells are pluripotent, while adult stem cells are partially lineage committed giving rise to specialized cells of the germ layer. (i.e., they are multipotent rather than pluripotent). Bone marrow stem cells include progenitor cells such as the multipotent adult progenitor cells, mesenchymal and haematopoietic stem cells. Endothelial progenitor cells are adult haemangioblast-derived cells. In addition to having all functional properties of endothelial cells, they secrete paracrine mediators, interleukins, growth factors (angiopoietins Ang1 and Ang2, vascular endothelial growth factor (VEGF)) and chemokines to encourage migration of endothelial and support cells and the further proliferation and differentiation of the progenitor cells. Likewise, mesenchymal stem cells stimulate endothelial cell migration and vessel tube formation. They too release factors, migrate to injured sites, differentiate and secrete further trophic factors for paracrine signalling, activating endothelial cells [5]. SCT in the treatment of PAD is relatively new. In 1997, Asahara et al. identified a class of bone marrow-derived, circulating endothelial progenitor cells that contribute to angiogenesis in ischemic tissues [6]. Thereafter, the first clinical trial of cell therapy for PAD was performed by Tateishi-Yuyama et al. in 2002 known as the Therapeutic Angiogenesis using Cell Transplantation Study (TACT),...
where bone marrow derived mononuclear cells were injected into the gastrocnemius muscle, and subsequent improvement in ankle-brachial index (ABI), transcutaneous oxygen tension, pain-free walking time, and rest pain was documented [7]. Since then, several studies have reported variations of stem cells usage, including embryonic, adult and induced pluripotent stem cells, examining the efficacy and safety of cell therapy while obtaining varying results [8,9]. The procedure has also been generally well tolerated, with the most frequent adverse reaction being local pain or anaemia. In this study, an initial experience with 4 patients who underwent SCT as an alternative therapy when standard revascularisation was no longer an option for them is described. The aim of the study is to see if these patients can avoid amputation through improved wound healing brought about by angiogenesis and vascular collateral formation.

**Materials and Methodology**

Approval from the Institutional Medical Board Ethics Committee was obtained prior to commencement of this study. Informed consent was sought from all patients undergoing this treatment. Patients selected had no evidence of underlying malignancy, with life expectancy of more than 6 months, not pregnant and were without known haematological disease. All patients selected had an extensive CLI history, multiple revascularisation procedures, failed revascularisation therapy or recalcitrant, recurrent, persistent disease not amenable to treatment for reasons including high surgical risk, multi-level distal disease or both as summarized in Table 1.

**SCT procedure**

Bone marrow aspiration from the iliac crest was carried out under regional anaesthesia in the operating theatre. The aspirate is then centrifuged with the Terumo SmartPRep2 system (Figures 1A-E) to allow concentration of Bone Marrow-Mononuclear cells (BM-MNC). This was then injected intramuscularly, adjacent to the affected vessels, 1 mL at 2 cm intervals under ultrasound guidance with the remaining around ulcers and wounds (peri-lesional). Wound surveillance was performed at both inpatient and outpatient settings post procedure.

Clinical improvement in terms of reduced or resolution of symptoms was also noted (Figure 1).

**Results**

SCT was well tolerated in all 4 patients and patients 1 and 2 were discharged well. None of them developed direct SCT-related complications such as anaemia or severe injection site pain. Patients 1 and 2 benefitted from SCT with improvement of rest pain, claudication symptoms and healing of ulcers. We achieved the best results in Patient 1 who had excellent clinical improvement and was well enough to go abroad half a year after treatment. Angiogenesis and neovascularization can be seen in her follow up angiography 3 months post SCT. In patients 3 and 4, results of the treatment were not as encouraging. Wound healing was not noted in either. Patient 3, whose wound deteriorated further, underwent further multiple amputations to cope with the sepsis. Patient 4 was readmitted for exacerbation of cardiac failure brought about by NSTEMI about a month after treatment. Prior to his demise, the patient had returned to smoking, neglected foot care and declined further intervention or amputation. Table 2 summarizes the results of the patients undergoing SCT.

