The Development of Diagnostics in Wound Care
Proteases and Chronic Wound Healing; Moving Beyond Clinical Inertia

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Learning Objectives

• Explain the role of proteases in normal and abnormal wound healing.

• Explore the implications of the lack of visual cues in formulating treatment decisions

• Learn how Point of Care diagnostics could improve the care of patients with chronic and stalled wounds.
“Think like an internist, act like a surgeon” Wm. Ennis, DO

- Wound management often requires a subtle balance between medical and surgical interventions
Sometimes Clinicians Become Confused
Core Healing Principles

- Patient factors
- Physical aspects
- MACROscopic environment
- MICROscopic environment
Wound Specialists Have to Be Medical Detectives
Wound Etiology

- Mechanical
- Arterial
- Venous
- Neuropathic
- Malignancy
- Vasculitic
- Other

All chronic wounds share a common biochemistry

Courtesy of Sharon Baranoski, RN.
Proposed Mechanisms for Chronicity in Diabetic Foot Ulcer

Robert Kirsner, MD

- Unresponsive and/or Senescent Cells
- Deficient and/or unavailable growth factors/receptor sites
- Proteolytic/Inflammatory environment
- Bacterial interference
- Non migratory, hyperproliferative edge epithelium
- MMP’s and Elastase
Matrix Metalloproteases (MMPs)

A Family of Protein-Degrading Enzymes

- 20 structurally related members
- Need calcium and zinc ions for proper shape
- Made by every cell in the wound
- Collectively, can degrade all components of the extracellular matrix
- Normally controlled at the tissue level by TIMPs (Tissue Inhibitors of Metalloproteases)

Proteases in Wound Healing

- **Matrix Metalloproteinases (MMPs)** are a family of enzymes (collagenases, gelatinases, elastase, etc.) that degrade proteins.

- MMPs play a physiological role in tissue remodeling and clearing of tissue debris during normal wound healing.

Role of Proteases in Wound Healing

Excessive Proteases in Chronic Wounds

- Numerous studies have found elevated levels of MMPs in chronic wounds:

<table>
<thead>
<tr>
<th>Venous Leg Ulcers</th>
<th>Diabetic Foot Ulcers</th>
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Infection Contributes to Various Complications Including Amputation

- Risk factors for infection:
  - Wounds that penetrate to the bone
  - Wounds with a duration >30 days
  - Recurrent foot wounds
  - Wounds with a traumatic etiology
  - Peripheral vascular disease

Infection plays a role in about 60% of the DFU cases that result in amputation

Secondary Signs and Symptoms
  - Pain
  - Foul odor
  - Deterioration of the Wound
  - 20% show no clinical signs

DFU = diabetic foot ulcer.
Using Visual Assessment

The Problem:

• Clinicians tend to treat inflammation with antibiotics when potentially no infection is present

• Clinicians fail to treat infection when it is present due to a lack of visual cues

• A Point of Care Diagnostic Test could help clinicians formulate a treatment plan

Stremitzer et al (2007) investigated the variety in judgments of clinicians when evaluating chronic diabetic foot wounds.

- Study data showed an extensive heterogeneity and a wide spread of results, like in no other field of medical diagnostics.
- In practice this can lead to suboptimal therapy and may translate into prolonged treatment and burden for the patient.

570 US wound care specialists recently were surveyed to determine their perceptions on wound healing:

- The specialists polled overwhelmingly agreed (81.6%/n = 465) that point of care diagnostic tests would be helpful or useful.

- Consensus panels of key opinion leaders globally (i.e. South Africa, US, Canada, UK, and Italy) agreed with this contention.


In a recent survey of more than 165 US wound care specialists:

- Overwhelmingly agreed that proteases were important in wound healing and that a POC diagnostic test to evaluate them would be important (> 90%)
- Specialists were asked to review several cases to ascertain elevated protease levels
- Preliminary data revealed that on average, >40% were unable to appropriately determine that protease levels were elevated
- Raw data indicated that many specialists reviewed the email survey but elected not to respond; several stated that they were not comfortable or did not feel qualified to answer questions about proteases
- This occurred despite the fact that more is currently known about proteases than any other biochemical marker involved in wound healing

Advanced therapies are often prescribed as “last resorts” because there is presently no qualitative or quantitative pathway to justify their usage

- Treatment choices are often based upon intuition
- The lack of objective biochemical and physiologic landmarks that can be used to assess wound status are a major hurdle in the progression toward improved treatment regimes (Yager et al 2007)
- Point of care (POC) tests are important
- POC tests have been successfully integrated into the medical mainstream especially in oncology and cardiology

Bacterial Burden, Inflammation or Infection?
“We are now entering an era in which therapies can be tailored to take advantage of (subtle) differences (in disease states), thus providing hope that this will translate into meaningful improvements in patient outcomes”

Robert Dreicer  
MD, Cleveland Clinic

Cleveland Clinic Newsletter. 2011.
Factors Affecting the Normal Healing Process

Damage to Tissue

- Angiogenesis
- Hemostasis
- Epithelialization
- Granulation tissue formation
- Remodeling

Healing/Repair ???
The Problem Behind Chronic Wounds

High Levels

- Proteases MMPs
- Bioburden/Infection
- Inflammatory Cytokines

Low Levels

- Growth Factors
- Cell Proliferation
What Causes Delayed Healing?

