

# Using a Diagnostic Tool to Identify Elevated Protease Activity Levels in Chronic and Stalled Wounds: A Consensus Panel Discussion

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## Abstract

Care of chronic and stalled wounds is hampered by the lack of diagnostic tools to help direct clinicians to specific treatments or diagnose specific conditions. Studies have shown a correlation between high protease levels and nonhealing wounds; a diagnostic protease test is under development. Seven wound care experts (two podiatrists, two vascular surgeons, a physician expert in hyperbaric oxygen therapy, a physical therapist with a specialty in home health, and a registered nurse) met to reach consensus on several aspects about a point-of-care protease test. They agreed that although disease states interfere with wound healing, such states do not automatically mean that wound healing will be impaired or that the wound becomes stalled after inception; and that patient comorbidities, patient factors, patient medications, and the microenvironment of the wound all affect the risk of nonhealing. They also agreed that: 1) appropriate protease activity was important in healing, 2) measuring just one individual protease would be unlikely to be representative of the proteolytic environment of the wound, 3) no diagnostic or theranostic tests to detect high protease activity levels in a wound is currently available, and 4) the development of a simple, widely available protease diagnostic test could dramatically change the provision of care, especially in outpatient settings. If subsequent research confirms that high protease activity levels delay healing, confirmation that a stalled wound has high protease activity levels could better target protease-modulating therapies and improve outcomes. Extensive validation of a protease test will be necessary from proof-of-concept pilot studies to controlled clinical trials to demonstrate that use of the test improves outcomes of care.

**Keywords:** consensus, chronic wounds, proteases, metalloproteases, wound healing trajectory

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Wound healing is a complex biochemical process with four sequential, overlapping processes: hemostasis, inflammation, granulation (proliferation), and maturation.<sup>1</sup> For acute wounds, the healing process follows an orderly progression through these steps. However, for certain groups of wounds to which the term chronic is applied, this process is disrupted and healing stalls, most typically in the beginning inflammatory phase.<sup>2</sup> Lazarus et al<sup>3</sup> defined a chronic wound in 1994 as one that fails to progress through a normal, orderly, and timely sequence of repair or one that proceeds through the repair process without restoring anatomic and

functional results. Although wounds may have different underlying etiologies such as diabetes or venous disease, they share a common biochemical milieu.<sup>4</sup> Preclinical research also suggests that if a wound occurs in patients with certain comorbidities such as diabetes<sup>5</sup> or in patients taking particular medications such as steroids,<sup>6–10</sup> it may be thought of as immediately stalled based on the underlying pathophysiology and biochemistry.<sup>11</sup>

Treatment challenges. Stalled wounds frequently do not respond to basic wound care (ie, debridement, offloading or compression, moisture balance, and control of bacterial

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burden). However, after confirming and removing all apparent causes of tissue damage, addressing the underlying etiology, ruling out malignancy, and complying with relevant guidelines for safe, effective wound management (eg, at [www.guidelines.gov](http://www.guidelines.gov)), clinicians have little direction with regard to choosing which advanced therapy to “jump start” healing, when to initiate therapy, and when to discontinue. Moreover, not all wounds respond to the same therapies. Ideally, advanced treatment would be directed at correcting the underlying biochemical defects. However, due to lack of visual cues, these defects cannot readily be identified without appropriate tests. Current wound healing assessment is based on physical tests and clinical observations, biological tests such as qualitative and quantitative culture, biochemical tests such as blood glucose or C-reactive protein (CRP), and other physiological tests such as local oxygen perfusion assessment or nutritional screening. These suggest factors that are likely to impede wound healing; however, they frequently do not identify the specific causes of delayed wound healing.<sup>12</sup>

**Assessment tools.** New diagnostic tests examining the underlying biological abnormalities of the wound bed could be of potential benefit in identifying these causes, and may support when, what, and how long to use therapies. In this context, a diagnostic tool is a test that requires little or no interpretation and provides unambiguous information regarding the diagnosis of a condition, usually without reference to other sources. By comparison, a theranostic tool is one that provides specific information in relation to the appropriateness of a particular therapy or intervention. The classic example of a diagnostic tool is the home-based pregnancy test based on the detection of human chorionic gonadotrophin in urine to confirm pregnancy. In wound care, a good example of a theranostic test would be the identification of beta-haemolytic streptococci from a wound sample, indicating the need for aggressive medical and/or surgical treatment, even if clinical signs of infection are absent.<sup>12,13</sup> (Currently this is performed using time-consuming culture techniques; a rapid, simple, cost-effective diagnostic test is not yet commercially available for wounds.) The World Union of Wound Healing Societies<sup>12</sup> has recognized that the development of appropriate diagnostic and theranostic tools in wound care will be an important step to advance treatment in the field.

