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THE ROLE OF PROTEASES IN WOUND DIAGNOSTICS

an expert working group review

Wounds International

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FOREWORD

An international group of experts met in Cape Town, South Africa in February 2011 to build on the 2008 World Union of Wound Healing Societies (WUWHS) consensus document *Diagnostics and Wounds*[1]. The goal was to explore the importance of protease activity in wound healing, and to gain consensus on the value of having an easy to use, point-of-care protease test in clinical practice.

The key to success for such a test will be for clinicians to know clearly when, how and why to use such a test. The expert consensus opinion of the meeting participants reaffirmed increased protease activity as currently the best available marker for impaired wound healing when other causes have been excluded, and that effective use of a protease test kit at the point of care has the potential to change wound care globally.

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What are proteases?

Proteases (also known as proteinases) play key roles in the normal wound healing process[2] (Table 1). Proteases are enzymes that act on proteins by breaking them down into peptides and amino acids. In wound healing, the major proteases are the matrix metalloproteinases (MMPs) and the serine proteases, eg elastase. In general, different wound-related proteases act on different proteins. These include extracellular matrix (ECM) and connective tissue proteins such as collagen, gelatin, proteoglycans and elastin.

In the normal wound healing process, proteases break down damaged ECM proteins and foreign material so that new tissue can form and wound closure can occur in an orderly fashion. However, when the level of protease activity is too high the delicate balance between tissue breakdown and repair is disturbed.

Excessive wound proteases lead to the degradation of newly formed ECM and other proteins, eg growth factors and receptors. As a result wound healing is impaired due to ECM damage and abnormal prolongation of the inflammatory stage of healing that prevents the wound from progressing to the proliferative phase[2].

Protease activity is an essential part of wound healing[3]. However, once out of control, and if left unchecked, proteases in wounds may cause sufficient damage to the extracellular matrix, growth factors and receptors to impair healing and destroy normal tissue

SOURCES OF PROTEASES

As well as being secreted by the cells involved in the repair process, eg fibroblasts and endothelial cells, proteases are produced by immune cells stimulated by an inflammatory process or infection. For example, human neutrophil elastase (HNE) is produced by neutrophils and is responsible for fibronectin degradation in non-healing wounds.

This is important because fibronectin degradation products stimulate the further release of MMPs[4,5]. Intact fibronectin (which is necessary for cell adhesion and growth factor signalling) is absent in non-healing wounds, but has been shown to reappear in the wound bed as healing begins[6].

Elevated tissue iron levels in chronic venous disease due to extravasation of red blood cells has been suggested to be another stimulus to over-expression of MMPs[7]. In addition, patients with chronic venous disease who also have a haemochromatosis gene mutation (C282Y) that causes abnormal iron metabolism have significantly increased risk of developing a venous leg ulcer[8].

In the future, testing for genetic variants may become part of the screening process for assessing risk of ulceration and probability of healing.

What are proteases?

Proteases are enzymes that break down proteins into peptides and amino acids. In wound healing, the major proteases are the matrix metalloproteinases (MMPs) and the serine proteases, eg elastase. In general, different wound-related proteases act on different proteins. These include extracellular matrix (ECM) and connective tissue proteins such as collagen, gelatin, proteoglycans and elastin.

Table 1 | Main roles of proteases in normal wound healing[2]

Main phase of healing	Role of proteases
■ Inflammation	■ Removal of damaged ECM (aids autolytic debridement)
■ Proliferation	■ Degradation of capillary basement membrane for angiogenesis ■ Aiding detachment and migration of cells
■ Remodelling	■ Contraction of scar ECM ■ Remodelling of scar ECM

Another source of proteases in wounds is bacteria. In addition to stimulating protease production via activation of the immune system, some bacteria in wounds may themselves secrete proteases. However, the impact of bacterially derived proteases on wound healing and their contribution to total wound protease activity remains to be determined.

