

# Protease Activity Levels Associated with Healing Status of Chronic Wounds

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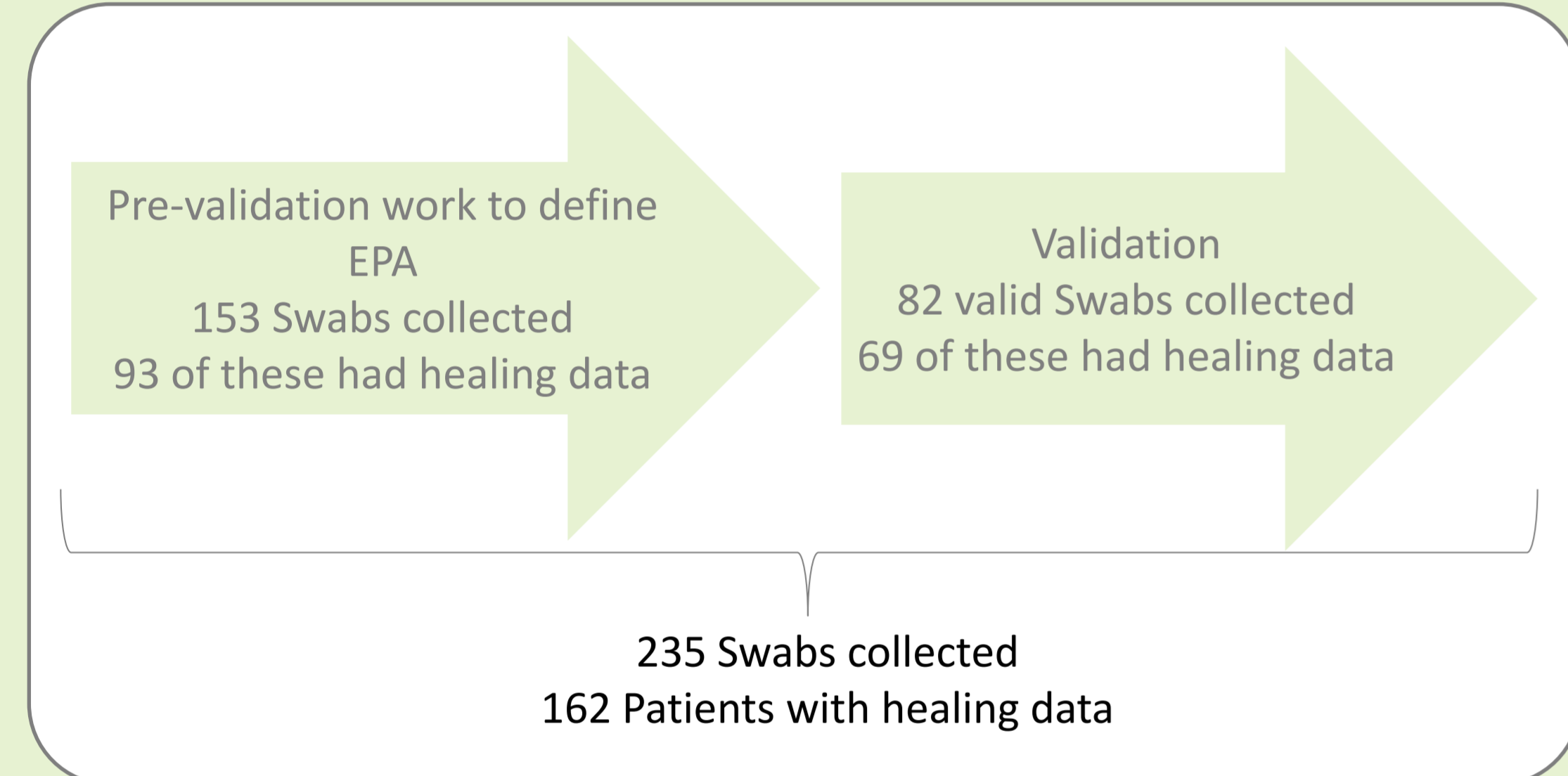


## BACKGROUND

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.

## METHODS

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.