Developing diagnostic or theranostic tools requires a detailed understanding of the dysfunctional wound microenvironment and how it might affect healing. A key concept gaining ground in this regard is dynamic reciprocity (DR), a term first coined by Bornstein et al.<sup>14</sup> In essence, DR describes the interaction between cells and the extracellular matrix (ECM) and has been applied to the processes of wound healing.

**Physical and chemical healing mediators.** During the normal wound-healing process, vascular damage causes extravasation of blood components and exposure of ECM proteins such as collagen, fibronectin, laminin, and the matricellular protein thrombospondin-1.<sup>15,16</sup> Binding of platelets to

#### Key Points

- Several studies have shown a relationship between high wound fluid protease levels and nonhealing.
- A panel of seven wound experts met to discuss a variety of statements related to the potential role of a simple protease diagnostic test on clinical practice.
- The panel agreed that such a test could change care, especially in outpatient settings.
- Studies to determine whether high protease levels are the cause or the result of nonhealing are needed to determine the potential role of protease-modulating treatments on chronic wound outcomes.

exposed collagen via the integrin family initiates an intracellular cascade, resulting in activation and degranulation that releases a variety of cytokines and other chemical mediators, as well as creating fibrin clots. The fibrin clot, along with bound fibronectin, serves as a repository for growth factors, proteases, and protease inhibitors, as well as a matrix that harbors cellular attachment points.<sup>16,17</sup> Immediately following hemostasis, in response to platelet-derived chemokines, neutrophils infiltrate the wound to begin the removal of cellular debris and bacteria, in addition to signaling initiation of other immune responses. This response includes the attraction of monocytes whose binding to the ECM proteins induces differentiation into macrophages and upregulation of growth factors, including transforming growth factor (TGF- $\alpha$ ) and platelet-derived growth factor (PDGF-B).<sup>16,18,19</sup>

Before the formation of new granulation tissue and blood vessels can proceed, the wound microenvironment must be sufficiently clean and organized to facilitate fibroblast migration and interaction via signals sent from the existing fibrin matrix. In addition, keratinocyte migration and proliferation following release of their attachment to the basement membrane is necessary — a course facilitated by matrix metalloproteases (MMPs).<sup>20</sup> It is important to understand at this point MMPs are beneficial. However, it is also clear from several prospective clinical and in vitro studies that pre-existing disease states (eg, peripheral vascular disease<sup>21</sup> or repeated ischemia-reperfusion<sup>22</sup>) interfere with the normal process of the wound-healing events just described or directly contribute to the formation of a wound in an internal rather than external mechanism of injury.<sup>23</sup> These factors buttress the theoretical concept that certain wounds can be characterized as healing-impaired in some fashion, and have the potential to eventually become chronic. For example, in a recent review based on several in vitro studies, Moore et al.<sup>24</sup> conjectured that in chronic wounds, lymphocyte function-associated antigen-1 (LFA-1) and very late antigen-4 levels (VLA-4) are elevated in neutrophils, macrophages, T lymphocytes, and

mast cells; intercellular adhesion molecule (ICAM-1) levels are raised in endothelial cells; inflammatory mediators are expressed at higher than normal concentrations; a pattern emerges of disordered growth factors in which receptor expression is downregulated and proteolytic degradation of growth factors advanced; and higher levels of proteases destroy the ECM, aided by opposing lower concentrations of tissue inhibitor of metalloproteases (TIMPs). Likewise, in a recent review of many clinical and in vitro studies, Schultz and Wysoki<sup>25-27</sup> have proposed that in patients with diabetes, potential abnormalities in ECM metabolism, including elevated expressions of MMPs 2 and 9 and TIMPs 1 and 2, as well as glycation of several ECM proteins (primarily collagen and fibronectin, which results in poor adhesion to cells), are factors that predispose wounds to healing difficulties.