More is currently known about proteases than any other biochemical marker involved in wound healing

ELEVATED PROTEASE ACTIVITY AS A MARKER FOR NON-HEALING

There is a large body of evidence from animal and human studies suggesting that protease activities (the MMPs and human neutrophil elastase [HNE] in particular) are elevated in wounds that fail to progress towards healing[9-20].

In the normal course of wound healing, there is a rapid initial increase in protease levels[21,22]. The levels peak at about day three and start to reduce by about day five (Figure 1). In non-healing wounds, however, not only do proteases reach higher levels than in healing wounds, but they persist far longer. The result is a highly destructive wound environment.

Although the relationship between proteases, inflammation and wound healing is broadly understood, there are other markers associated with inflammation (such as TNF-alpha) that may warrant further investigation as potential candidates for diagnostic tests[23]. However, data to date suggest that proteases may be the most promising biomarker for assessing wound healing at a biochemical level.

The reasons for the imbalance between increased protease production and a lack of protease inhibition in non-healing or chronic wounds is not fully understood, but regular monitoring of protease activities during treatment may help to guide appropriate management

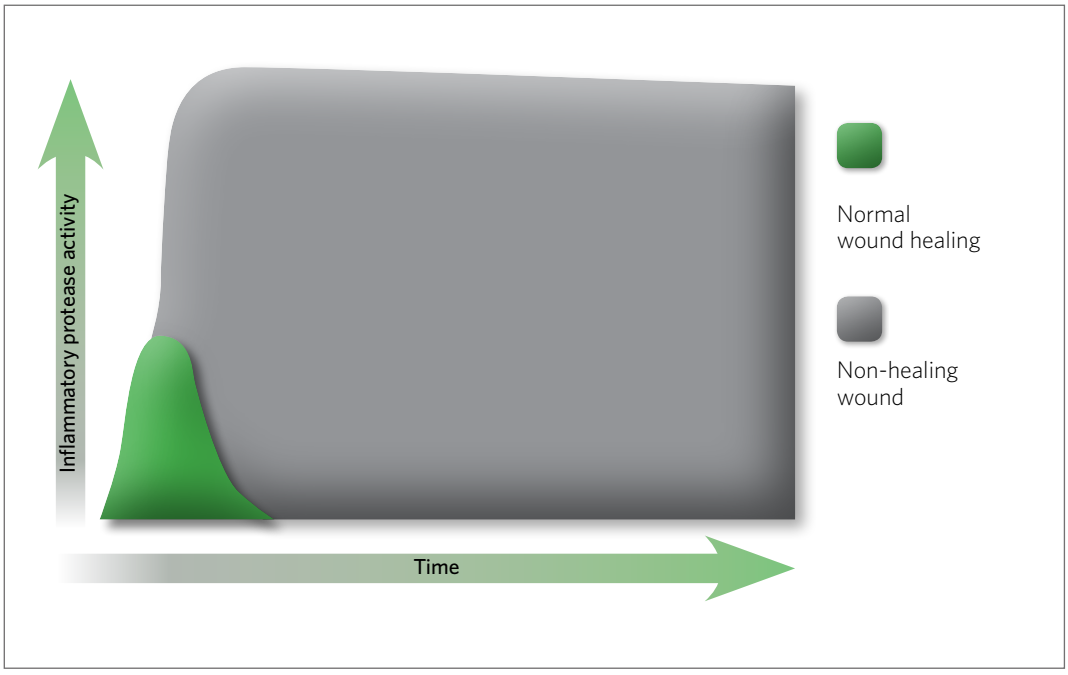


Figure 1 | Changes in protease activity in wounds

Understanding the role of proteases

When educating clinicians about proteases, it is important to teach the concept of balance and imbalance in healing, ie the balance between synthesis and degradation of ECM. The main learning points are:

- Proteases are important for organising and remodelling new ECM. There is a burst of protease activity at the start of acute wound healing. In normally healing wounds, the activity peaks in the first couple of days and then declines to very low levels by one week[21]
- Stimuli that may prolong high protease activity include the presence of damaged tissue, foreign material, bacteria and biofilms
- If protease activities are too high they start degrading and destroying the ECM and damage newly formed tissue, harm the wound bed and delay healing
- Interventions that reduce harmful proteases and correct the imbalance may aid healing[24].

