EPA made easy

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Introduction

Elevated protease activity (EPA) may disrupt wound healing. This Made Easy describes the types of wounds that may have EPA and how wounds with EPA may be identified. The methods that may be used to reduce protease activity to a level where healing can progress are also discussed.

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What are proteases?

Proteases are a group of enzymes that act on proteins. They generally cut a protein into two or more pieces, and so change its structure. For some proteins this results in loss of function, but for others it may result in activation of a molecule that interacts with other molecules, eg another enzyme or a receptor. Proteases may have a specific substrate, ie they may act on only one specific protein, or they may be able to act on a range of proteins.

Some proteases have key roles in wound healing. However, in high levels some have been implicated in delayed healing. The major groups of proteases involved in wound healing are the matrix metalloproteases and the serine proteases.

Matrix metalloproteases

Matrix metalloproteases (MMPs) all contain a zinc atom (hence the metallo- prefix). They preferentially break down the proteins comprising the extracellular matrix (ECM), and together can act on all ECM components, eg collagens, elastin and glycoproteins. So far, 23 human MMPs have been identified, with MMP-1, MMP-2, MMP-8 and MMP-9 the particular focus of wound-related research[1].

Serine proteases

There are numerous serine proteases involved in wound healing, with human neutrophil elastase (HNE) predominating[2]. This enzyme is able to act on a wide range of proteins in the ECM and also on inflammatory mediators[3].

What are the roles of proteases in wound healing?

Proteases have a number of functions in the inflammatory, proliferative and remodelling phases of normal wound healing. Broadly, in normal wound healing they break down damaged ECM and foreign material, aiding new tissue formation and orderly wound closure. It is widely recognised, however, that in excess, proteases can have a detrimental effect on wound healing.

What affects protease activity?

The production and regulation of proteases is complex. MMPs are produced by tissue cells involved in healing, eg neutrophils, fibroblasts, endothelial cells and epithelial cells. They are also produced by immune cells as part of the inflammatory process or in response to infection. As the name suggests, HNE is produced by neutrophils.

When first produced, MMPs are usually in an inactive (pro-MMP) form. They are subsequently activated by other proteases and by serine proteases such as HNE.

Tissue inhibitors of metalloproteases (TIMPs), which are produced by a variety of tissue cells, inhibit the activation of pro-MMPs and also the activity of activated MMPs. The main inhibitor of HNE is α-1 protease inhibitor (also known as α-1 antitrypsin), which is secreted by macrophages and liver cells[4].

The presence of bacteria in wounds may increase protease activity. The bacteria induce an inflammatory response that stimulates protease production. In addition, bacteria may also produce proteases[5].

How do proteases sometimes cause problems with healing?

Proteases are essential for normal healing. However, it is widely recognised that protease activity, including that of the MMPs and HNE, is elevated in wounds that are failing to progress[6–11].

In normal wound healing, an initial rapid rise in protease activity starts to reduce by about day five. In non-healing wounds, protease activity reaches higher levels and persists for longer[12].

Elevated protease activity may result in 'off target' destruction of proteins that are essential for healing, such as growth factors, receptors and newly formed ECM. This may disturb the balance between ECM deposition and destruction[5].

The damaging effects of the proteases may further stimulate the inflammatory response and release of damaging reactive oxygen species. The resulting excess protease activity causes the wound to enter a vicious circle (Cullen's circle) that ultimately delays healing (Figure 1, see page 2). High wound bioburden may augment the circle through the production of bacterial proteases that further stimulate the inflammatory response.

Which sorts of wounds are affected by high protease activity?

Studies have noted high levels of protease activity in chronic wounds

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of widely differing aetiology, eg venous leg ulcers, diabetic foot ulcers, pressure ulcers and trauma wounds[11,13-15]. This suggests that high protease activity is related to a problem with the healing process itself rather than with the aetiology of the wound.

How high is too high?

For clinicians to effectively target high protease levels, they need to know at what level protease activity is likely to start to cause harm, and to be able to easily identify affected wounds.

A recent study has examined the correlation between HNE and MMP activity and healing rates in a range of chronic wounds[16]. Likelihood of healing was determined by measuring changes in wound area over two to four weeks. A reduction of 50% or more for diabetic foot ulcers, or of 30% or more for venous leg ulcers and pressure ulcers, was considered indicative of healing.