### 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-NH<sub>2</sub>.

### Classification of healing

- Wounds were classified as healing / non healing based on changes in wound area over the previous 2 - 4 weeks

### Development of optimal Sample collection Technique:

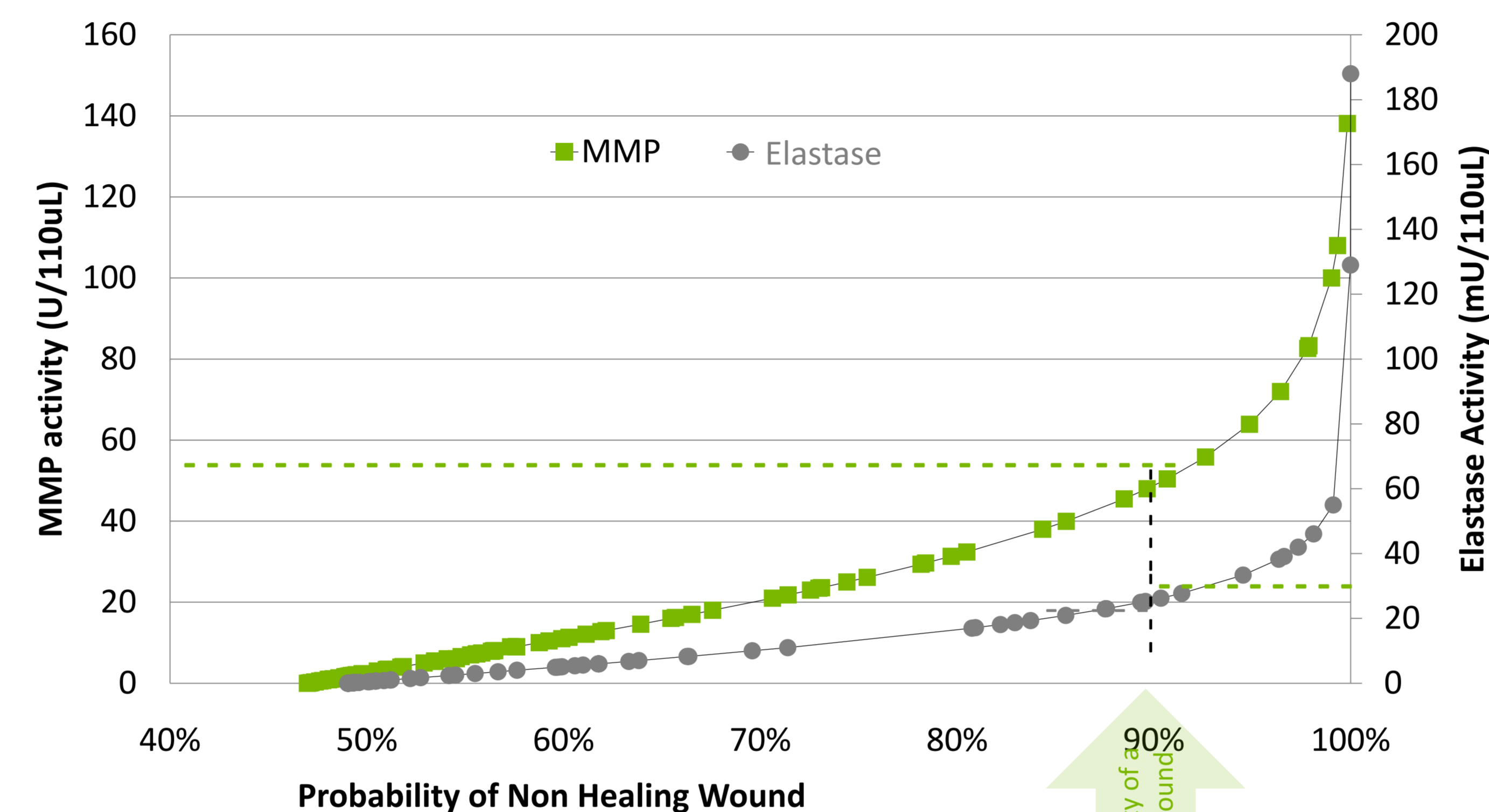
Serena's sample collection technique was developed prior to commencement of this study:



- ✓ 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

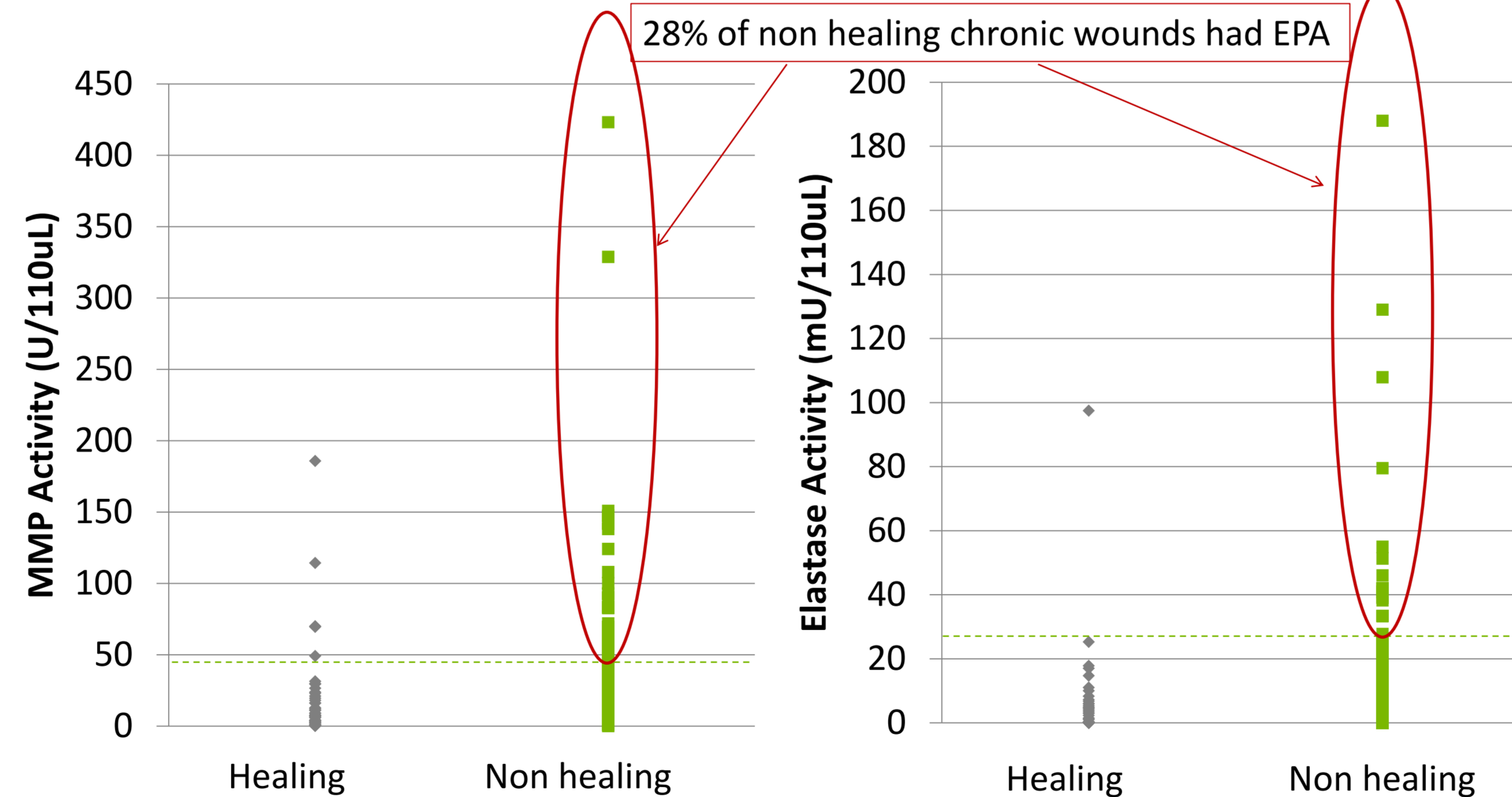
A Binary Logistical Regression model was used to predict the probability of non healing associated with each protease activity level (n=93)