Proteases. One recurring theme from a review of the literature<sup>28-33</sup> is the persistence of excessive proteases in stalled wounds, although no published data exist on the percentage of wounds having elevated levels. Neutrophils — ie, phagocytes recruited to the site of injury in the early stages of the inflammatory phase — often have been observed in excessively large numbers in stalled wounds; therefore, the high content of proteases produced and stored in them could be a factor contributing to chronicity under such circumstances. However, whether high protease levels (including serine-containing, metallo- and, cysteine-containing, aspartic-based proteases, and myeloperoxidases) under these circumstances are direct agents in causing damage to cellular communication factors or tissues, or whether they represent a biochemical signal that underlying pathological conditions are causing dysfunctions in normal wound healing mechanisms, is currently unknown for a given wound and will likely be a focus of future research.

Elastase. Several investigations have probed concentrations of elastase, the serine protease in neutrophils that degrades several ECM proteins, cytokines, growth factors, and cell surface receptors.<sup>28,34-37</sup> Work in the mid-1990s based on in vitro and clinical research<sup>34-37</sup> suggested that in many stalled wounds, the degradation of proteins such as fibronectin by elastase is common and is caused by elevated levels of elastase and cleavage of the proteinase inhibitors alpha 1-antitrypsin and alpha 2-macroglobulin. Other prospective clinical comparative and noncomparative investigations<sup>38-40</sup> during the same time period revealed other elastase-related proteases in both acute and stalled wounds including tissue plasminogen activator, urokinase, and proteinase-3. More recent prospective clinical comparative and noncomparative research<sup>41-44</sup> also has confirmed that neutrophil elastase degrades plasminogen in leg ulcers<sup>45</sup> and is significantly elevated in stalled wounds. Furthermore, the process of aging in healthy human subjects is associated with upregulation of this serine protease in acute wound healing.

These associations suggest these proteases signal wound deterioration. Because they are upregulated in both acute

healthy wounds and chronic venous ulcers,<sup>43</sup> they appear to be related to the tissue response to wounding. What is not known with any certainty is why the downregulation of proteases fails to occur in some wounds, a factor in the conversion of an acute to a stalled wound.<sup>43</sup> Regardless, these proteases clearly signal tissue damage. Although prospective quasi-experimental and longitudinal clinical studies<sup>33,35,37-43,45-55</sup> have shown a strong correlation of elevated protease activity in stalled, nonhealing chronic wounds, it has yet to be established what proportion of these wounds have elevated protease activity and how these might be identified, hence the need for testing.

MMPs. MMPs are a family of at least 20 mammalian, neutral pH, zinc-dependent endopeptidases that have the capability to degrade the majority of ECM proteins. Neutrophils contain large amounts of MMP-9.<sup>37</sup> Several prospective comparative/noncomparative, quasi-experimental and longitudinal clinical studies<sup>41,42,46-52</sup> also have demonstrated higher quantities of these MMPs in stalled wounds compared to healing or surgical wounds, sometimes as much as 10-fold or even 25-fold (most studies have measured levels of activated MMPs, but some have measured both activated and total levels). The level of neutrophil collagenase MMP-8<sup>37</sup> also has been found to be significantly higher in stalled compared to healing acute wounds. In addition, it is interesting to note that levels of this enzyme are higher than MMP-1 or MMP-13 in both acute and stalled wounds.<sup>28,46,51,53</sup> These latter findings might be related, in part, to the fact that collagen is the largest component of the structural protein family, and therefore require higher levels of collagenase in the normal first phase of healing. Other MMPs also have demonstrated elevated levels in stalled wounds, such as MMP-3, MMP-10, and MMP-11.<sup>28</sup>

In a prospective analysis of traumatic extremity combat wounds, Utz et al<sup>54</sup> observed that impaired wound healing was significantly ( $P < .001$ ) associated with MMP-2 and MMP-7. The authors hypothesized that the presence of these MMPs, as well as MMP-3, could effectively predict the outcome of traumatic war wounds. The prospective case series results of Ladwig et al<sup>48</sup> were framed in terms of ratios of MMPs to TIMPs with the mean MMP-9/TIMP-1 ratio in fluids decreasing significantly ( $P < .05$ ) as the ulcers healed. In addition, the mean MMP-9/TIMP-1 ratio was significantly lower for wound fluids collected at baseline from wounds that ultimately healed well compared with wounds that healed poorly. The implication, as the authors propose, is that it is the ratio of specific MMPs to TIMPs that provides the best healing prognostic. Other clinical case series and comparative longitudinal clinical studies suggest that higher levels of myeloperoxidase are present in stalled wounds,<sup>55,56</sup> but much more research will be required to determine whether myeloperoxidase levels can be used in a diagnostic fashion.