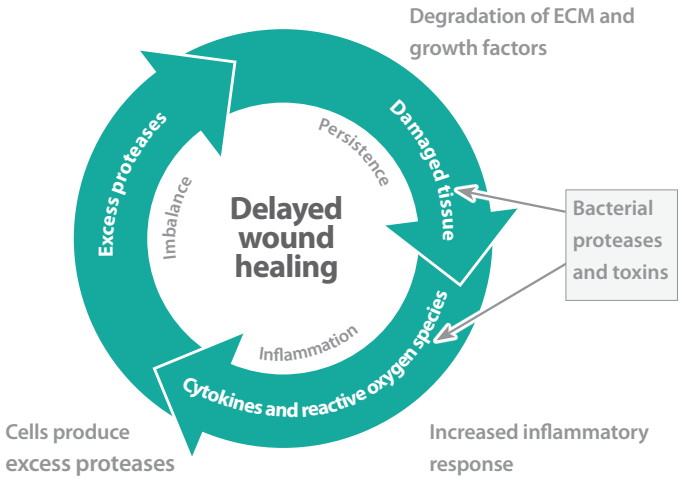
The study found that a wound had a 90% probability of being classified as non-healing when HNE activity was ≥25mU/110µl and/or total MMP activity was ≥48U/110µl[16]. Protease activity at or above these levels has therefore been determined to indicate elevated protease activity (EPA) and a 90% probability of non-healing.

How many wounds are affected by EPA?

Multicentre studies in the USA found the prevalence of EPA to be 25-28% of non-healing wounds[15,16]. The wounds included in these studies were classified as non-healing according to clear criteria related to changes in wound area over two to four weeks. When healing wounds were included in the analysis from the first study, the prevalence of EPA was 22%.

In addition, wounds of any duration may have EPA and EPA may be present in all common chronic wound types, ie leg ulcers, diabetic foot ulcers, pressure ulcers and trauma wounds[15].

Figure 1 Cullen's circle – the vicious circle of delayed wound healing[17]



What might affect prevalence of EPA?

The prevalence of any condition is subject to variation between studies as a result of a number of factors, eg differences in the population studied, the criteria for inclusion and the measurement methods used. So the prevalence of EPA measured in different care settings may be affected by a range of issues including:

- **whether healing wounds are included**
- **whether the criteria for non-healing have been applied consistently**
- **the treatment regimen being used prior to testing, eg whether protease modulating dressings have been used**
- **whether sample collection and test procedures have been followed correctly.**

Testing for EPA

Not all wounds with delayed healing have EPA. Therefore, clinicians need to be able to identify which wounds have EPA to be able to use protease modulating strategies effectively. However, there are no visual clues specific to EPA and it is not possible for clinicians to determine EPA from a visual examination alone[18,19].