Box 1: **Terminology of wounds with healing problems (adapted from[25])**

- Chronic
- Delayed
- Hard to heal
- Stalled
- Recalcitrant
- Difficult
- Complex
- Fail to respond

For further discussion on non-healing wounds, go to the Chronic Wound Debate in the Wounds International Journal, Vol 1; Issue 2 (www.woundsinternational.com)

PREDICTING HEALING PROBLEMS

Wounds that are difficult to heal are often labelled as 'chronic'. However, this may not be helpful because the term 'chronic' implies long duration and the need to wait to see if a wound is slow to heal. Clinicians know that wounds in patients with certain comorbidities, eg diabetes mellitus, or in patients on certain medications, eg steroids, can be identified from the time they occur as being difficult to heal.

Furthermore, the term 'chronic' may exclude acute wounds, eg surgical wounds, which have healing problems. As a result a number of terms are used to describe wounds that may be slow or difficult to heal (see Box 1, left).

There are numerous factors other than duration that influence ability to heal. Initial assessment of all wounds should include assessment for these factors (Table 2). The regimen of care for patients with a wound of any type should, therefore, include management of any correctable factors. Early recognition of correctable factors provides clinicians with the opportunity to implement care that can speed healing.

Equally important, however, is that clinicians recognise when a wound is unlikely ever to heal, eg in patients with malignancy or advanced disease, on chemotherapy or on high dose steroids.

POINTS FOR PRACTICE

The importance of protease activity

- High protease activity is the best available biochemical marker for predicting poor wound healing of both acute and chronic wounds
- If wound protease activity and ratios are appropriate for the stage of wound healing, healing will be more orderly and timely
- Research is required to identify and clarify:
  - what level of protease activities are appropriate during autolytic debridement
  - why and at what specific point on the healing trajectory protease activity may become imbalanced
  - the protease activity typical of stalled and healing wounds for different wound types, eg pressure ulcers, vasculitic wounds
  - how patient factors such as age, hormone levels and co-morbidities affect protease activity
  - the synergistic and chronological relationships between different proteases, ie how the different MMPs and elastases work together to degrade ECM proteins
  - the impact of bacterial contributions to protease activities
- Establishment of a registry that collects data on protease activities in different wound types at different stages of healing would provide useful data on wound healing prognosis

REDUCTION IN WOUND AREA AS A PREDICTOR OF HEALING

Research has suggested that a reduction in wound area by weeks two to four is a good predictor of ability to heal by week 12. For venous leg ulcers, a 20–40% reduction in wound area within two to four weeks has been found to be predictive of healing[27], whereas for diabetic foot ulcers, a reduction of ≥50% by week four is predictive of healing[28–30].

It is logical, therefore, that wounds that do not show these levels of healing within these time frames trigger the need for reassessment and re-evaluation of the care regimen.

However, rather than waiting until problems develop, it would clearly be very beneficial to be able to identify even earlier when advanced interventions may assist with healing. Testing for markers of healing, such as protease activity, may help clinicians predict which wounds will have problems[2].

