ELEVATED LEVELS OF MATRIX METALLOPROTEINASES AND CHRONIC WOUND HEALING: AN UPDATED REVIEW OF CLINICAL EVIDENCE

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Normal wound healing is organized into sequential and overlapping processes: Hemostasis/inflammation, granulation, epidermization, and tissue remodeling.

Matrix MetalloProteinases (MMPs) are present during all of these processes. First discovered for their role in the degradation of extracellular proteins, they are known to ensure much various functions.
Protease levels are increased:
- with age [Ashcroft 97, Herrick 07]

[Background – MMP Expression & Specific Conditions]

- [Aschroft 97]

- [MMP Expression & Specific Conditions]

- [20 to 39-year-old patients]

- [60 to 96-year-old patients]
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- with chronic venous insufficiency [Beidler 08, Saito 01]
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This imbalance may lead to a pro-ulcer forming environment [Saito 01, Dinh 12]
BACKGROUND – DETECTION OF MMPs

- Clinical signs do not always predict the presence of high protease activity [International consensus 11]
  → No visual cues

- Several advanced techniques are used to analyze levels, types and activities of MMPs in wound fluid and in wound biopsy:
  - *In situ* hybridization, Q.RT-PCR
  - ELISA
  - Zymography
  - Fluorimetric assays
  - WoundChek Protease Status

- The different biological samples assessed bring complementary information, not equivalent information
  
<table>
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<tr>
<th>Wound fluids</th>
<th>Wound biopsies</th>
<th>Blood samples</th>
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This review is focusing on the following issues:

1. Do chronic wounds present higher levels of MMPs than acute wounds?
2. Are the higher MMP levels observed in chronic wounds really correlated with delayed wound healing time course?
3. Is there such a thing as a MMP threshold?
4. How could MMP-related indicators be used as wound healing prognosis?
5. Considered individually, are all chronic wounds concerned by an elevated level of MMPs? What proportion of patients is concerned?
**Question 1. Do chronic wounds present higher levels of MMPs than acute wounds?**

- YES! On average, **MMP levels in chronic wounds are higher** than those recorded at any time in normally healing acute wounds.

- **Twenty one clinical trials**
  - Chronic wounds of various etiologies: VLUs, PUs, DFUs, arteriosclerotic ulcers, dehiscent surgical wounds (spinal, abdominal, back), chronicized traumatic war wounds, rheumatoid ulcers, vasculitis wounds and pyoderma gangrenosum
  - versus “surgical” wounds: healthy volunteers biopsies, mastectomies, abdominoplasties, seborrhoic wart ablations, donor sites of skin grafting ...

Wysocki 93, Bullen 95, Yager 96, Wecroth 96, Vaalamo 96, Vaalamo 97, Herrick 97, Grinnel 98, Barone 98, Nwomeh 99, Trengove 99, Wysocki 99, Tarlton 99, Saito 01, Lobmann 02, Pirila 07, Beidler 08, Rayment 08, Wiegand 10, Serra 13 and Krisp 13
**Question 1. Do chronic wounds present higher levels of MMPs than acute wounds?**

- The increased level of MMP activity seems to be due to a *combination* of an *increased expression of MMPs*:
  - Gelatinases MMP-2 and MMP-9
  - Collagenases MMP-1, MMP-8 and MMP-13
  - Stromelysin MMP-3 [Vaalamo 96, Beidler 08]
  - Matrilysin-2 (MMP-26) [Pirila 07]

  and a possible down-regulation of their inhibitors: TIMP-1 §, TIMP-2 [Lobmann 02]

- Other proteases, like serine proteases, are also implicated in the extracellular matrix degradation (*e.g.* neutrophil elastase) [Tarlton 99, Wiegand 10]

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¥ [Yager 96, Grinnel 98, Weckroth 96, Vaalamo 96, Vaalamo 97, Barone 98, Nwomeh 99, Lobmann 02, Beidler 08, Pirila 07, Wiegand 10 and Krisp 13]

§ [Bullen 95, Vaalamo 1996, Yager 96, Nwomeh 99]
**Question 2. Are the higher MMP levels observed in chronic wounds really correlated with delayed wound healing time course?**

- Yes, high levels of MMPs correlate with delayed wound healing

- **Thirteen clinical trials**
  - VLUs [Wysocki 99, Trengove 99, Tarlton 99, Beidler 08, Rayment 08, Serra 13]
  - PUs [Ladwig 02]
  - DFUs [Liu 09/Muller 09, Dinh 12, Li 13]
  - Dermal graft integration [Izzo 14]
  - Traumatic war wounds [Utz 10]
Question 2. Are the higher MMP levels observed in chronic wounds really correlated with delayed wound healing time course?

- In well-defined studied groups of wounds, treated according good standards of care, MMPs negatively correlate with the wound healing [Li 13, Liu 09, Beidler 08]
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  - The more severe the prognosis of the wound, the highest the MMP levels, and inversely [Singh 14, Menghini 13, Rayment 08, Beidler 08]
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  - Even in wounds with less severe prognosis or in wounds considered “improving”, “high healing ulcers”, or “good healers”, the MMP levels are **still higher** than in acute wounds [Serra 13, Beidler 08, Rayment 08, Tarlton 99]
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  - Elevated protease levels decrease during wound healing [Serra 13, Beidler 08]
QUESTION 3. IS THERE SUCH A THING AS A MMP THRESHOLD?