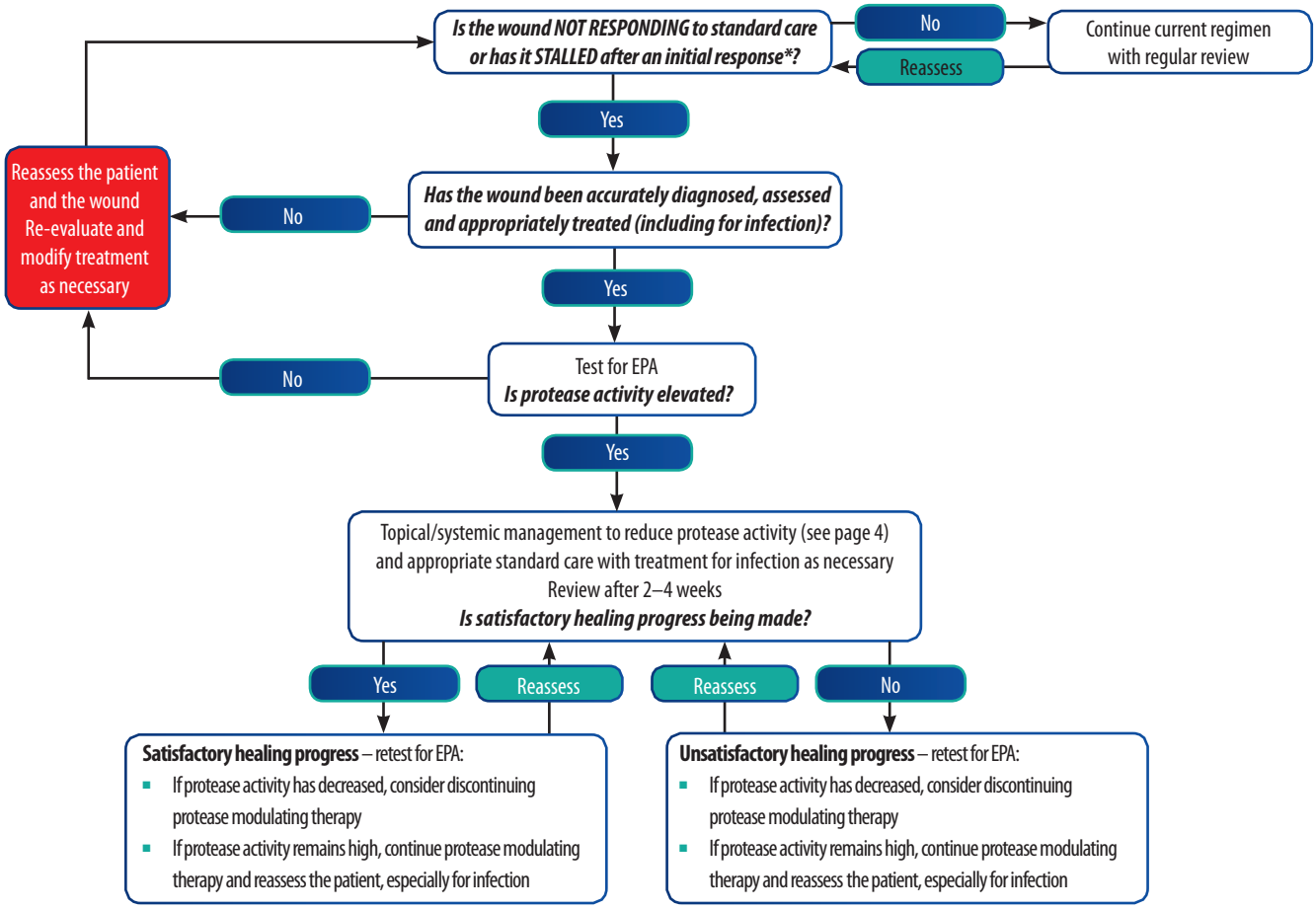
The availability of a diagnostic test that clinicians can use to reliably detect EPA and to indicate appropriate treatment has the potential to have a considerable impact on clinical and economic outcomes[20-22].

Until recently, measurement of protease activity has been a laboratory procedure that has been used for research purposes. However, an easy to use point of care test for EPA — WOUNDCHEK™ Protease Status (Woundchek Laboratories, previously Systagenix) — is now available and in clinical use.

How to use WOUNDCHEK™ Protease Status

WOUNDCHEK™ Protease Status is a point of care test that uses wound fluid swabbed from chronic wounds. The test takes about 15 minutes.

Figure 2 Pathway for the use of an EPA test (adapted from[12])



*Healing status should be identified through early comprehensive assessment that includes detecting and correcting the cause of the wound. Healing problems are more likely to occur in patients who are compromised or who have comorbidities such as diabetes or malignancy.

Specimen collection protocol (The Serena Technique©)

- **Prior to swabbing, cleanse the wound with sterile saline to remove all loose debris, remains of therapeutic agents (eg enzymatic debriders, gels, dressings, etc) and necrotic tissue. Do not perform sharp wound debridement prior to sample collection.**
- **Ensure that complete haemostasis has been achieved before obtaining the specimen.**
- **Apply additional saline to the wound area to be swabbed, such that the area is visibly moist. Care should be taken not to flood the wound with excessive saline. Avoid pooling of saline. Solutions other than saline should not be used as they can alter the test results.**
- **Avoid swabbing areas that contain blood, necrotic material, thick slough or fibrinous tissue.**
- **Press the head of the 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 it is fully coated and discoloured (tan/yellow) by wound fluid.**

The Serena Technique is the copyright of Dr Tom Serena.