Even when wound and patient care is optimal and infection has been excluded, some wounds do not heal. These wounds may have persistent inflammation with high protease activity that is preventing progression to the proliferative stage of healing

Table 2 | Factors that may influence wound healing ability[26]

Area	Factors
■ Patient	■ Aetiology ■ Comorbidity, eg diabetes mellitus, autoimmune disease ■ Allergy ■ Medication, eg steroids ■ Psychosocial status ■ Pain ■ Concordance
■ Wound	■ Duration ■ Size ■ Wound bed condition ■ Ischaemia ■ Inflammation/infection ■ Anatomical site ■ Treatment response
■ Care provision	■ Skill and knowledge of clinicians ■ Healthcare system (availability, cost/reimbursement)

Assessing protease activity

CURRENT METHODS FOR ASSESSING PROTEASE ACTIVITY

Laboratory analysis

Currently, it is very difficult to assess the level of proteases in wounds. Research studies have analysed types, levels and activities of proteases in wound fluid derived from biopsies obtained in laboratory conditions. These studies have involved several different techniques, eg gelatin zymography that primarily detects MMP-2 and MMP-9, ELISAs that use antibodies to measure levels of proteases, and assays that measure the enzymatic activity of proteases.

The results of these studies show a consistent trend of low levels of protease activity in acute healing wounds, and high levels in stalled or poorly healing wounds that decrease when the wounds begin to heal[10,17]. However, for most clinicians, laboratory evaluation of protease activity is not feasible.

Clinical assessment

In wounds that are not healing as expected, excess protease activity may be suspected in a wound that has not responded to treatment or that has stalled after initially successful treatment if:

- comprehensive treatment has included correction of the underlying cause (eg compression for venous stasis), management of patient concerns (eg pain) and optimal local wound care (debridement and provision of a moist wound environment), and;
- infection is not suspected or has been ruled out.

Clinical signs of inflammation, which may be indicative of high protease activity may be difficult to distinguish from those of infection. Signs may include a red wound bed, absent or diminished granulation that may bleed easily on contact, and increased exudate and pain levels.

However, although the majority of uninfected non-healing wounds will have excess proteases, a percentage of non-healing wounds will not have excess proteases.

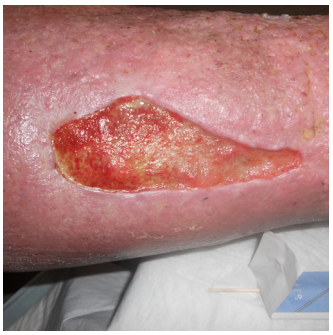
Consequently, clinicians may wrongly assume that a chronically inflamed uninfected non-healing wound, which has received appropriate care and management of underlying causes, has high protease activity.

An on-going study of over 100 acute and chronic wounds has shown that skilled clinicians using the clinical criteria for chronic inflammation have not been able to accurately predict which wounds have high protease activity. In fact, the only wounds in which protease activity has correlated with clinical examination are vasculitic ulcerations[31]. Figure 2 (overleaf) demonstrates the difficulty of predicting high protease activity based on clinical examination alone.

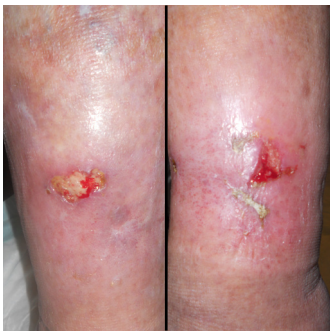
Clinical signs do not always predict the presence of high protease activity. Accurate detection of elevated protease activity would aid in the appropriate use of treatments aimed at modifying protease activity and would help to avoid inappropriate use of advanced wound care products

Relevance of protease levels to healing
Studies show a consistent trend of low levels of protease activity in acute healing wounds, and high levels in stalled or poorly healing wounds that decrease when the wounds begin to heal[10,17]. However, for most clinicians, laboratory evaluation of protease activity is not feasible and clinical assessment is necessary.

