- Critical limb ischemia (CLI) is a severe form of PAD
- Rutherford grading

<table>
<thead>
<tr>
<th>Grade</th>
<th>Category</th>
<th>Clinical Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>Asymptomatic – no hemodynamically significant occlusive disease</td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>Mild claudication</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Moderate claudication</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Severe claudication</td>
</tr>
<tr>
<td>II</td>
<td>4</td>
<td>Ischemic rest pain</td>
</tr>
<tr>
<td>III</td>
<td>5</td>
<td>Minor tissue loss – nonhealing ulcers, focal gangrene with diffuse pedal ischemia</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Major tissue loss – extending above TM level, functional foot no longer salvageable</td>
</tr>
</tbody>
</table>

**Incidence:** 0.05-0.1% per year

**Prognosis**
- High risk for limb loss and for fatal and nonfatal vascular events
- Fate after 1 year

- 25% CLI resolved
- 20% Continue CLI
- 30% Alive amputated
- 25% Death

*Norgren et al. Eur J Vasc Endovasc Surg, 2007*
Treatment of CLI

CLI confirmed

Candidate for revascularization

Imaging (Duplex, angiography, MRA, CTA)

Revascularization as appropriate

Not candidate for revascularization

Stable pain and lesion

Medical treatment (non-operative)

Not-tolerable pain, spreading infection

Amputation

Revascularization

Methods:
- Endovascular (PTA)
- Surgical

Tendera et al. Eur Heart J, 2011
- Approximately 40% of CLI patients are ineligible for revascularization.

- Six months outcome for “no-option” patients is even worse.
Patients who are not candidates for conventional revascularization

Novel means of revascularization

*Stem cell based therapy*
What are stem cells?

Unspecialized cells capable of:
- **Proliferation:**
  division and self renewal

- **Differentiation:**
  giving rise to cell types of the same tissue where they reside

- **Trans-differentiation or plasticity:**
  giving rise to cell types of a completely different tissue

http://stemcells.nih.gov/info/basics
Where can one find stem cells?

- **Embryo:**
  * Inner cell mass of the embryonal blastocyst
  * They give rise to the multiple specialized cell types that make up different organs

- **Adult:**
  1. Bone marrow (Sternum, Iliac bones, ribs, skull and spine)
  2. Fat (Adipose tissue)
  3. Peripheral blood
  4. Blood vessel
  5. Some organs (e.g., heart, liver, brain, skin, GIT)

* They generate replacement for cells lost through wear and tear, injury or diseases

- **Cord blood:**
  Banked for future need

*Zhang et al. Circulation, 2002*
Bone Marrow Mononuclear Stem Cells

http://stemcells.nih.gov/info/basics
Stem cell therapy in CLI

• **Hypothesis:** stem cells have been reported to contribute to neoangiogenesis
  
  *Burt et al. Bone Marrow transplantation, 2003*

• **Mechanism:** they provide endothelial progenitor cells (CD34+, CD133+) and angiogenic cytokines. Neoangiogenesis and collateral vessels formation

  *Tateishi et al. Lancet, 2002*
Clinical Applications

- Patients’ selection criteria
- Source of stem cells
- Cell population
- Optimal dose
- Routes of delivery of stem cells
- Clinical evaluation after cell delivery
- Single Vs multiple injections
- Function of injected cells
Patients’ selection

- TAO patients did better than ASO patients
  
  Sprengers et al. Eur J Vasc Endovasc Surg, 2010

- Patients with lower Rutherford grade did better than those with higher grades

- Patients with Rutherford category 6; gangrene, show no benefit at all

  Walter et al. Circ Cardiovas Interv, 2011
Sources of stem cells

Embryonic:

- Embryonic cells research is restricted to in vitro studies

Cord blood: Could be banked at birth and used later in life

Adult:

- Advantages:
  - No rejection
- Disadvantages:
  1. Only certain types of cells could be grown in labs (till now)
  2. Number of cells grown are relatively lower than embryonic ones

http://stemcells.nih.gov/info/basics
Sources of adult stem cells

- **Bone marrow**
  - Most frequent source used in clinical trials as
  - It’s easy to obtain
  - No complex purification steps
  - Contains variety of cells

- **Peripheral blood**
  - G-CSF mobilized cells

*Results have been disappointing and reported only limited success*

*Sprengers et al. Eur J Vasc Endovasc Surg, 2010*
Cell population

- Unpurified mononuclear cell population of bone marrow
  - Aspirated from the bone marrow
  - Mobilized from BM to PB after G-CSF injections

- Single isolated cell fractions: CD133+, CD34+

Optimal dose

No relation found between varying cell doses, or angiogenic cell fractions, and obtained clinical response