When should we test for EPA?

An international consensus has recommended that testing for EPA in wounds with delayed healing should be used in the context of continued re-evaluation and optimisation of care in accordance with local wound management protocols (Figure 2)[12].

How do we know when a wound is non-responsive or has stalled?

Studies have indicated that ability to heal, ie whether a wound is

responding to treatment or has stalled, can be determined by reduction in wound area over two to four weeks.

For venous leg ulcers and pressure ulcers, an area reduction of 20-40% in two to four weeks has been found to be predictive of healing[23-26]. For diabetic foot ulcers, a wound area reduction of ≥50% by week four is predictive of healing[27-30].

How soon can we test for EPA?

Non-response to treatment may become apparent within two to four weeks, depending on wound aetiology. Therefore, testing for EPA may be useful after two to four weeks of standard care as part of the process of re-evaluating the wound, the patient and the treatment.

Currently, it is not known how soon EPA may occur after a wound develops and therefore when it may be appropriate to test for EPA. However, a study of the prevalence of EPA found that wounds of any duration may have EPA[15].

Once a wound has been shown to have EPA, an appropriate strategy for reducing protease activity can be implemented. The strategy should take into account the care setting and the needs of the patient and the wound, eg whether treatment for infection is also necessary (Figure 2, see page 3).

What can be done about EPA?

Recognising EPA in a wound with delayed healing will help the clinician to identify which treatments are appropriate. Treatment to reduce elevated protease activity should take place in the context of a full assessment and an appropriate local wound care protocol[12].

Treatment of the underlying cause of the wound and any factors or comorbidities that may be contributing to perpetuation of the wound, along with optimisation of the wound bed and patient, must underpin treatment to reduce protease activity.

Approaches to reducing protease activity may include:

- **reducing protease production** by reducing inflammation, eg where appropriate:
  - removing necrotic wound tissue (eg debridement)
  - reducing wound bioburden (eg antimicrobial dressings)
  - dampening the immune response (eg oral/topical doxycycline[31] or steroids)
- **removal of proteases from the wound bed** eg cleansing, absorbent dressings and negative pressure wound therapy (NPWT)[32]
- **reducing protease activity** eg collagen/oxidised regenerated cellulose (ORC) dressings (evidence discussed below).

Protease modulating dressings

There are many dressings marketed as modulating protease activity. Some reduce protease activity by absorbing wound exudate and so removing proteases and/or inflammatory mediators from the wound bed; others also act directly by binding or inactivating proteases[19].

There are varying levels of clinical evidence for protease modulating dressings. The action of some dressings is supported by *in vitro* studies only, while other dressings have a wide range of evidence including the results of randomised controlled clinical trials[33,34].

When choosing which dressing to use to modulate protease activity, clinicians need to choose a formulation or a combination of primary and secondary dressings that also meets the other needs of the wound and the patient. For example, should the dressing be suitable for use under compression, does the dressing also need to have antimicrobial activity because the wound is infected, does the patient have fragile skin that requires a dressing with low adhesive fixation, or does the dressing have the right absorbency for the level of exudate production?

An international consensus recommended that protease modulating dressings should be used for short courses of two to four weeks, followed by a full reassessment[12].

Collagen/ORC dressings

Dressings containing collagen/ORC have been shown to reduce the activity of MMPs and serine proteases, as well as levels of inflammatory cytokines in a range of chronic wounds[2,34-37].

An *in vitro* study of the effect of a variety of dressings against MMP and elastase activity in chronic wound fluid taken from wounds with EPA, found that collagen/ORC and collagen/ORC/silver dressings performed significantly better than dressings containing collagen only or nano-oligosaccharide factor (NOSF)[38].

A recent retrospective analysis of venous leg ulcers treated with a collagen/ORC dressing +/- silver showed that wounds which had EPA at the start of treatment had a 22% higher response rate at 4 weeks (77% of wounds with EPA responded vs 63% of all wounds in the study)[39]. This suggests that response rates to collagen/ORC dressings may be improved by targeting treatment to wounds shown to have EPA. The case study (see page 5) is an example of the use of targeted treatment of EPA with a collagen/ORC dressing in a patient with a venous leg ulcer.