In general, cells regulate protease levels in acute wounds through genetic expression augmented by production of

counterbalancing serine protease inhibitors in the liver (eg, alpha1-antitrypsin; AAT),<sup>28</sup> protease inhibitors linked with wound healing (eg, plasminogen activator inhibitor type-1; PAI-1),<sup>57</sup> and TIMPs in proliferating keratinocytes spindle-shaped, fibroblast-like, and plump, macrophage-like stromal cells, as well as by endothelial cells.<sup>58</sup> However, like homeostasis is severely disrupted in some stalled wounds. Prospective quasi-experimental clinical studies<sup>48-53</sup> suggest that the release of proteases by large numbers of invading neutrophils overwhelm existing protease inhibitors in the local area. The inability to produce enough protease inhibitors, excessive activation of latent proteases, and breakdown of protease inhibitors by bacterial proteases present in wounds associated with high bioburden are likely responsible for impaired wound healing in some cases. However, when the immune system is responding to a high biobacterial burden, larger-than-normal release of proteases may initially be an appropriate response. It is possible that when physiological and oxidative stress is factored in, a quasi-equilibrium process can develop in which continual production of granulation tissue and its simultaneous degradation results in no new tissue being produced. Alternatively, new tissue may fail to grow because the biochemical mechanisms involved in its creation have become dysfunctional. Thus, excessive protease activity could be one of many causes of impaired wound healing. However, local or systemic existing diseases or conditions (eg, venous reflux disease, high glucose levels associated with diabetes mellitus, pressure, or ischemia) are often the primary cause that results in disruption of the relevant homeostatic processes involved in normal wound healing, including the elevation of proteases. It is also likely that in some chronic wounds tissue is damaged by mechanisms other than excessive levels of proteases.

To further understanding of the observed correlations, many questions must still be answered. Specifically, future studies must explore under what circumstances protease levels are raised — ie, if they are the result of underlying pathological conditions that should be addressed (if they can be) or if they are a primary cause of nonhealing and levels should be lowered. Although systemic glucocorticoid use impairs wound healing, a 3-month prospective study found topical application beneficial.<sup>59</sup> Per in vitro study,<sup>60</sup> one mechanism by which this might occur is reduction of MMP activity. Prospective, quasi-experimental clinical studies<sup>61,62</sup> have documented the use of doxycycline, an MMP inhibitor, to lower excessive MMP levels. Additionally, a review and an in vitro study design<sup>63,64</sup> suggest that dressings containing oxidized regenerated cellulose also may have the potential to lower protease activity levels as well as oxygen-free radicals and excessive levels of metallic ions while minimizing the degradation of growth factors.

With the knowledge that excessive protease activity levels either can be directly harmful to wound healing or signal major dysfunction in the wound healing process, and because

the possibility that approaches to bring them back into balance might facilitate the healing process, a method is needed to easily identify wounds that have large protease imbalances. A point-of-care (POC) diagnostic protease test to measure inflammatory proteases is currently under development. Because a variety of advanced treatments are available that can potentially address existing protease activity imbalance and perhaps facilitate healing, these advanced treatments might be more cost-effective if they can be used in a timely fashion and on appropriate wounds. In addition, discontinuing or not initiating treatments that might not be useful under such circumstances could save costs.

To address how a POC test that measures inflammatory proteases should be employed, a group of wound care experts (the Protease Diagnostic Consensus Panel) gathered in Miami, FL on May 14, 2011 to reach consensus on a number of issues regarding use of such a tool. The format of the discussion followed a series of questions that were posed to the panel for several topics with the discussion moderated by Robert Snyder, DPM.

The goal of this paper is to present the issues discussed and the consensus reached on each topic, which builds on the international consensus meeting held in February 2011 regarding the role of POC protease tests in clinical practice.<sup>11</sup>

### Consensus Process

The internationally recognized panel of wound care experts comprised seven members: two podiatrists, two vascular surgeons, a physician expert in hyperbaric oxygen therapy, a physical therapist with a specialty in home health, and a registered nurse. The panel was provided relevant literature in preparation for the consensus meeting. Using a pre-specified agenda that contained several topics and subtopics relating to the role of protease tests, the moderator presented one topic at a time for the panel's consideration. Following initial discussion, a consensus statement for each topic was proposed by the moderator. (For the purposes of this article, the literature supporting the panel's consensus statement is noted.) If the consensus was not unanimous, further discussion ensued to refine the statement(s) until consensus was unanimous. Topics that took the most time to reach consensus were: 1) what constitutes a chronic wound, 2) which factors make a wound hard to heal, and 3) how should protease imbalances be managed.

