Protase Activity Levels Associated with Healing Status of Chronic Wounds

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Background

❖ It is widely accepted that an elevated level of protease activity (EPA) in chronic wounds impedes healing.

❖ However, there has been little progress in quantifying an actual level of protease activity that is detrimental to wound healing.

❖ The aim of this study was to determine the relationship between inflammatory protease activities and wound healing status.

❖ Through clinical studies we have established the level of inflammatory protease activity (EPA) above which matrix metalloproteinases (MMPs) and elastase activities correlate with non-healing chronic wounds.

❖ This is the first time that a level of protease activity has been published which shows that above which the wound status is considered to be non-healing.
Objectives

- To define elevated protease activity (EPA) associated with non healing chronic wounds
- To show the correlation between EPA and healing status of chronic wounds
- To establish an optimal sample collection technique for prospective tests for EPA
The study was carried out across 4 wound healing centres in the USA. Wound swabs were taken from chronic wounds including diabetic foot ulcers, pressure ulcers and venous leg ulcers. The swabs were frozen and sent to an in-house laboratory for measurement of inflammatory protease activity. Human neutrophil elastase and MMP activity were measured using fluorogenic substrates, using a “for research only” method. The healing trajectory of the wounds was retrospectively calculated according to the percentage reduction in wound area over the previous 2-4 weeks. Wounds were defined as healing if the wound area reduced by at least 50% for diabetic foot ulcers or 30% for both pressure ulcers and venous leg ulcers over 2-4 weeks. Any wounds not meeting this percentage reduction in wound area were classed as non-healing.

**Methods**

- **Pre-validation work to define EPA**
  - 153 Swabs collected
  - 93 of these had healing data

- **Validation**
  - 82 valid Swabs collected
  - 69 of these had healing data

- 235 Swabs collected
  - 162 Patients with healing data

**Measurement of Protease activity**
- Elastase activity was measured using the fluorogenic substrate MeOSuc-Ala-Ala-Pro-Val-AMC
- MMP activity was measured using the broad spectrum MMP substrate Mca-Lys-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH2.

**Classification of healing**
Wounds were classified as healing / non healing based on changes in wound area over the previous 2 - 4 weeks.
Serena’s sample collection technique was developed prior to commencement of this study:

- Prior to swabbing: cleanse with sterile saline, do not perform sharp wound debridement, ensure that complete haemostasis has been achieved before obtaining the specimen.

- Moisten wound with a few (up to five) drops of saline. Care should be taken not to flood the wound with excessive saline.

- Avoid swabbing areas containing blood, necrotic material, thick slough or fibrinous tissue.

- Press head of swab flat against the base of the wound and gently roll it back and forth several times while applying pressure. Continue rolling the swab head until fully coated and discoloured by wound fluid.
Determining the level of protease activity
Associated with non-healing wounds

A Binary Logistical Regression model was used to predict the probability of non-healing associated with each protease activity level (n=93)

Statistical analysis associated a 90% probability of a wound being non-healing with Elastase activity of 25mU/110uL or Total MMP activity of 48U/110uL, with the probability increasing as protease activity increases.

Samples included in binary logistical model: Elastase n=92, MMP n=91
Clinical range of protease activity

Chronic wounds (n=235)
RESULTS

28% of non healing chronic wounds had EPA = 90% probability will not proceed to heal

This demonstrates that not all non-healing wounds are due to elevated protease activity as there were a number of non-healing wounds with low protease activity.

This supports the hypothesis that there are a range of other factors that could also lead to delayed wound healing.
What does a wound with EPA look like?

There are **no visual signs** that can be used to identify wounds with EPA.

Click here to find out which of these wounds are **EPA**.
Inflammatory protease activity, namely MMP and elastase activity, is indicative of non-healing wounds when assessed to be above the levels identified in this study, and these are clinically relevant.

The use of a diagnostic test able to assess protease activity in clinical practice could enable clinicians to identify wounds that are non-healing due to elevated protease activity (EPA).

This could in turn aid treatment decisions and enable the targeted use of protease modulating dressings.

Further Clinical Studies are ongoing to learn more about the prevalence of EPA and how a point of care diagnostic could aid treatment decisions*

A chronic wound with EPA, as defined by this research, has a **90% probability it won’t heal**.*

**28% of non healing chronic wounds have EPA.** A test that identifies which wounds have EPA would identify wounds that are non healing due to elevated protease activity, which could lead to targeted treatment with protease modulating therapies, to increase the chance of healing in these wounds.

**Wounds with low protease activity may be non-healing for other reasons**

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* These references demonstrate that the percentage reduction in wound area over 4 weeks is a good prognostic indicator of healing status