Cells produce excess Proteases

Degradation of ECM and growth factors

Bacterial Proteases and toxins

Increased Inflammatory response

Passive and Active Dressings

• **Passive Dressings:** Covers the wound, creates a barrier, allows exchange of gases and liquids, absorbs fluid, may create a moist healing environment.

• **Active Dressings:** Promotes a favourable micro-environment to stimulate healing. Often more effective and economical over the whole course of care.
History of Foot Ulcer Increases among Individuals with Diabetes

Ten Year Follow-up of the Nord-Trondelag Health Study, Norway

- A large population based study examined the association between foot ulcers in patients with diabetes and mortality risk while controlling for disease factors
- Foot ulcers were independently associated with increased mortality risk
  - Patients with diabetes and a foot ulcer had an increased mortality risk of 2.3-fold (229%) compared to non-diabetic subjects
  - In patients with diabetes, presence of a foot ulcer alone increased mortality risk by 47%

<table>
<thead>
<tr>
<th>Population</th>
<th>Mortality Rate (%)</th>
<th>Hazard Risk</th>
<th>Hazard Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Db+HFU vs Non-Db</td>
<td>Db+HFU vs Db-HFU</td>
</tr>
<tr>
<td>Non-Db (N = 63,632)</td>
<td>10.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Db+HFU (N = 155)</td>
<td>49.0</td>
<td>229</td>
<td>1.47</td>
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<tr>
<td></td>
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<td>[95% CI 1.82-2.88]</td>
<td>[95% CI 1.14-1.89]</td>
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<tr>
<td>Db-HFU (N = 1339)</td>
<td>35.2</td>
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<td></td>
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</tbody>
</table>

Db = Diabetic; HFU = History of foot ulcer.
Hazard risk calculated with compression analyses, adjusted for chemographic and lifestyle factors.
Just Having a Neuropathic Foot Ulcer Is a Marker for Death!

Treatment Approach

Which is an emergency?  Which has the highest mortality rate?

A Paradigm Shift in Wound Management Protocols:

Understanding the wound micro-environment may lead to better choices

Snyder RJ. 2005. A holistic approach to understanding and addressing the wound microenvironment to facilitate healing. WOUNDS. Supplement: S12-S17
Standard of Care?

- Treatments are often chosen on the bases of local traditions.
- Wet-to-dry gauze dressings are the most widely used primary dressing material in the United States and may be erroneously considered a standard of care.
- In a review of the literature, Ovington notes that impeded healing due to local tissue cooling, disruption of angiogenesis by dressing removal, and increased infection risk from frequent dressing changes, strike through, and prolonged inflammation represent good reasons to abandon this traditional dressing technique.

Healing Neuropathic Ulcers: Results of a Meta-Analysis

Weighted Mean Healing Rates

- These data provide clinicians with a realistic assessment of their chances of healing neuropathic ulcers.
- Even with good, standard wound care, healing neuropathic ulcers in patients with diabetes continues to be a challenge.

Continuing Research: Healing of Diabetic Foot Ulcers after 4 Weeks

Percentage of Patients in Whom Ulcers Healed During the 12-Week Period

- Wounds achieving less than 53% closure at week 4 have minimal chance of healing with conventional therapy

A Post-hoc Analysis of Reduction in Diabetic Foot Ulcer Size at 4 Weeks as a Predictor of Healing by 12 Weeks

Robert J. Snyder, DPM, CWS; Matthew Cardinal, ME; Damien M. Dauphinée, DPM, FACFAS, CWS; and James Stanocky, DPM

Abstract:
Percent area reduction (PAR) after 4 weeks of diabetic foot ulcer (DFU) treatment has been suggested as a clinical monitoring parameter to distinguish DFUs that will heal within 12 weeks from those that will not despite standard wound care. The purpose of this post-hoc analysis of control DFUs treated outcomes from two published, randomized, controlled studies was to assess the relationship between PAR during early standard wound care and ulcers closure by week 12. The proportion of DFUs treated after 12 weeks were: 42% were PAR of 0-49%, 41% were PAR of 50-99%, 16% were PAR of 100%.