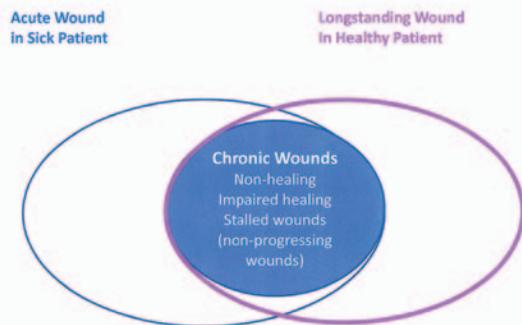
### Concept of the Protease Test

Based on unpublished data from Sibbald et al, the panel agreed wound etiologies and patient-centered concerns must be assessed by performing a complete history and physical examination before considering the use of a protease test.

Some of the test's keys attributes should<sup>12</sup>:

- Be clinically relevant
- Be able to measure a single protease (or a set of proteases) and present a single result

## Venn diagram describing non-healing wounds



**Figure 1.** Stalled wounds can form from acute wounds in patients having certain comorbidities or long-standing wounds in a healthy patient in which the local wound biochemistry is abnormal. In this context, long-standing could mean a wound age of 1 month to several years. Although healthy and sick are relative terms, a sick patient would have one or more significant comorbidities, while a healthy patient would not have any significant comorbidities.

- Clearly indicate the need for (or inappropriateness of) a specific therapy
- Be appropriate for use by clinicians with different skill sets, as well as community nurses or possibly the patient/caregiver
- Be quick, easy to use, and easy to interpret
- Be used at the point of care
- Have the potential to support enhancing the standard of care by aiding the clinician in selecting the most cost-effective type of treatment, treatment duration, and reasonable stopping points
- Be accurate, reliable, sensitive to change, provide reproducible results in normal use, be unaffected by other substances present in the test sample, and be easily correlated with reference test methods
- Be cost-effective (ie, decreased cost of failure to heal by way of decreasing inappropriate therapies; this will encourage cost appropriate/effective versus the most expensive therapy).

### Protease Science

Definition of a stalled or chronic wound. The consensus panel agreed that defining a stalled or chronic wound is challenging but proposed the following as an operational definition: A stalled or chronic wound is a wound in which the severity of a single comorbidity or multiple systemic comorbidities interfere with the normal healing process. Chronicity is not necessarily dependent on the time since the wound was first formed.<sup>11,65-68</sup>

Although disease states interfere with wound healing, such states do not automatically mean wound healing will

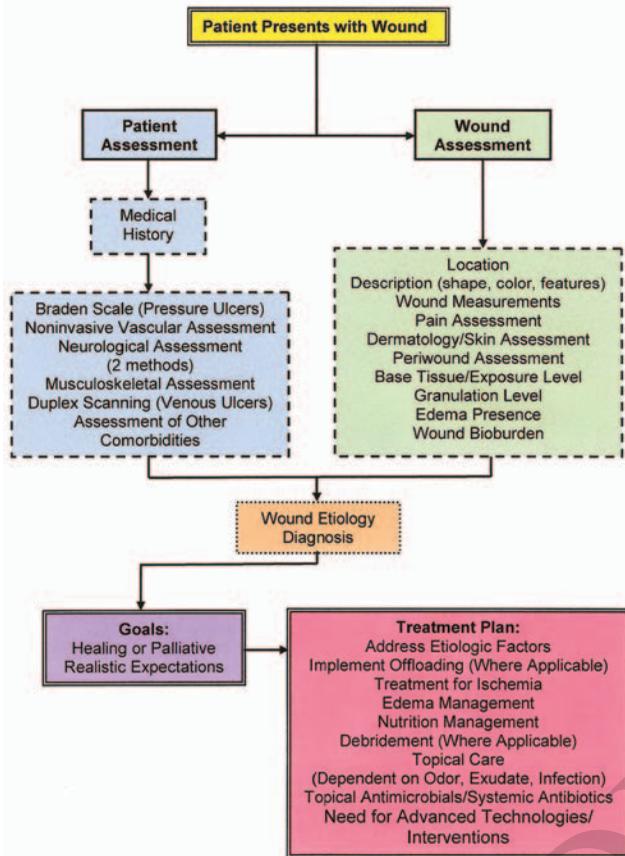
be impaired or that the wound becomes stalled after inception. However, an acute wound can become stalled because a patient has certain comorbidities or the local wound biochemistry is abnormal in an otherwise healthy patient (see Figure 1). Many diabetic foot ulcers heal within a reasonable period of time, which means that having diabetes is not sufficient by itself to create wound-healing impairment; rather, such factors as blood glucose control, HbA1c levels, and duration of diabetes are likely to determine the likelihood of wound-healing problems.<sup>69-71</sup> Operationally, this has been translated to whether a diabetic foot ulcer achieves a 50% reduction in percent area reduction within 4 weeks. Wounds that do not meet this target are likely to have an unsatisfactory wound healing trajectory and may be stalled wounds.<sup>72,73</sup> Nevertheless, in individual patients who have comorbidities that can be translated to pathological processes at the cellular level, it can be difficult to ascertain whether a cutaneous injury can be considered an acute wound that will heal relatively quickly or stall.