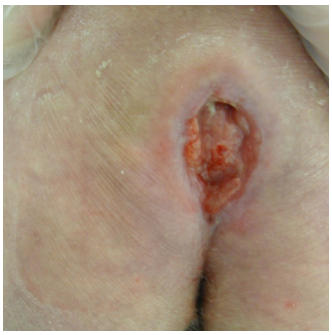
Figure 2 | Clinical observation cannot detect high protease activity. Do these pictures show wounds with high or low protease activity? (*See answers at the bottom of the page — photos courtesy of Tom Serena*)



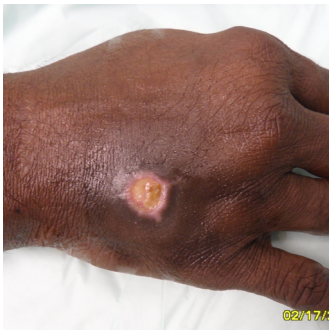
A: A 52-year-old non-diabetic male with a long-standing venous leg ulcer. There is minimal exudate after treatment with a topical silver agent. Recently treatment has been changed to a collagenase and compression wrapping. He has minimal pain.



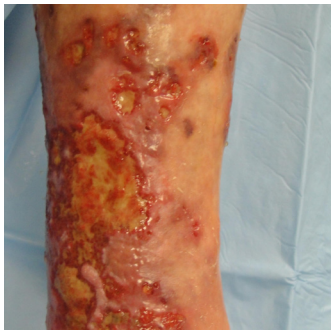
B: A 40-year-old non-diabetic woman with bilateral lateral gaiter chronic venous leg ulcers currently being treated with topical alginate and compression. The right leg ulcer (above left) has a clean granulating base. The left leg ulcer has repeatedly developed tan slough requiring curettage.



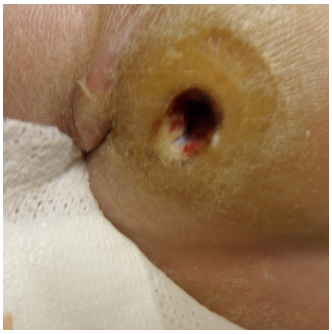
C: Stage III pressure ulcer treated with ORC-silver/collagen.



D: A non-diabetic patient with an acute wound on the dorsum of her hand after an intravenous line infiltrated. The wound is healing.



E: A patient with known vasculitis of the lower extremity.



F: Diabetic plantar neuropathic ulcer treated with topical silver alginate and hyperbaric oxygen.

Answers

Positive test for elevated protease activity: A, D, E
Negative test for elevated protease activity: B (both legs), C, F

Management of high protease activity

Wounds that are not healing despite correction of underlying causes, exclusion of infection and optimal wound care may be stuck in a persistent inflammatory state with high protease activity. Treatment of such wounds requires a systematic, often interdisciplinary approach that focuses on correcting the underlying cause of inflammation. Care should be provided in keeping with appropriate local protocols that use the basic tenets of good wound management.

The use of a point-of-care diagnostic test for protease activity may assist the clinician in the use of advanced therapies by indicating clearly which treatments are and are not appropriate, and when to start and stop treatment

Principles

There are a number of interventions that may reduce protease activity in a wound. The three key principles involved in treating wounds with suspected excessive protease activity are:

- **treat** the underlying cause and any factors that may aggravate the wound, eg compression, pressure relief, correct ischaemia and suboptimal nutrition
- **optimise** the wound bed and patient condition, eg wound bed preparation (including debridement), negative pressure wound therapy (NPWT), modulate bacterial load
- **modulate** protease activity, eg protease-modulating dressings.

Caution should be exercised when using skin/dermal equivalents in complex non-healing wounds where protease activity is high because degradation of the matrix is likely to occur

REDUCING EXCESS PROTEASE ACTIVITY

The following techniques may reduce protease activity in wounds:

- **Cleansing:** regular wound cleansing may help to reduce protease activity by removing surface debris that could act as an inflammatory stimulus. Cleansing may also help to reduce protease activity by removing protease-containing wound fluid. Studies that examine the effects of cleansing on protease activity are awaited
- **Debridement:** removal of slough at every dressing change, or surgical or sharp debridement at appropriate intervals, may help to reduce excessive protease activity by removing necrotic tissue and reducing bacterial load that may be acting as inflammatory stimuli. Again, studies that examine the effect of debridement on protease activity are awaited
- **Protease inactivators:** protease-modulating dressings (eg collagen/oxidised regenerated cellulose [ORC]) that bind to and inactivate proteases (MMPs and elastase)[32,33]
- **Antiseptic dressings (eg iodine or silver):** a reduction in bacterial levels may reduce protease activity by reducing host and bacterial protease production[34]. It has been postulated that silver may also reduce MMP activity by displacing the zinc ion necessary for MMPs to function from the enzymes[35]
- **Anti-inflammatories:** oral or topical doxycycline is a potent anti-inflammatory and antimicrobial that inhibits protease activity[36] — steroid therapy has an anti-inflammatory effect by upregulating the expression of anti-inflammatory proteins and downregulating the expression of pro-inflammatory proteins. Experience of these treatments to date is mainly in vasculitic wounds and pyoderma gangrenosum. Studies in other wound types and the possible use of these treatments in combination are awaited
- **Dressings and devices that absorb/remove wound exudate:** absorbent dressings and materials may reduce protease activity by removing protease-containing wound fluid[37], although this has yet to be demonstrated in a clinical context. An effect of NPWT in stimulating healing may be to reduce protease activity[38,39].

When using advanced therapies such as protease-modulating dressings or infection control products, a point-of-care test to measure changes in protease activity may be useful in monitoring the effectiveness of treatment and indicating whether there is a need to modify therapy

PROTEASE-MODULATING DRESSINGS

As with all advanced wound care products, the use of protease-modulating dressings should be carefully integrated into the overall care plan, which needs to be appropriate to the condition of the wound bed, bacterial load and exudate level.

In general, protease-modulating dressings, eg ORC/collagen, are used for short courses of two to four weeks, followed by a full reassessment of the effectiveness of treatment. Intermittent or pulsed treatment is also sometimes used, eg two weeks of treatment with the protease-modulating dressing followed by two weeks without the dressing.

A collagen/ORC dressing has been shown to reduce protease activity and to have a positive effect on healing in a variety of non-healing wounds[24,33,40].

The use of a protease-modulating dressing should be a clearly timed intervention, ie the proposed duration of treatment should be clearly documented with a review date. It is essential that regular assessments of healing progress, eg of wound margin, base and wound area, are conducted during treatment. For venous leg ulcers, a guide for improvement indicating that healing is likely would be a 20–40% reduction in wound area at four weeks[27].

The introduction of a point-of-care test for excessive protease activity would allow more targeted use of protease modulators to reduce excessive protease activity. A protease activity test may also provide an opportunity to examine other potentially beneficial effects of continued use of protease modulators on healing once proteases are under control.

Current knowledge of the role of proteases in delayed healing suggests that every stalled or slow to heal uninfected wound in a patient that has been adequately assessed and managed is a potential candidate for a point-of-care test for protease activity

The role of a point-of-care protease test

Ideally, a new diagnostic tool for use in wounds will indicate specific modifications to practice or treatment that will move the wound towards healing[1].

A point-of-care protease test will be an innovation in wound care. It is anticipated that it will be used to detect whether protease activity is elevated in wounds that are not healing as expected.

USING A PROTEASE TEST

A point-of-care protease test may help clinicians to make informed, cost-effective decisions about which treatment is or is not appropriate. For example, it would not be appropriate to use a protease-modulating dressing in a wound with low protease activity, and tissue-engineered products, scaffolds and skin grafts would be inappropriate in a wound with elevated protease activity.

Advantages of guiding therapy in this way may include less frequent dressing changes, avoidance of unnecessary interventions, reduced nursing time, fewer clinic visits, shorter overall treatment duration, earlier recognition and prevention of complications, improved quality of life, faster healing and earlier return to work. These potential benefits may lead regulators in the future to require the test before the use of specific treatments.

Monitoring proteases, eg through weekly testing, may allow clinicians to recognise whether care is effective in reducing protease activity and, therefore, whether the current treatment approach is appropriate. It has been speculated that the results of a point-of-care protease test may eventually be used to identify successful treatment and may become an alternative outcome measure to healing.