Key Words: post-hoc analysis, diabetic foot ulcer, wound assessment, wound measurement, outcome predictor

Index: Ostomy Wound Management 2016;56(3):44-50

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Diabetic foot ulcers (DFUs) are among the most common complications of diabetes mellitus, with an annual incidence of 1% to 4% and The incidence of DFUs in patients with diabetes mellitus is estimated to be 1%. The morbidity associated with DFUs is high — 40% of patients suffering from DFUs develop amputations. The mortality of patients suffering from DFUs is also high — approximately 10% of patients with DFUs die within 5 years of diagnosis.

In a post-hoc analysis of the control groups from two DFU treatment studies showed that with standard good wound care 24% of ulcers healed after 12 weeks of care and 31% after 26 weeks of care. It was also found that even with good standard wound care, DFUs remain difficult to heal. Proprietary indicators of wound closure can assist in selecting patients who might benefit from more intensive treatment approaches. Further research is needed to determine the potential role of other wound care practices in the treatment of DFUs.
Association Between PAR at Week 4 & DFU Closure at Week 12

Data was dichotomized by PAR of <50% or ≥50% by week 4 to assess the association of PAR with DFU closure by 12 weeks.
For years the wound care community has clamored for a “LITMUS TEST” that would aid in the diagnosis and treatment of chronic wounds!

Consensus Opinion

“The development of specific diagnostic tests for use in wounds has the potential to revolutionize their treatment…and help improve standards of wound care (while) aiding in the cost effective use of limited resources”

Diagnostics versus Theranostics

Diagnostic tools

- Provide information that requires little or no interpretation and clearly indicates the diagnosis (usually without reference to other sources)
- A classic example of a diagnostic tool is the home-based pregnancy test, which uses detection of human chorionic gonadotropin (hCG) in urine to confirm pregnancy

Theranostic Tools

- Do not necessarily provide or lead to a diagnosis, but provide very specific information that indicates the need for (or unsuitability of) a particular therapy
- Of particular interest to payers/funders of healthcare because they have the potential to ensure that treatments are targeted (early) specifically at the patients who will benefit most from them

Diagnostic Tools

- In patients with wounds, new diagnostic tools are most likely to be used to detect:

  - Substances (such as molecules involved in healing)
  - Biological factors (such as infecting micro-organisms)

These tests often take time to process therefore clinicians are relegated to treating empirically

- White cell count (infection)
- Erythrocyte sedimentation rate (ESR) (inflammation, infection)
- Wound histology and cytology (vasculitis, malignancy)
The Ideal Diagnostic Tool

To maximize their usefulness and potential for improving the treatment of wounds, new diagnostic tools must be:

- Clinically relevant
- Appropriate for use by clinicians with different skill sets, from the specialist physician to the community nurse or, in some instances, perhaps the patient/care-giver
- Accurate, reliable, sensitive to change and with reproducible results in normal use, unaffected by other substances present in the test sample, and easily correlated with reference test methods
- Quick and easy to use
- Easy to interpret – ie capable of providing unambiguous guidance on the significance of there result and the need to initiate or continue treatment
- Cost-effective – e.g. reduce time taken to heal, staff time, hospital or clinic costs and/or need for interventions
- Produced and disposed of after use in ways that minimize environmental impact.

The Ideal Diagnostic Tool

• Ideally, these diagnostic tools will also:
  – Measure a single marker or molecule (or a set of markers) and present a single result
  – Predict healing
  – Clearly indicate the need for (or inappropriateness of) a specific therapy
  – Be non-invasive and make use of a consistent (non-variable) sample that is easy to collect, permits repeat testing if required and requires no (or minimal) sample preparation
  – Be used at the point of care (ie near the patient rather than in a laboratory)
  – Be sufficiently cost-effective to encourage widespread adoption into everyday practice
  – Be self-contained and not require expensive dedicated hardware
Potential Markers

- Bacterial load/specific microbial species/biofilms
- Cytokine release in response to specific microbial antigens
- DNA – e.g. gene polymorphisms to indicate susceptibility to disease, poor healing or infection
- Immuno-histochemical markers – e.g. integrins, chemokine receptors and transforming growth factor, beta II receptors to monitor healing
- Inflammatory mediators – e.g. cytokines and interleukins to monitor healing status and guide use of anti-inflammatory treatments
- Exposed bone
- Growth factors and hormones – e.g. platelet-derived growth factor (PDGF), sex steroids (androgens/estrogens), thyroid hormones
- Nitric oxide
- Nutritional factors – e.g. zinc, glutamine, vitamins
- pH of wound fluid
- Reactive oxygen species
- Temperature
- Trans-epidermal water loss from periwound skin