The term chronic implies a long duration and may not include acute wounds that have healing problems or encompass wounds that have healing problems immediately after formation. Alternate terms that have been used in the literature include delayed, stalled, hard-to-heal, recalcitrant, difficult, complex, or fail to respond,<sup>74</sup> although none of these terms individually convey additional useful information. Thus, while the term stalled wound is retained here, the panel redefined it in broader terms.

Factors that impair wound healing. The consensus panel discussed that many factors can make the wound hard to heal when considered in the context of proteases. The factors can be divided into four categories: patient comorbidities, patient factors, patient medications, and wound microenvironment (see Table 1).

Importance of proteases to healing. The panel agreed that appropriate protease activity was important in healing. Under normal phases of healing, several different proteases are involved in degrading ECM-based wound debris, as well as in digesting bacteria. In addition, certain MMPs are necessary to allow keratinocytes to migrate by releasing them from basement membranes, and through ECM degradation proteases play a role in ensuring that increased cell density, changes in cell shape, and changes in tension occur so granulation tissue can form.<sup>16</sup> In simple terms, protease activity is an essential part of wound healing.<sup>11,113</sup> The panel agreed that when protease homeostasis is disrupted, leading to excessively high activity levels of proteases, wound healing can stall.

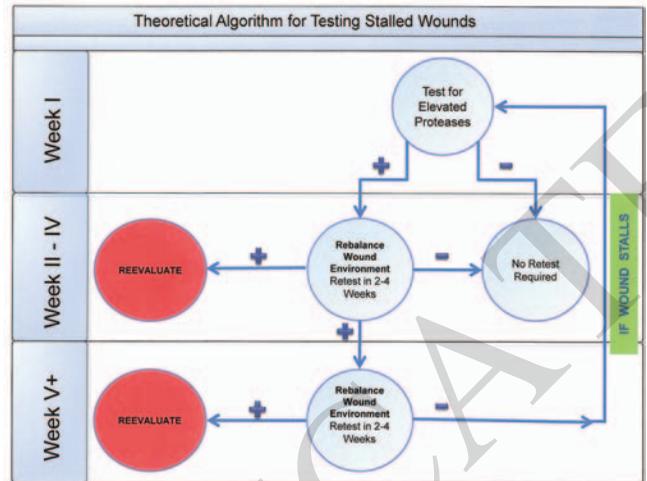
Point-of-care wound testing: Are proteases a good place to start? Consensus also was reached that a simple protease test would be extremely useful in clinical practice. Although clinicians often suspect that many nonhealing, stalled wounds have gross imbalances of protease activity in opposition to inhibitors, it cannot be known for certain unless the levels of key biochemical entities are measured. In the words of one



**Figure 2.** Typical pathway of care for patient presenting with a wound at first visit.

panel member, “One cannot manage what one cannot measure.” The results of such biological chaos mean the wound bed cannot be properly prepared and that most wound healing alone is unlikely to be successful, so a need to address this underlying problem remains. Furthermore, the use of some expensive advanced therapies, such as bioengineered human skin equivalents (HSE) or negative pressure wound therapy (NPWT) might be inappropriate because they are more likely to fail. This consensus panel, as well as previous international consensus panels,<sup>11,12</sup> agreed that simple tests based on key enzymatic markers — ie, proteases — would be good choices. Moore et al<sup>24</sup> endorsed the concept more than 4 years ago. However, it also must be remembered that a test of proteases as a general marker of inflammation does not constitute a specific diagnosis of the cause of wound healing failure or delay; rather, high activity levels of proteases are responses to an underlying stimulus that must be specifically identified.