Point-of-care protease testing may be shown to have the potential to aid early identification of wounds that may be potentially hard to heal, thus avoiding the delays and associated risks caused by 'waiting long enough' before classifying a wound as hard to heal. This may also help to avoid more expensive diagnostic tests, eg invasive tests such as wound biopsy, and could be used to help confirm the diagnosis of inflammatory conditions, eg vasculitis.

An easy-to-use point-of-care protease test may present an opportunity for remote management of wounds, ie clinicians/patients may be able to test for high protease activity in a wound and then gain advice or make decisions about referral based on the results. For example, a less experienced clinician could use the test as a signal to refer to specialist or not, dependent on the result of the test.

To gain acceptance of a point-of-care protease test, payers will need evidence of cost-effectiveness, there will need to be widespread adoption by key opinion leaders, and clear evidence of benefits.

For a point-of-care test to become integral to practice, data will be required that demonstrate the validity of the test in a spectrum of wound types in clinical practice. A key unanswered question is how to deal with wounds with high proteases that go on to heal without complication and those with low proteases that fail to heal

Potential pathway for the use of a point-of-care protease test

The use of a point-of-care protease test in wounds that fail to respond must be in the context of continued re-evaluation and optimisation of care in accordance with local wound care practices and policy. Figure 3 and Table 3 illustrate how such a test might be used in practice once introduced.

Further research will be required to fully characterise the role of a point-of-care protease test in clinical practice