• Only three trials have proposed an MMP threshold (ROC Analysis)
  - In specific cohort of patients with DFUs
  - Different “best threshold” in each study
  - A combination of markers (MMPs + TIMPs +/- TGF-β)
  - No analysis on the cost of decisions, nor real-life application yet

• Not enough clinical evidence for a global and universal threshold with acceptable specificity and sensibility, discerning for all kind of wounds or for heterogeneous groups of wounds of various etiologies
  - Still numerous unanswered questions [International consensus 11]
QUESTION 4. HOW COULD MMP-RELATED INDICATORS BE USED IN WOUND HEALING PROGNOSIS?

- With caution!
  - Different MMPs:
    - Different roles during the wound healing process,
    - Expressed by ≠ cells, in ≠ locations, at ≠ levels, at ≠ times and for ≠ durations.
    - Affected by various finely-shaded levels of regulation.
  
- Chronic wounds have different etiologies.
  - Different cellular and biochemical mechanisms lead to MMP dysregulation [Ren 14]
  - Possible various types or intensities of protease imbalance [Trengove 99, Menghini 13 and Edsberg 14]

- Any strong correlation established between MMP and wound healing has required specific markers in well-defined cohort of patients

- Any generalization or adaptation of these correlations would make the conclusions unreliable.
On average, MMP levels in chronic wounds are higher than those recorded at any time in healing acute wounds [Nwomeh 99, Tarlton 99]

Nonetheless, as numerous other biological markers, MMP levels present an important inter-individual heterogeneity [Weckroth 96, Trengove 99, Rayment 08, Krisp 13, Nwomeh 99, Lobman 02 and Wiegand 10]
QUESTION 5. CONSIDERED INDIVIDUALLY, DO ALL CHRONIC WOUNDS HAVE AN ELEVATED MMP LEVEL? IN WHAT PROPORTION OF PATIENTS IS THIS THE CASE?

- The MMP level heterogeneity may be due to
  1. Different wound etiologies [Ren 14, Trengove 99, Menghini 13, Edsberg 14]
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  2. Different sub-groups of wounds due to specific characteristics of the patient, the wound or the treatment \[\text{Marchesi 12, Rayment 08, Beidler 08, Fisher 06, Saito 01, Aschroft 97, Serra 14, Singh 14}\]
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-1562 C>T polymorphism within promoter region of MMP9
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  4. Different studied markers (MMP species) and methods of detection [Weckroth 96, Kriskova 11]
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4. **Different studied markers (MMP species) and methods of detection** [Weckroth 96, Kriskova 11]

5. **Different phases of the wound healing process** [Serra 13, Wiegand 10, Ladwig 02, Wysocki 99, Tarlton 99]
Some sub-group of patients have higher MMP levels:

- Ischemic DFU \cite{Mengini13},
- Wounds with a PUSH score $\geq 12$ \cite{Rayment08},
- Bad VLU responders under compression \cite{Beidler08},
- Patients with hypertension \cite{Marchesi12}, elderly patients \cite{Ashcroft97},
- And, as healed ulcers still present higher MMP levels than acute wounds, it could be hypothesized that recurrent VLUs may also exhibit high levels of MMPs \cite{Saito01}.

All those patients should be seriously considered in priority for possible treatment of their MMP issue.
Is it still possible to improve the wound healing course in chronic wounds?

* Despite of good standards of care (compression, offloading, debridement...)

4. Liu 2009, Dinh 2012, Beidler 2008 in DFU, in VLU the % of wound healing could be higher (60-75%).
5. Gibson 2013 – WoundChek Protease Status Poster Harrogate
MODULATING PROTEASE DRESSINGS, THE ANSWER?

• In a recent review on dressings improving venous leg ulcer healing, Raffetto has pointed out that “therapies directed at modulating MMPs may have promise in ulcer healing”

• No exhaustive list of these modulating protease treatments published to date.
  - UrgoStart : TLC-NOSF (Technology LipidoColloid – NanoOligoSaccharide Factor)
  - Promogran : ORC/Collagen (Oxidised Regenerated Cellulose/Collagen)
  - ...

• Selection of best treatments need to be based on clinical evidence of high level
  - Numerous published RCTs have assessed the efficacy of protease modulating treatments in chronic wounds: Meaume 2012, Schmutz 2008, Smeets 2008, Lazaro 2007, Vin 2003, Veves 2002, ...
  - A Cochrane review on the use of protease-modulating dressings in VLUs is currently underway

7 Key Take-Home Messages

1. Groups at risk: Certain sub-group of patients present higher MMP levels: elderly, patients with CVI, diabetes, hypertension ...

2. Chronic wounds & high MMP level: In chronic wounds, MMP levels are, on average, higher than those recorded at any time in normal healing acute wounds

3. MMP & delay wound healing: In well-defined cohorts of patients treated according good standards of care, MMPs negatively correlate with the clinical course of wound healing

4. No consensus threshold: Not enough clinical evidence for a global and universal threshold, with acceptable specificity and sensibility, discerning for all kind of wounds

5. Groups with highest MMP levels: Some patients should be considered seriously for possible MMP issue treatment in priority: Ischemic DFUs, wounds with a PUSH score ≥12, bad VLU responders under compression and possibly recurrent VLUs, patients with hypertension, elderly patients ...

6. Prevalence of wounds with high MMP levels: It depends on what we consider an optimal healing rate. It could be evaluated between 50% to 80%, almost 100% if we assume that we can still optimize the healing course of what are, today, considered “good healers”

7. The best treatment: At the end of the day, to treat a wound with protease issues, we still have to choose the best protease modulating treatment, based on published clinical evidence of high level
THANKS FOR YOUR ATTENTION