What proteases should be measured? The panel generally agreed that because proteases have overlapping functions, measuring just one individual protease would be unlikely to provide a good overview, and therefore might not be representative of the proteolytic environment of the wound. Measuring the key proteases known



**Figure 3.** Theoretical algorithm for testing stalled wounds.

to be present and in excess in stalled wounds would be the correct approach and would give the clinician more valuable information.

### Products that Modulate Proteases: Utilizing Test Results

Available treatments/therapies/modalities. The panel agreed that a variety of treatments are available that could be used to reduce excess protease activity (see Table 2).<sup>11</sup>

Management of protease imbalance. The panel’s consensus on managing protease imbalance is to first assess wound bioburden and prepare the wound bed through appropriate standard measures such as cleansing and debridement. If bioburden is high or an infection is present, topical antimicrobials and systemic antibiotics, respectively, should be used, because bacteria have proteases that may contribute to impaired wound healing. Specific therapies, such as MMP inhibition or use of oxidized regenerated cellulose dressings, also can be utilized to bring protease activity levels into balance.<sup>120</sup>

Some wounds may need protease modulation: which ones? The panel agreed that no diagnostic or theranostic tests currently are available to detect high protease activity levels in a wound. Panelists likened the situation to a process of trial and error or intuition in selection of and response to more advanced therapies such as NPWT, more absorptive dressings, or oxidized regenerated cellulose dressings to confirm that a protease imbalance exists. The difficulty in establishing whether high activity levels of proteases are interfering with wound healing based on mere clinical observation and the absence of visual cues already has been discussed in the most recent international consensus document.<sup>11</sup> Moreover, if the clinician is unaware of the issue of proteases and healing, healing a stalled wound is likely to present very challenging problems. Finally, the ability to detect and possibly reduce high levels of proteases if they are found does not

Table 1. Some factors that might be implicated in impaired wound healing

Patient comorbidities	Patient factors	Medications	Microenvironment
Diabetes <sup>75-77</sup>	Old age (≥80-85 years of age) <sup>76,77,95</sup>	Steroids <sup>6-10</sup>	Senescent cells <sup>108</sup>
Autoimmune diseases <sup>76,78</sup>	Poor or no insurance <sup>86a</sup>	Methotrexate <sup>105</sup>	Wound bioburden/infection (in particular biofilms, bacterial types, and virulence factors) <sup>109,110a</sup>
Hemodialysis <sup>87,88a</sup>	No social/family support <sup>96,97</sup>	Hydroxyurea <sup>106a</sup>	High cytokine levels <sup>28,111</sup>
Obesity <sup>76,77,81</sup>	Problems with activities of daily living (ADL) <sup>98</sup>	Chemo/modulatory drugs <sup>105,107</sup>	Abnormal enzyme and substrate levels <sup>23,28</sup>
Malnutrition <sup>76,82,83</sup>	Smoking <sup>80a,98,99,100a</sup>	Radiation (eg, cancer therapy) <sup>107</sup>	Necrosis (ie, necrotic tissue) <sup>86a</sup>
Malabsorption (nutrients) <sup>84</sup>	Use of alcohol <sup>102</sup>		Hypoxia <sup>76,80a,88,98a</sup>
Weight-bearing (on wound) <sup>83,85,86a</sup>	Poor hygiene <sup>103</sup>		Reactive oxygen species (ROS; excessively high levels) <sup>23,28</sup>
Hepatitis <sup>87</sup>	Lack of adherence or compliance with prescribed treatments <sup>104</sup>		Presence of immunohistochemical markers <sup>23,28</sup>
Peripheral arterial disease (PAD) <sup>88,89a</sup>			Nitric oxide (NO) levels <sup>112</sup>
Vasculitis <sup>90</sup>			Growth factors and receptor sites (abnormal concentrations or dysfunction) <sup>24,25</sup>
Chronic obstructive pulmonary disease (COPD) <sup>86a</sup>			
Sleep apnea <sup>91a</sup>			
Sickle cell disease <sup>92</sup>			
Neuromuscular diseases and deformities, including idiopathic neuropathy <sup>93,94</sup>			

<sup>a</sup> Includes a formal analytical procedure that evaluated risk factors for impaired wound healing or clinical study with outcomes suggestive of a factor that could be implicated in wound healing

automatically mean that the wound will heal. Rather, it is hoped the additional data gathered will assist in determining what kinds of chronic wounds might benefit from specific advanced therapeutics once it is learned that: 1) high protease levels delay healing, and 2) high levels of proteases are present.

