Biomarker Discovery: Prognosis and Management of Chronic Diabetic Foot Ulcers

Joseph Colasurdo, BS
Dr. William M. Scholl College of Podiatric Medicine
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Disclosure of Conflicts of Interest

- No financial relationships to disclose
Learning Objectives

- Deliver insight into the proteomic, biochemical, and molecular mechanisms which instigate diabetic foot ulcerations
- Establish a foundation for the significance of translational research within the field of podiatric medicine
- Fashion a framework for further investigation into biomarkers for diabetic foot ulcerations
Diabetes, Chronic Wounds and U.S. Healthcare

- Cost of caring for chronic wounds exceeds $50 billion each year\(^1\)

- Over 29 million individuals in the U.S. suffer from Diabetes\(^2\)
  - \(~25\%\) of all diabetics will develop at least one foot ulcer in their lifetime
    - From these patients, 12\% will require a lower extremity amputation
    - 50\% 5-year survival rate after a lower extremity amputation
    - Cost of treating a single chronic wound \(\approx\) $70,000
Intact Skin

Phases of Acute Wound Healing

**Injury**
- Slough tissue
- Excess exudate

**Inflammation**
- Wound closure
- Scar tissue
- Granulation tissue
- ECM synthesis
- New blood vessel formation
- Re-epithelialization

**Migration – Proliferation**
- Slough tissue
- Excess exudate

**Remodeling**
- Wound closure
- Scar tissue
Phases of Chronic Wound Healing

Injury

Chronic Inflammation
- Slough tissue
- Excess exudate
The Complicated Story of Diabetic Foot Ulcers

- Trauma
- Biomechanical Abnormalities
- Soft tissue Infection
- Edema
- Motor Neuropathy

- Nutritional Status
- Neuropathic Osteoarthropathy (Charcot foot)
- Arterial disease
- Limited joint mobility
- Sensory Neuropathy

- Microvascular disease
- Allergens
- Hyperglycemia
- ???
- Autonomic Neuropathy

- Lifestyle
- Macrovascular disease
- Immunodeficiency
- Arterial Disease
- Bone Infection
### Cytokines in Acute Wound Healing

#### Cell Types

<table>
<thead>
<tr>
<th>Inflammation</th>
<th>Migration-Proliferation</th>
<th>Remodeling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets</td>
<td>Keratinocytes</td>
<td>Myofibroblasts</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Endothelial cells</td>
<td>Macrophages</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Fibroblasts</td>
<td></td>
</tr>
<tr>
<td>Macrophages</td>
<td>Macrophages</td>
<td></td>
</tr>
</tbody>
</table>

#### Cytokines

- TGF-beta
- TNF-alpha
- IL-1 beta
- IL-4
- IL-6
- MCP-1
- TGF-beta
- MMPs
- EGF
- KGF
- VEGF
- FGF
- FN
- MMPs
- TIMPs
Prior research has demonstrated that different cell types are present throughout different phases of wound healing.

We understand that each cell type produces specific cytokines.

Cytokine abundance within the wound environment can predict the prognosis of the wound.
**Aim**

- Prognosis of chronic foot ulcer healing through identification of biomarkers
- Use of prognostic cytokine biomarkers in the clinical treatment of chronic ulcers to better patient quality of life
## Methodology

<table>
<thead>
<tr>
<th><strong>Inclusion Criteria</strong></th>
<th><strong>Exclusion Criteria</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>• Diagnosed with Diabetes Mellitus</td>
<td>• Unable to provide informed consent</td>
</tr>
<tr>
<td>• Chronic diabetic foot ulcer (&gt;4 weeks) with an area &gt;1cm²</td>
<td>• Active Neuropathic Arthropathy</td>
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<tr>
<td></td>
<td>• Soft tissue infection</td>
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<tr>
<td></td>
<td>• Untreated Osteomyelitis</td>
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</tbody>
</table>
Patient/Wound Overview

- 36 wounds from 29 patients
- Average Age 59 (30-73)
- 82% Male, 18% Female
- Ulcer locations: Sub 1\textsuperscript{st}, 2\textsuperscript{nd} and 5\textsuperscript{th} metatarsal heads, plantar aspect of the calcaneus, Chopart’s amputation, anterior aspect of the tibia, distal to the medial malleolus
Methodology

1. **Diabetic Foot Ulcer**
2. **Debridement**
3. **Centrifugation**
4. **EDTA Coated Test Tube**
5. **Immunodepletion**
6. **In-solution Digestion**
7. **UPLC Separation**
8. **Processing at the Midwest Proteome Center**
9. **Serum**
10. **Luminex -18 Cytokines Analysis**
11. **Mass Spectrometer**
12. **Data Analysis**

**Experimental Workflow Scheme**
Luminex 18 Cytokine Analysis

- Performed at the Cytoplex Multiplex-Core Facility, Department of Obstetrics and Gynecology at Yale Medical Center

- Flow cytometry analysis
  1. Polystyrene microspore bead with capture antibody
  2. Capture antibody binds analyte
  3. Fluorescence labeled reported antibody binds to captured analyte
  4. Bead identification and reporter quantity analyzed by laser detection
Luminex Results

Cytokines analyzed:
- GROa
- IL-1b
- IL-6
- IL-8
- IL-10
- IL-12
- IL-17
- G-CSF
- GM-CSF
- IFN-g
- MCP-1 (MCAF)
- MIP-1a
- MIP-1b
- RANTES
- TNF-a
- VEGF
Luminex Cytokine Analysis
Increased levels of the following cytokines in healing ulcerations

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Function</th>
</tr>
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<tr>
<td>IL-6</td>
<td>Secreted by macrophages and T-cells to stimulate immune response</td>
</tr>
<tr>
<td>IL-8</td>
<td>Induces chemotaxis and promotes angiogenesis</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Induces chemotaxis of stem cells for production of RBCs, WBCs, platelets</td>
</tr>
<tr>
<td>GROa</td>
<td>Secreted by melanoma cells. Mitogenic properties; neutrophil chemotaxis, and mediator of angiogenesis</td>
</tr>
</tbody>
</table>
UPLC-MS Analysis

- Performed at the Midwest Proteome Center, Rosalind Franklin University of Medicine and Science
# UPLC-MS Proteomic Analysis
Quantitative differences of proteins in healing versus non-healing wounds

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCL7</td>
<td>Chemokine (C-X-C motif) ligand 7</td>
<td>Stimulates mitogenesis and glucose metabolism, and synthesis of extracellular matrix and plasminogen activator</td>
</tr>
<tr>
<td>GDIR2</td>
<td>Rho GDP-dissociation inhibitor 2</td>
<td>Regulates the GDP/GTP exchange reaction and reorganization of the actin cytoskeleton</td>
</tr>
<tr>
<td>ALDOA</td>
<td>Fructose-bisphosphat aldolase A</td>
<td>Glycolysis and gluconeogenesis Scaffolding protein</td>
</tr>
<tr>
<td>FRIL</td>
<td>Ferritin light chain</td>
<td>Iron homeostasis</td>
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Study Limitations

- Low patient enrollment
- Limited resources prevented inclusion of additional, potentially important, parameters (ex. pH of the wound, HbA1c, patient’s Rx involvement)
Future Work

- Inclusion of chronic wounds of various etiologies
- Longitudinal study following patients over time
- Biomarker identification and quantification to alter treatment modalities
- Biomarker incorporation into pharmaceutical treatment options
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Questions