**Discussion**

The experience with injection of BM-MNCs for CLI in these 4 patients, while early, has been invaluable. Healing of ischemic ulcers demands an improvement in blood supply, good glycaemic control as well as tissue that is infection free. The treatment of CLI is often fraught with despair and the disease process is known to take a toll both physically and psychologically. Many patients develop fatalistic attitudes as they lose their limbs, give up on good glycaemic control...
### Patient 1

74/Chinese/Female  
Non-smoker  
Pre-morbidly ambulant, good social support  

**Past Medical History:** Hypertension, hyperlipidaemia, ischaemic heart disease with triple vessel disease s/p CABG 2006

| Signs/Symptoms | Investigations | Treatment/Interventions |
|----------------|---------------|-------------------------|
| **First Presentation** | | |
| **61 years old** | **Right Duplex US and Angiography** | **Medical therapy** with statins and anti-platelets |
| Bilateral calf claudication | Diffuse atherosclerotic changes in distal abdominal aorta, R CIA, EIA, bilateral SFA, popliteal, ATA, PTA | Claudication exercises |
| Intermittent rest pain | | |
| **13 Years Later** | **Further decreased claudication distance** | **Left LL Angioplasty** |
| **Rest pain** | Left- 0mmHg, Right- 2mmHg | Severe long segment stenosis and occlusion bilateral CIA, EIA, SFA reconstituted at P2 level with run off via PTA and peroneal artery |
| Left mid shin and dorsal foot non-injected ulcers with exposed tendons, absent pedal pulses | US Duplex and Angiography (Figure 2) | Brisk flow to profunda via collaterals |
| **CT Peripheral Angiography** | Bilateral EIA, CFA, SFA severely calcified and occluded to popliteal, peroneal and L DP occluded. <1mm trickle to R PTA | DEB to L CIA and EIA performed |
| **3 Months Later** | | |
| Left lower limb cellulitis and distal ulcers with wet gangrene and exposed mid-foot tendon | No investigations done | Declined amputations or surgical bypass for fear of risks |
| Pre-morbidly wheelchair mobile, good social support | | Treated with IV antibiotics till ulcers stable |

### Patient 2

66/Chinese/Male  
Non-smoker  
Pre-morbidly wheelchair mobile, good social support  

**Past Medical History:** Hypertension, hyperlipidaemia, diabetes mellitus, ischaemic heart disease with triple vessel disease s/p CABG >10 years ago, congestive cardiac failure (EF 27%), Left neck of femur fracture conservatively managed with straight leg traction

| Signs/Symptoms | Investigations | Treatment/Interventions |
|----------------|---------------|-------------------------|
| **First Presentation** | | |
| **64 years old** | **CT Peripheral Angiography** | **Right CFA end-arterectomy, EIA and CIA stenting** |
| Rest Pain | Diffuse atherosclerotic changes of aorta, iliac, femoral, popliteal and trifurcation | |
| Right anterior shin ulcer | **Bilateral Lower Limb Angiography** | |
| Severe calcified atheromatous disease affecting aorta, bilateral CIA, EIA, CFA, SFA, reconstituting at popliteal via profunda collaterals | | |
| | **Immediate Post-operatively** | |
| Developed bilateral dusky toes | Repeat Angiography | Repeat Angioplasty of Right EIA, stenting of Left EIA, Right SFA |
| Dry gangrene of right big toe and pus involving tendon sheaths | Narrowed profunda artery | Right BT Ray amputation and debridement |
| **4 Months Later** | | |
| Poor healing of Right BT amputation wound | | |
| Dry gangrene with slough, heel ulcer with exposed bone | | |
| Left heel ulcer with dry gangrene | Debridement of Right BT amputation site and heel wound | |
| **5 Months Later** | | |
| Right heel bone and Left TA exposed | US Duplex | Bilateral wound debridement |
| Increased rest pain | Bilateral in-stent stenosis | Bilateral LL Angioplasty |
| | | Done mainly to stent and popliteal arteries |
| 4 Months Later                                                                 |
|--------------------------------------------------------------------------------|
| **Increased frequency of Right LL rest pain**                                |
| **Left leg angioplasty, EIA stent, CFA, popliteal plasty**                     |
| **Left foot 4 x 4 cm dry ulcer**                                              |
| **Recurrent SFA in-stent stenosis**                                            |
| **Unable to tolerate R LL angioplasty in prone position, rescheduled ATA plasty**|
| **Right foot 8 x 4 cm ulcer with dry gangrene**                               |