Retesting for EPA

An international consensus has suggested that retesting for EPA should take place two to four weeks after initial detection of EPA (Figure 2, see page 3). If EPA remains, then the wound, patient and treatment regimen should be reassessed, with an emphasis on determining if infection is present. If EPA has resolved and healing progress is satisfactory, it is not yet clear at what point

discontinuation of protease modulating therapy can be considered. If EPA is no longer present, but the wound is still not healing, a full review should be conducted.

What do we know about EPA and bioburden?

Although wound bioburden may raise protease activity, diagnosis of wound infection is made clinically and detection of EPA (ie human inflammatory protease activity) cannot be taken to be confirmation of increased wound bioburden or wound infection.

Further research is required to clarify the impact of raised protease activity resulting from wound bioburden, and whether distinguishing this activity from EPA due to delayed wound healing is feasible and relevant.

What are the benefits of testing for EPA and of targeted treatment?

Wounds with delayed healing are enormously costly to healthcare systems and to patients. It is logical that a test for EPA, which can direct the clinician to appropriate protease modulating treatment, will bring financial and social benefits and improved utilisation of healthcare resources through:

- **fewer dressing changes**
- **reduced nursing time and fewer clinic visits**
- **avoidance of unnecessary interventions**
- **avoidance of more invasive and expensive diagnostic tests, eg wound biopsy**
- **earlier identification and prevention of complications**
- **shorter overall treatment duration**
- **improved quality of life**
- **earlier return to work[12].**

An economic model based on the UK healthcare system in which 100 chronic wounds were not tested for EPA has estimated that undiagnosed EPA could waste about £126,000[40].

The future of testing for EPA

As research continues, the role for an EPA test in clinical practice will evolve. Specific areas for further investigation include:

- **Why and at what point on the healing trajectory protease activity may become imbalanced**
- **If there are any differences in the protease profile of wounds of different aetiologies and in the various phases of wound healing**
- **The relationship between EPA and wound bioburden**
- **The impact on healing and economic outcomes of testing for EPA and protease management**
- **The relationship between wound duration and EPA in different wound types**
- **If there is a role for testing for EPA in acute wounds**
- **How patient factors such as age and co-morbidities affect protease activity**
- **Why some healing wounds have EPA and what implications this has on management**
- **Which are the most effective treatments for EPA.**

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CASE STUDY: NON-HEALING VENOUS LEG ULCER WITH EPA

Background

Mr F, aged 69 years, presented with a non-healing venous leg ulcer on the left medial malleolus that had been unresponsive to treatment with compression therapy and various dressings. There was no evidence of wound infection. A WOUNDCHEK™ Protease Status test showed elevated protease activity (EPA).

Treatment

The wound was treated with a protease modulating collagen/ORC dressing (PROMOGRAN®) and multi-layer compression bandaging.

Outcome

After two weeks of treatment, the condition of the wound bed had improved and the wound had reduced in size. The protease modulating dressing was discontinued and the wound was fully healed six weeks after treatment started.

Acknowledgement: Dr Caroline Dowsett, Newham, London

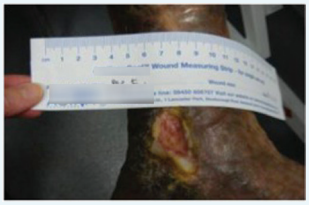

Fig 1: Venous leg ulcer at baseline

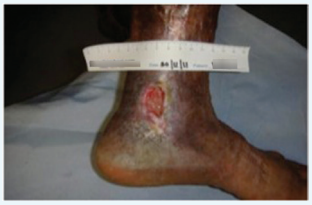

Fig 2: Venous leg ulcer after two weeks of treatment with a protease modulating dressing

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Summary

Wounds with elevated protease activity (EPA), and therefore at risk of delayed healing, can now be readily identified using the WOUNDCHEK™ Protease Status point of care test. The wounds identified as having EPA can receive targeted treatment to modulate protease activity with the expectation that both clinical and economic outcomes will be improved.

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