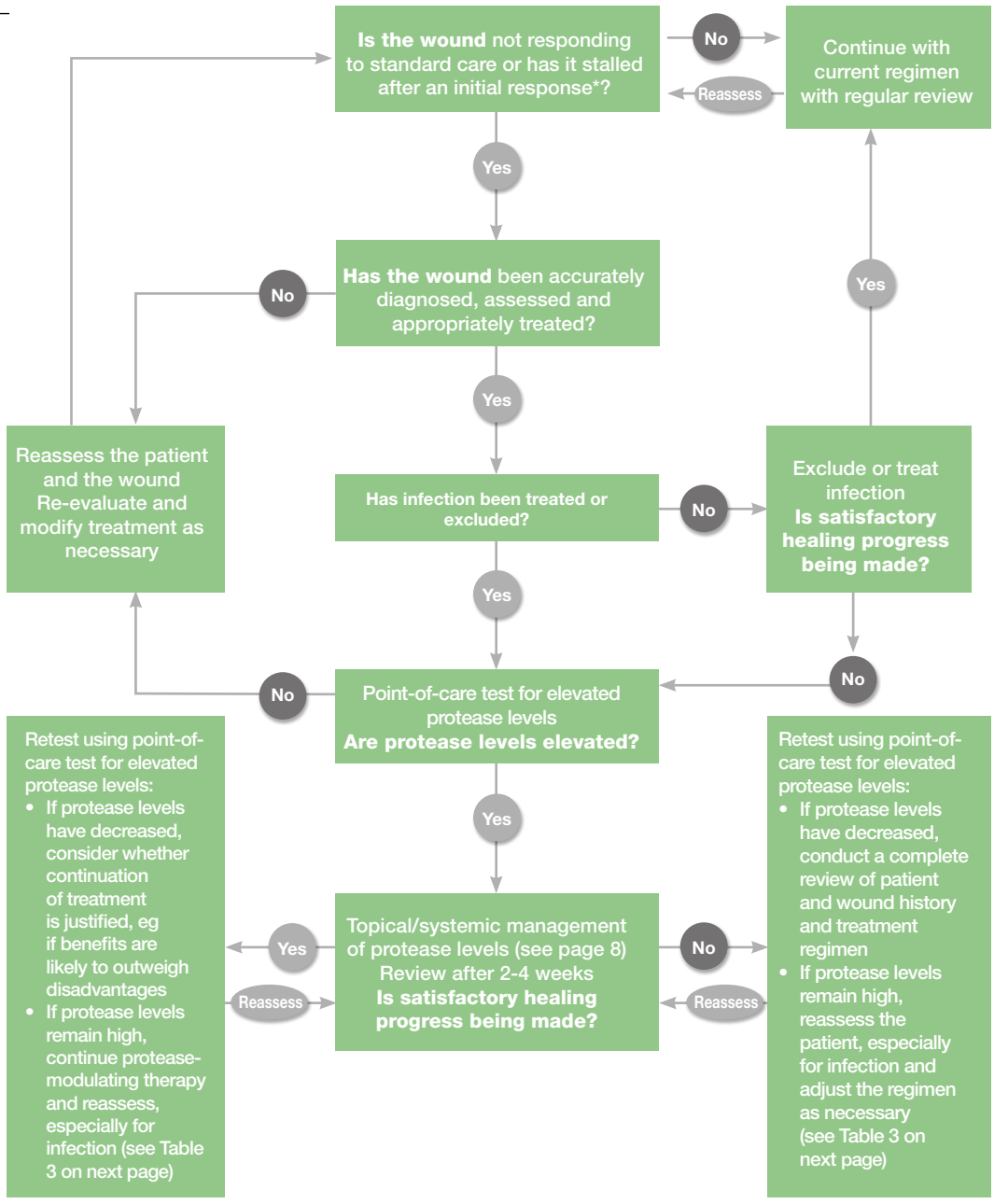


Figure 3 | Potential pathway for use of a point-of-care protease test

* *Identify healing status through early comprehensive assessment that includes examining and correcting the cause of the wound. Healing problems are more likely to occur in patients who are compromised or who have co-morbidities such as diabetes or malignancy.*

Table 3 | Relationship between protease activity, bacteria/infection, and treatment modality

	SIGNS/SYMPTOMS OF INFECTION	PROTEASE ACTIVITY	
		Low protease activity	Elevated protease activity
LOW BACTERIA	No signs of infection	Moisture balance dressing	Protease-modulating dressing
HIGH BACTERIA	**Superficial infection** Three of: ● Non-healing ● Exudate ↑ ● Pain ↑ ● Red friable wound ● Debris, smell	Antimicrobial dressing	Protease-modulating dressing with antimicrobial activity; +/- systemic antibiotic
	Deep/systemic infection: Three of: ● Size ↑ ● Temperature ↑ ● Pain ↑ ● Fistulae/bone exposed ● New/satellite wounds ● Erythema/oedema ● Smell ● Elevated acute phase proteins	Systemic antibiotics	Systemic antibiotic Systemic anti-inflammatory Antimicrobial dressing Protease-modulating dressing with antimicrobial activity

Payers are likely to embrace the availability of an objective measure to guide treatment if it allows clinicians to treat early and for a shorter duration to achieve healing and that testing can be shown to reduce costs overall

POINTS FOR PRACTICE

Using a point-of-care protease test

- Only use a point-of-care protease test if the results will influence clinical decisions on topical wound care
- Avoid user-induced false positives and false negatives by carefully following the instructions
- Know how a positive result and a negative result are shown by the test
- Understand what these results mean and what implications they have for care
- Know whether to test before or after debridement or cleansing
- If cleansing should take place before testing, know which solution(s) may be used, and how the solution used may affect test results
- Know what type of wound fluid to use for the test and how much is needed
- Know how to collect the wound fluid
- Know how soon after collection of the wound fluid the test needs to be done
- Know whether and how the presence of blood or necrotic tissue may affect the test results
- Know what to do if wound fluid is difficult to access or is not present
- Know why, when and how often to retest
- Understand the validity of the test in different wound types, ie how accurately the test measures protease activity in different wound types
- Understand the sensitivity and specificity of the test and how these may affect test interpretation, ie how often true positives (reported high protease activity when activity is high) and true negatives (reported low protease activity when activity is low) are indicated by the test

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