| 5 Months Later                                                                 |
|--------------------------------------------------------------------------------|
| **Increased rest pain**                                                        |
| **Stable ulcers, no gangrene**                                                  |
| **Developed CCF secondary to NSTEMI, underwent Coronary PCI**                  |
| **No investigations done**                                                     |
| **Left LL angioplasty with L Iliac stent plasty**                               |
| **Simultaneous with PCI**                                                      |

| 6 Months Later                                                                 |
|--------------------------------------------------------------------------------|
| **Deterioration of bilateral foot ulcers**                                    |
| **US Duplex**                                                                 |
| **Bedside debridement (in view of recent NSTEMI)**                            |
| **Exposed bone with pus**                                                     |
| **Severe calcification bilaterally**                                           |
| **- Right EIA stent only trickle of flow**                                    |
| **Left BKA, Right foot wound debridement**                                    |

| 6 Months Later                                                                 |
|--------------------------------------------------------------------------------|
| **Stable symptoms**                                                           |
| **US Duplex**                                                                 |
| **Right SFA full length in stent stenosis and occlusion, ATA, DP occluded**   |
| **Right LL angioplasty to EIA, SFA and ATA**                                   |

| 4 Months Later                                                                 |
|--------------------------------------------------------------------------------|
| **Worsening Right foot ulcers**                                                |
| **US Duplex**                                                                 |
| **Right LL angioplasty to Right SFA in-stent stenosis, DEB applied**           |
| **2nd toe gangrene**                                                          |
| **Persistent SFA in-stent stenosis**                                           |
| **Failed ATA angioplasty**                                                     |

| Patient 3                                                                      |
|--------------------------------------------------------------------------------|
| **81/Malay/Female**                                                            |
| **Non-smoker**                                                                 |
| **Pre-morbidly wheelchair mobile, good social support**                       |
| **Past Medical History:** Hypertension, hyperlipidaemia, End stage renal failure on haemodialysis, ischaemic heart disease with NSTEMI, Left knee fracture managed conservatively** |
| **Signs/Symptoms**                                                             |
| **Investigations**                                                            |
| **Treatment/Interventions**                                                    |
| **First Presentation**                                                         |
| **78 years old**                                                               |
| **Cold toes, rest pain, no tissue loss**                                       |
| **Declined investigations**                                                    |
| **Medical therapy with anti-platelet and statins**                            |
| **Declined vascular imaging or intervention**                                  |

| 5 Months Later                                                                 |
|--------------------------------------------------------------------------------|
| **Wet gangrene of Right lateral foot 4th and 5th toes**                        |
| **Angiography**                                                               |
| **Right 4th and 5th toe amputation**                                          |
| **Increased rest pain**                                                       |
| **Multifocal SFA stenosis and popliteal, PTA occluded.**                       |
| **Absent distal pulses**                                                      |
| **Collaterals seen.**                                                          |
| **Bilateral Angioplasty**                                                     |
| **R SFA, popliteal, TP trunk and peroneal, L SFA, L popliteal artery plastied**|

| 2 years later                                                                 |
|--------------------------------------------------------------------------------|
| **Wet gangrene of Right 3rd toe**                                              |
| **Angiography**                                                               |
| **Bilateral BT Amputation, Right 2nd toe debridement**                        |
| **Bilateral dry gangrene with small ulcers**                                  |
| **Bilateral SFA, popliteal, R TP trunk, proximal ATA, L ATA, L PTA L DP multiple stenosis** |
| **Left 5th MT debridement**                                                   |