Most is known about enzymes and their substrates – e.g.: matrix metalloproteinases and extracellular matrix – e.g. platelet-derived growth factor (PDGF), sex steroids (androgens/estrogens), thyroid hormones
Potential Diagnostic Based on Enzyme Activity

• The research suggests that wounds that heal well have a lower overall Protease activity than those that heal poorly

• As a result, a test that readily provides this information might be clinically useful in predicting how well a wound is likely to heal and whether an intervention that modifies net Protease activity may be useful

Current Treatment Plan

1. Patient Presents with a Wound
2. Medical History / Wound History / Physical Examination
3. Assessment
   - Specific Tests (APBI / Doppler)
   - Blood Tests / Microbiological Tests
   - Imaging / Wound Size
4. Diagnosis Aetiology / Co-Morbidities / Wound Status
5. Management Plan / Therapy Selection
6. Review
How Best Can We Use Existing Therapies?

**Antimicrobial Products**
- Infection status
- Bacterial bioburden
- Species specific
- Resistance
- Host response
- Biofilm presence

**Infection Diagnostics**
When to use & when not to

**Active Wound Healing Products**
- Proteases,
  - Protease / Inhibitor
- Cell receptor markers
- Urinary nitrate / nitrates
- Cortisol
- T-lymphocytes
- Fibronectin
- Fibrin deg. Products

**Status Markers**
Right wound, Right time

**Preventive Measures**
- Genetic markers
- Genetic polymorphisms
- Neuropathy
- Pressure damage

**Prognostic Markers**
Potential Markers for Chronic Wounds

**Infection**
- Bacterial load
- Microbial species
- Biofilms
- Host response
- Bacterial resistance

**Healing**
- Proteases
- ECM proteins
- Inflammatory mediators
- Nitric oxide
- Growth factors

**Physical / Chemical**
- Temperature
- pH
- Size
- Pressure
- Colour
- Smell

**Preventive Measures**
- Genetic markers
- Genetic polymorphisms
- Neuropathy

New Treatment Plan

1. **Patient Presents with a Wound**
2. **Medical History / Wound History / Physical Examination**
3. **Assessment & Diagnostic Test**
   - Specific Tests (APBI / Doppler)
   - Blood Tests
   - Imaging / Wound Size
4. **Diagnosis Aetiology / Co-Morbidities / Sub-Categorize Wound**
5. **Management Plan / Targeted Therapy Selection**
6. **Review Progress / If Unimproved / Deteriorated – Retest**
After much research, wound specialists are on the cusp of having the first **Inflammatory Protease Test** consistent with the aims and ideals of the World Union of Wound Healing Societies’ Consensus document for use in clinical practice.

The results could adjunctively help lead clinicians to an appropriate algorithm of care including the use of wound therapies *early* in the treatment regime.

Results may also foster the use of sequential therapy in the treatment of chronic/stalled wounds.

The test has the potential to create a paradigm shift in the diagnosis and subsequent treatment chronic wounds.
Abscessed Foot in a Neuropathic Patient with Diabetes: A Stepwise Approach

Could this have been avoided with early intervention and diagnostic tools that could aid in expediting the utilization of targeted therapies?
Conclusions

- Proteases are important for wound healing however at elevated levels can create an imbalance in wound biochemistry leading to a chronic wound.

- Protease modulating therapies can aid in ‘rebalancing’ the wound biochemistry.

- Visual cues are not available to help the clinician in making a diagnosis.

- Point of Care Diagnostic tests can aid the clinician in choosing appropriate and targeted therapies early in the treatment regime for the right patient, the right wound, at the right time.
What We Are Trying to Prevent
At the End of the Day

It’s all about the patient
Welcome to 21st Century Wound Care!
Wound Diagnostics

Thomas E. Serena
MD FACS FACHM MAPWCA
Current Treatment Plan

Patient Presents with a Wound

Medical History / Wound History / Physical Examination

Assessment
- Specific Tests (APBI / Doppler)
- Blood Tests / Microbiological Tests
- Imaging / Wound Size

Diagnosis Aetiology / Co-Morbidities / Wound Status

Management Plan / Therapy Selection

Review
Multi-Center Clinical Trials
for Diagnostic Test in Development

St. Vincent’s Health Center, Erie, PA
Armstrong County Memorial Hospital, Kittanning, PA
Miami Valley Medical Center, Dayton, Ohio
Orangeburg Regional Medical Center, Orangeburg, SC.
Can you predict the level of protease activity based on clinical examination?
63 yr old with a Venous Leg ulcer of 6 months duration. Low level of exudate.
Pressure Ulcers

Elevated or Low
Pressure Ulcers with Tunneling

Elevated or Low
Venous Leg Ulcer
(Same Patient)
Diabetic Foot Ulcers