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

### Relationship between healing status and EPA



### 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.

### CONCLUSIONS

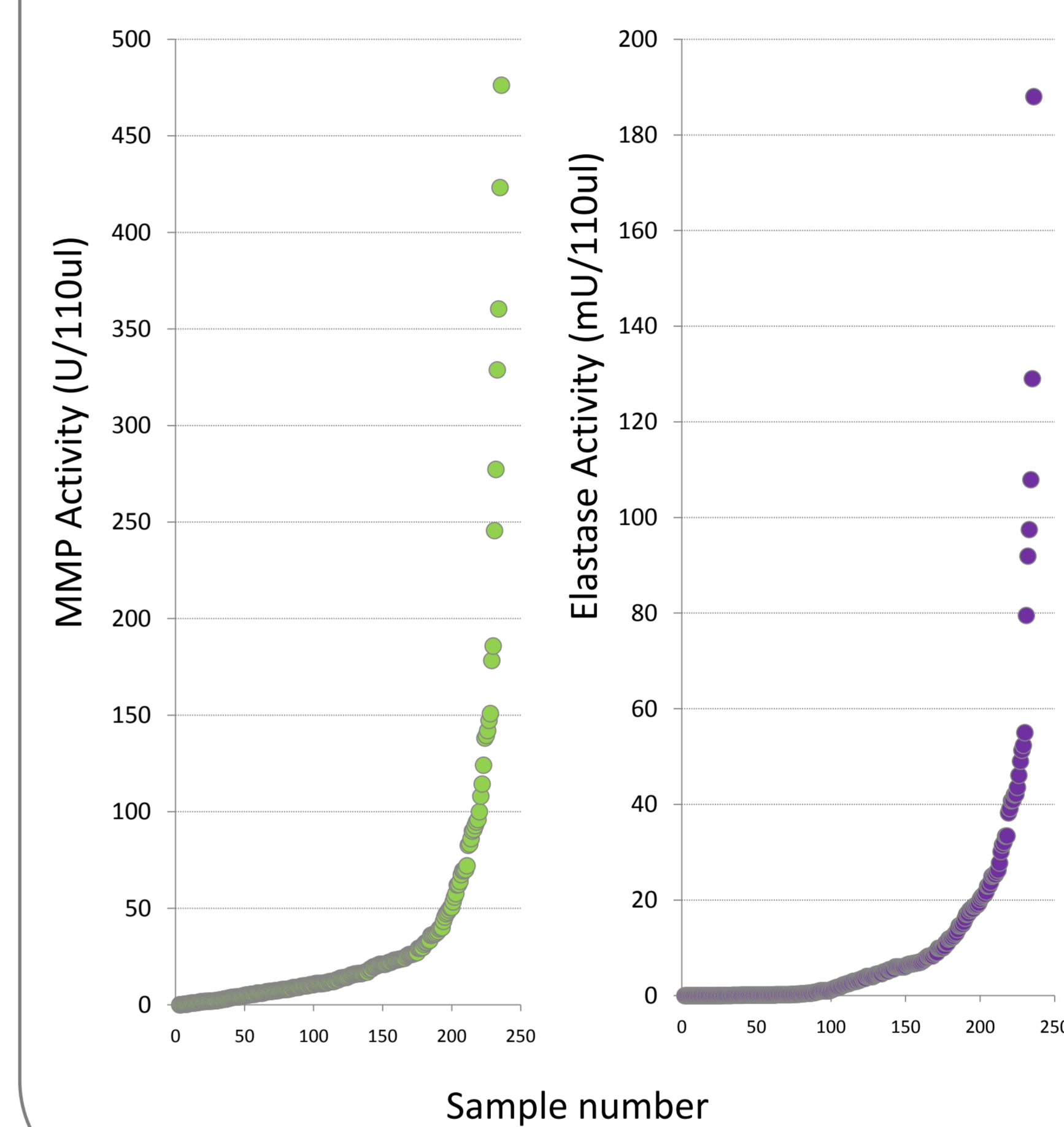
- A chronic wound with EPA, as defined by this research, has a 90% probability it won't heal\*
- 28% of non healing chronic wounds have EPA
- A test that identifies which wounds have EPA would identify wounds that are non healing due to elevated protease activity, which could lead to targeted treatment 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

## 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

### Clinical Range of Protease activity

Chronic wounds (n=235)



### What does a wound with EPA look like?

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



Non healing chronic wounds with EPA

Non healing chronic wounds, without EPA

## DISCUSSION

Interestingly we also noted that, wounds with high levels of elastase activity did not necessarily have high levels of MMP activity and vice versa. This shows that an individual protease is not causative of the excessive protease activity (EPA) found in a wound and that a wound does not need to have high levels of all proteases to be non-healing. Instead individual proteases seem to be able to compensate for one another in providing a highly proteolytic wound environment. This highlights the need to measure multiple proteases in order to determine if proteolytic activity is causing a problem in the wound and preventing it from healing.

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.

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

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