| Patient 4                                                                      |
|--------------------------------------------------------------------------------|
| **81/Chinese/Female**                                                          |
| **Smoker**                                                                    |
| **Pre-morbidly wheelchair mobile, good social support**                       |
| **Past Medical History:** Hypertension, hyperlipidaemia, triple vessel disease s/p CABG 1997, NSTEMI 2014 s/p multiple PCIs, congestive cardiac failure, paroxysmal atrial fibrillation on Warfarin** |
| **Signs/Symptoms**                                                             |
| **Investigations**                                                            |
| **Treatment/Interventions**                                                    |
| **First Presentation**                                                         |
| **75 years old**                                                               |
| **R LL Angiography**                                                          |
| **Right LL Angioplasty**                                                       |
| **Right leg rest pain and cold feet**                                          |
| **Sub-acute popliteal artery occlusion extending into peroneal and PTA, reformed with collaterals** |
| **Unable to cross into peroneal and PTA despite multiple attempts**           |
| **Debilitating claudication distance**                                         |
| **Covered stent placed across popliteal occlusion and coronary stent placed into proximal PTA with single PTA run off eventually.** |
| **No tissue loss**                                                            |
| **Thrombolysis to acute stent thrombosis**                                    |
| **Check angiography POD 2 showed satisfactory recanalization of SFA and popliteal artery** |

| POD 1: Acute stent thrombosis                                                 |
|--------------------------------------------------------------------------------|
| **3 months later**                                                            |
Recurrent cold right leg

**US Duplex**

- Increased rest pain
- Recurrent subacute occlusion

**Right LL Angioplasty**

- PTA stent angioplasty with good results
- Self-expanding Stent inserted in mid SFA

**Angiography**

- In stent thrombosis
- Occlusion from mid-thigh SFA with slow distal flow and no reconstitution of PTA
- Slow flow to DP

**6 years later**

- Right heel dry ulcer
- US Duplex

**Angioplasty of popliteal artery stent and P2 region and TP trunk and peroneal artery with DEB**

**Right 3rd toe dry gangrene**

- Occluded popliteal artery stent with occluded PTA and ATA, monophasic flow to foot

**Increased rest pain**

**Angiography**

- Offered bypass surgery - declined

**Post angioplasty: Persistent cold heel and toe pain**

- Complete popliteal artery in stent occlusion
- TP trunk occluded into proximal peroneal artery, occluded ATA and PTA

ATA: Anterior Tibial Artery; BKA/AKA: Below Knee Amputation/Above Knee Amputation; BT: Big Toe; CABG: Coronary Artery Bypass Graft; CFA: Common Femoral Artery; CIA: Common Iliac Artery; DEB: Drug Eluting Balloon; DP: Dorsalis Pedis; EF: Ejection Fraction; EIA: External Iliac Artery; L: Left; LL: Lower Limb; MI: Myocardial Infarction; PTA: Posterior Tibial Artery; R: Right; SFA: Superficial Femoral Artery; TA: Tendo-Achilles.

**Table 1:** Summary of patients’ characteristics, co-morbidities, signs/symptoms, vascular investigations and Interventions Pre-SCT.

| Patient 1 | POD | Outcomes (Figures 3A-J, Figures 4A-D) |
|-----------|-----|---------------------------------------|
| Length of hospitalization post SCT: 5 days | 15 | L shin and dorsal foot ulcers healing and granulating |
| | 36 | L shin wound healed, foot defect smaller, healing |
| | 78 | Continued healing seen |
| | 89 | Left shin ulcer slight dehiscence < 1cm |
| | 91 | Angiogram performed: Patent CIA up to CFA, occlusions seen along SFA with neovascularization seen and multiple collaterals at adductor canal. PTA reconstituted by collaterals, dorsal foot perfused by PTA |
| | 6 months | Completely healed ulcers, able to travel abroad for holiday with no rest pain or significant claudication |

| Patient 2 | POD | Outcomes (Figures 5A-C) |
|-----------|-----|-------------------------|
| Length of hospitalization post SCT: 3 days | 34 | Granulation tissue seen over dorsal foot wound |
| | 74 | Further reduction in wound size and granulation |
| | 109 | Healing of dorsal foot wound |
| | 8 months | Wound size less than 1cm, No complains of rest pain |