Sprengers et al. Eur J Vasc Endovasc Surg, 2010
Routes of cell delivery

• **Intra-muscular:**
  - Into the calf muscles of ischemic limbs

• **Intra-arterial:**
  - Distal SFA in pts with infra-popliteal disease
  - Deep femoral artery if additional fem-pop disease

• **Intra-venous:**
  - Pulmonary passage is a major obstacle for intravenous cell delivery

---


Walter et al. Circ Cardiovas Interv, 2011

Fischer et al. Stem Cells and Development. 2009
Clinical assessment of vascular status

• ABI alone is a poor predictor of vascular improvement


• Multiple measures should be combined

  Norgren et al. Eur J Vasc Endovasc Surg, 2007
Single Vs Multiple injections

• Single injection may have a limited duration of effect

• Multiple injections show better results (3 months at least between injections)

Walter et al. Circ Cardiovas Interv, 2011
Functionality of injected cells

- Stem cell proliferation and function are affected when:
  - Multiple risk factors
  - Increased severity of PAD
  - Older patients
  - Presence of chronic ischemic heart disease

Sprengers et al. Eur J Vasc Endovasc Surg, 2010
Role Of Autologus Bone Marrow Adult Stem Cell Transplantation In Neoangiogenesis And Lower Limb Salvage In Patients with Critical Limb Ischemia

By
Ghada Sayed Mahmoud Yousef; MSc

Under supervision of

Hussein Rizk, MD
Prof. of Cardiology, Cairo University

Mohamed Hosni, MD
Prof. of Vascular Surgery, Cairo University

Hala Gabr, MD
Prof. of Clinical Pathology, Cairo University

Waleed Ammar, MD
Lecturer of Cardiology, Cairo University

Cardiovascular Department
Cairo University
Aim of the work

- Use of BM-MNC in patients with CLI who are ineligible for conventional revascularization aiming at
  - improving the clinical status of the limb,
  - reducing ischemic rest pain,
  - inducing healing of ulcers and/or
  - delaying the decision for amputation
Study population

30 CLI patients, ineligible for revascularization

Group 1
(15 patients)
Active ttt group

Group 2
(15 patients)
Control group

Follow up
Methodology

Peripheral vascular disease assessment

- Ankle pressure
  * using a hand-held Doppler probe and a blood pressure cuff
  * CLI level: < 60 mmHg
- Ankle-Brachial index (ABI)
  * How to measure?
  * CLI level: ≤ 0.6
Methodology

Patients’ questionnaire
- At first presentation
And
- At follow up visits
Methodology

Bone marrow aspiration (Group 1)
- Patients admitted, received sedation
- Strict aseptic condition
- Aspiration site: posterior superior iliac spine
- Amount: 250 ml
**Methodology**

**BM-MNC separation:**
- Using centrifugation
- Cells are cultured for 48 hours
- Sterility check
- A concentrate volume of 40 ml of BM-MNC
- Total no. of cells, CD34+, CD133+, CD34+/CD133+ cells were counted and cell viability was checked
Methodology

**BM-MNC injection (Group 1)**

- Patients should take a prone position
- Calf muscles’ area was sterilized and marked for 40-50 injection sites
Methodology

**BM-MNC injection (Group 1)**

- Outpatient basis
- Using 25-gauge needle
- Needle pushed 1.5 cm deep into the calf muscles
- BM-MNC concentrate injected, 1 cm in each site
- Procedure: 20-30 minutes
Methodology

Follow up
- Monthly for 6 months, or till an endpoint is reached
- Clinical (questionnaire, size of the wound, level of gangrene) and ABI assessment

Primary endpoints
- Major amputation (above the ankle) of the index limb
- Persistent critical limb-threatening ischemia of the index limb after 3 months

Secondary endpoints
- Wound healing
- Improved Rutherford grade
- Improved quality of life
- Improved absolute ankle perfusion pressure/ABI
## PVD presentation

<table>
<thead>
<tr>
<th>Clinical</th>
<th>CLI presentation</th>
<th>Group 1 No. (%)</th>
<th>Group 2 No. (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest pain</td>
<td>3 (20.0)</td>
<td>2 (13.3)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Unhealed ulcer</td>
<td>3 (20.0)</td>
<td>4 (26.7)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Gangrene</td>
<td>9 (60.0)</td>
<td>9 (60.0)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Absent peripheral pulsations (index limb)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Femoral</td>
<td>6 (40.0)</td>
<td>7 (46.7)</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Popliteal</td>
<td>7 (46.7)</td>
<td>10 (66.7)</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>ATA and/or PTA</td>
<td>8 (53.3)</td>
<td>9 (60.0)</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Infection</td>
<td>7 (46.7)</td>
<td>6 (40.0)</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Median ankle perfusion pressure (range)</td>
<td>40 (26→60)</td>
<td>36 (20→50)</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Median ABI (range)</td>
<td>0.38 (0.20→0.50)</td>
<td>0.28 (0.20→0.45)</td>
<td>0.06</td>
</tr>
</tbody>
</table>
### Results