| Patient 3 | POD | Outcomes |
|-----------|-----|----------|
| Length of hospitalization post SCT: NA | 16 | No improvement of foot ulcers. Developed purulent discharge |
| | 26 | Further deterioration requiring bilateral forefoot amputation and right heel debridement |
| | 51 | Forefoot stump infection. Underwent R AKA and L forefoot wound debridement |
| | 75 | AKA stump dehiscence secondary to infection with purulent discharge and poorly felt femoral artery pulses, Underwent stump revision and debridement |
| | 4 months | Developed altered mental status secondary to sepsis, requested terminal discharge to Nursing Home, no follow up since |

| Patient 4 | POD | Outcomes |
|-----------|-----|----------|
| Length of hospitalization post SCT: 5 days | 8 | Readmitted for CCF sec to NSTEMI, stable right 3rd toe and heel gangrene, no infection, persistent rest pain |
| | 17 | New R BT wound from accidental trauma at home |
| | 17 | Increasing right foot duskniness and progression of gangrene |
| | 25 | Restarted smoking, further increase in rest pain. Developed R BT wet gangrene, declined amputation |
| | 47 | Passed on from NSTEMI with cardiac failure contributed by pneumonia and critical limb ischaemia |

**Table 2:** Summary of SCT and patient outcomes.

or return to smoking as with patient 4. Improvement in quality of life as seen in patients 1 and 2 can only take place with both ulcer healing and reduction of symptoms like rest pain and claudication. The varied response in the 4 patients suggests that while SCT can work, either the factors which influence its efficacy or the proper patient selection has not been truly determined. The exact science behind or conditions required to promote neovascularization demonstrated in the angiogram of Patient 1 (see Photos) is not fully understood. SCT as a new therapeutic option have generally been offered only to end-stage PAD patients with CLI without other therapeutic options. Most clinical trials of cell therapy for PAD have consisted of uncontrolled patient series, with few randomized, properly controlled studies. Sample sizes have been small, with most studies enrolling fewer than 50 patients. The therapeutic product for nearly all of the trials has been bone marrow-derived
mononuclear cells and/or peripheral blood-derived mononuclear cells harvested with or without granulocyte colony-stimulating factor mobilization. Cells were delivered by direct intramuscular injection at multiple sites of the affected limb or by intra-arterial injection via
the femoral artery. Reported endpoints of these studies have included ABI, transcutaneous oxygen tension, and angiography examined at baseline and following cell therapy, with an average follow-up period of approximately 6 months to 1 year. Subjective outcomes have also been reported, including patient-perceived rest pain and pain-free walking time or distance. Collectively, results were promising and the procedures were well tolerated by the patients, with few adverse events reported (Figures 2-4). Haemodialysis, diabetes mellitus and complications with coronary arterial disease are all known factors affecting amputation-free survival in patients as these risk factors can negatively affect angiogenesis [10-12]. Matoba et al. postulate that severity of rest pain and number of repeated vascular interventions, in particular bypass procedures may negatively intervene with neo-

Figure 2J: Patient 17 months post SCT Left shin ulcer.

Figure 3A: Patient 1 PRE SCT angiography.

Figure 3B: Patient 13 months Post SCT increased collaterals.

Figure 3C: Patient 1 ATA 3 months Post SCT increased collaterals.

Figure 3D: Patient 13 months POST SCT Hint of new vessel.

Figure 4A: Patient 2 PRE SCT Right dorsal foot ulcer.

capillary formation sprouting from collateral vessels retarding angiogenesis [13]. While severe rest pain from ischemic limbs may lead to an active production of cytokines promoting angiogenesis, multiple attempts at revascularisation may retard this and render the patient unable to achieve any healing of ischemic wounds. SCT might thus work best through delivery of these angiogenic factors.
Conclusion

This study, likely the first of its kind in Singapore and Southeast Asia, has proven no different from others that SCT may eventually provide hope to patients and surgeons that have exhausted conventional treatment options save for amputation(s). The science is not perfect but it remains a potential treatment for chronic limb ischaemia recalcitrant to standard of care techniques. Perhaps more widespread use of SCT can help determine a specific group of patients that will benefit most from it.

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