**Injected BM-MNC features**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of BM-MNC</td>
<td>67.1 ± 33.7 x 10^7</td>
</tr>
<tr>
<td>Viability of BM-MNC (%)</td>
<td>94.1 ± 2.7</td>
</tr>
<tr>
<td>CD34+ (%)</td>
<td>4.5 ± 1.0</td>
</tr>
<tr>
<td>CD133+ (%)</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>CD34+/CD133+ (%)</td>
<td>1.2 ± 0.2</td>
</tr>
</tbody>
</table>
Results

Fate of patients at the end of follow up period

<table>
<thead>
<tr>
<th>Fate of the patient</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improvement</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Continued CLI</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Amputation</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Death</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

P value = 0.7
P value = 1.0
### Results

**Subjective data at the end of follow up period**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 No. (%)</th>
<th>Group 2 No. (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mobility</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improved</td>
<td>3 (20.0%)</td>
<td>3 (20.0%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Deteriorated</td>
<td>10 (66.7%)</td>
<td>9 (60.0%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Median time to</td>
<td>120 (60→150)</td>
<td>120 (90→120)</td>
<td>0.8</td>
</tr>
<tr>
<td>improvement (range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Self-care</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improved</td>
<td>3 (20.0%)</td>
<td>3 (20.0%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Deteriorated</td>
<td>10 (66.7%)</td>
<td>9 (60.0%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Median time to</td>
<td>130 (30→140)</td>
<td>110 (90→120)</td>
<td>0.5</td>
</tr>
<tr>
<td>improvement (range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Usual activities</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improved</td>
<td>3 (20.0%)</td>
<td>3 (20.0%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Deteriorated</td>
<td>10 (66.7%)</td>
<td>9 (60.0%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Median time to</td>
<td>140 (50→150)</td>
<td>110 (80→120)</td>
<td>0.5</td>
</tr>
<tr>
<td>improvement (range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pain</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improved</td>
<td>2 (28.6%)</td>
<td>2 (25.0%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Deteriorated</td>
<td>4 (57.1%)</td>
<td>5 (62.5%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Median time to</td>
<td>65 (50→80)</td>
<td>75 (60→90)</td>
<td>0.4</td>
</tr>
<tr>
<td>improvement (range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Anxiety</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improved</td>
<td>3 (20.0%)</td>
<td>3 (20.0%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Deteriorated</td>
<td>10 (66.7%)</td>
<td>9 (60.0%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Median time to</td>
<td>30 (20→90)</td>
<td>80 (60→90)</td>
<td>0.4</td>
</tr>
<tr>
<td>improvement (range)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Results**

**Objective data at the end of follow up period**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 No. (%)</th>
<th>Group 2 No. (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Use of analgesics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased</td>
<td>2 (28.6%)</td>
<td>2 (25.0%)</td>
<td>1.0</td>
</tr>
<tr>
<td>The Same</td>
<td>1 (14.3%)</td>
<td>1 (12.5%)</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Median time to improvement (range)</strong></td>
<td>70 (60→80)</td>
<td>100 (90→110)</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Size of unhealed ulcer</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased</td>
<td>2 (66.7%)</td>
<td>1 (25.0%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Deteriorated</td>
<td>1 (33.3%)</td>
<td>1 (25.0%)</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Median time to improvement (range)</strong></td>
<td>100 (80→120)</td>
<td>120</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Level of gangrene</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The same</td>
<td>1 (11.1%)</td>
<td>3 (33.3%)</td>
<td>0.6</td>
</tr>
<tr>
<td>Deteriorated</td>
<td>8 (88.9%)</td>
<td>5 (55.6%)</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>Median Walking distance (range)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At the beginning of the study</td>
<td>50 (38→66)</td>
<td>58 (42→60)</td>
<td>0.8</td>
</tr>
<tr>
<td>At the end of the study</td>
<td>103 (98→112)</td>
<td>95 (84→104)</td>
<td>0.3</td>
</tr>
<tr>
<td>P value</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td><strong>Median ABI (range)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At the beginning of the study</td>
<td>0.38 (0.2→0.5)</td>
<td>0.28 (0.2-0.45)</td>
<td>0.06</td>
</tr>
<tr>
<td>At the end of the study</td>
<td>0.40 (0.20→0.60)</td>
<td>0.30 (0.23→0.48)</td>
<td>0.2</td>
</tr>
<tr>
<td>P value of the change of ABI</td>
<td>0.03</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td><strong>Median time to improvement (range)</strong></td>
<td>90 (80→150)</td>
<td>110 (90→150)</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Results

Number of BM-MNC injected

- Number of BM-MNC injected: 823.3
- P value is 0.9

<table>
<thead>
<tr>
<th>Fate of the limb</th>
<th>No. of Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improvement</td>
<td>823.3</td>
</tr>
<tr>
<td>Continuing CLI</td>
<td>610</td>
</tr>
<tr>
<td>Amputation</td>
<td>658.9</td>
</tr>
<tr>
<td>Death</td>
<td>586.7</td>
</tr>
</tbody>
</table>

R&M Solutions
www.rmsolutions.net
Results

Viability of injected BM-MNC

Viability of cells

Fate of the Limb

- Improvement: 93%
- Continuing CLI: 95%
- Amputation: 94%
- Death: 94.7%

P value is 0.8
**Results**

**Progenitor cells percentages**

![Bar chart showing mean percentages of CD34+ cells, CD133+ cells, and dual CD34+CD133+ cells for different stages of limb fate: improvement, continuing CLI, amputation, death.](chart)

- **Mean percentage of CD34+ cells**
  - Improvement: 5
  - Continuing CLI: 4
  - Amputation: 3
  - Death: 2

- **Mean percentage of CD133+ cells**
  - Improvement: 1
  - Continuing CLI: 1
  - Amputation: 1
  - Death: 1

- **Mean percentage of dual CD34+CD133+ cells**
  - Improvement: 2
  - Continuing CLI: 1
  - Amputation: 1
  - Death: 1

**P values**:
- P value (CD34+ cells): 0.4
- P value (CD133+ cells): 0.7
- P value (CD34+CD133+ cells): 0.1
Conclusion

• Bone marrow aspiration and intramuscular injection were safe, well tolerated procedures

• We experienced lower total no. of BM-MNC, yet higher % of CD34+ cells

• Most of the patients had a multi-level of vascular obstruction

• No statistically significant difference between the active treatment and control groups

The proposed therapeutic benefit of BM-MNC injection could not be demonstrated in our CLI patients
Why negative results?

1- Small no. of patients → Unreliable statistical results

2- Choice of patients → Higher Rutherford grades do not respond to therapy

3- Stem cell dose → Less than other studies

4- Functionality of injected cells → Dysfunctional because of clustering of atherosclerotic risk factors

5- Route of delivery → Poor survival of cells in ischemic media
6- Site of cell delivery  The calf muscles might not be the optimum site for BM-MNC injection in patients with more proximal vascular obstructions

7- Methods of assessment of limb ischemia  ABI alone is a poor predictor of vascular improvement

8- Presence of multiple risk factors  Reduce no. and functionality of cells

9- Publication bias  Positive results are easier to publish
Recommendations

- Apply therapy to thromboangitis obliterans patients younger and healthier

- Apply therapy to patients with lower Rutherford grade

- Multiple cell injections especially in patients with inadequate response after a single injection

- Combine measures for vascular assessment Ankle pressure, toe pressure, TcO2, microcirculation investigations and anatomic imaging

- Define optimal stem cell dose, type and route of delivery

- Larger no. of patients and longer follow up periods monitor effects and define complications
Take home message

• CLI is an advanced stage of PAD with grave prognosis

• About 40% of CLI patients are ineligible for traditional revascularization procedures

• BM-MNC therapy is emerging as a novel therapy for such patients

• BM-MNC injection is a safe and feasible procedure

• Study results are being inconsistent as many issues are still to be solved

• Larger randomized, placebo-controlled trials are needed to evaluate the angiogenic therapeutic potentials of BM stem cells
4.5.3.4 Stem cell and gene therapy for revascularization

The development of novel therapies to stimulate neovascularization, known as therapeutic angiogenesis, is based on the use of angiogenic factors or stem cells to promote revascularization and remodelling of collaterals with the aim of ameliorating symptoms and preventing amputation.

While several trials reported relief of ischaemic symptoms, functional symptoms, and hard endpoints (ulcer healing and amputation). Patients with thromboangiitis obliterans showed larger benefits than patients with atherosclerotic LEAD. The TAMARIS study is the largest randomized placebo-controlled trial of gene therapy in CLI, including >520 patients from 30 countries with CLI and skin lesions, unsuitable for standard revascularization. This study found no statistical difference between the two groups regarding the primary efficacy endpoint of death or first major amputation on the treated leg, whichever came first (37.0% vs. 33.2%, P =

At present angiogenic gene and stem cell therapy are still being investigated and it is too early to give firm recommendations.

for clinical repair trials, because it is easy to obtain and no complex purification steps are required. Another advantage is that it contains a variety of stem and progenitor cells with suggested superiority over one selected type of progenitor cell. With the many different cell types that can be used for stem cell therapy, it is not yet clear which ones are the most promising. In a recent meta-analysis of 37 trials, autologous cell therapy was effective in improving surrogate indexes of ischaemia, subjective
Thank You