The consensus of the panel was that the development of a simple, widely available protease diagnostic test could dramatically change the current standard of care.

### When to Start and Stop Treatment

Current pathway of care and optimal time for protease testing. The general approach agreed upon by the consensus panel is that when a patient first presents with a wound, both patient and wound assessment occurs (see Figure 2). During this process (see dashed boxes, Figure 2), a wound may be recognized as chronic or stalled, and therefore the determination of protease activity becomes important. For certain kinds of wounds, a test could be taken as a baseline measurement (ie, before treatment), to establish whether the proteolytic environment is in balance.

### Diagnostic Protease Tools to Change Practice: Clinical and Economic Impact

Factors governing use. The consensus panel suggested that timing of when to use a POC protease test depends on several factors. Generally, when a wound does not have a satisfactory wound healing trajectory, a protease test would be

appropriate to rule in or rule out excessive protease activity.<sup>11</sup> Based on clinical findings, for some wounds this could be 4 weeks after the initial visit, but for other wounds an immediate test could be warranted — for example, when it already has been established that the wound is not healing despite best care or if the patient is at high risk for complications. If a protease test is performed and the findings are positive and a protease modulating therapy is instituted, 2 weeks could be allowed to elapse before retesting and evaluation in order to give the therapy time to work (see Figure 3). The panel decided that retesting every 2 weeks with adjustment of treatment is appropriate until it is established that the wound environment is in balance with regard to protease activity levels. This initial recommendation may be modified by the acquisition of further data.

Setting. The panel discussed several settings and grouped them as acute care facilities, outpatient clinics, home care, or long-term care facilities.

Acute care facilities. A protease test may be appropriate in this setting in the chronically ill patient or patient who is immunocompromised and whose wound is nonhealing and/or noninfected. The test would be administered after cleansing the wound. The panel agreed that, in general, such patients have been admitted for reasons unrelated to the wound — for example, an elderly patient admitted for pneumonia who also has a pressure ulcer on admission that developed in a nursing home. The panel also agreed protease testing would be limited in this setting, it would be

Table 2. Commonly used treatments to remove devitalized tissue and/or reduce wound exudate levels<sup>11</sup>

Treatment	Action/potential direct or indirect effect on protease levels
Cleansing Irrigation, jet lavage, etc.	Removes surface debris and protease-containing wound fluid
Debridement	Removes necrotic tissue and bacterial load
Protease modulators <sup>62,63,113,114</sup>	Protease-modulating biomaterials that bind to and inactivate proteases (MMPs and elastase)
Anti-inflammatories Steroids <sup>58,59,115</sup> Doxycycline <sup>60,61</sup>	Upregulates anti-inflammatory proteins, downregulates pro-inflammatory proteins Protease inhibitor
Antimicrobial dressings <sup>116,117</sup>	Silver-impregnated dressings may reduce host/bacterial protease production; may reduce MMP activity
Dressings/devices that absorb/remove wound exudate <sup>118,119</sup>	May reduce protease activity by removing proteases in wounds

essential for the physician and nurses to understand the nature of the protease test, and that appropriate action based on the result would be taken.

**Outpatient clinic.** Several members of the panel suggested a protease test might be of benefit in a wound care or outpatient clinic in choosing an advanced dressing or before use of split-thickness skin grafts, NPWT, or human skin equivalents in which it is important to verify not only absence of infection before proceeding,<sup>121</sup> but also that the wound microenvironment is balanced with regard to inflammation and protease activity levels. In other words, a POC test could serve as an objective quality check of the wound bed.<sup>122</sup> Another use might be to use the test before any major change of care (ie, the use of another advanced therapy).

**Home care.** The panel agreed that, at present, the test as contemplated would be performed by a healthcare professional (ie, a nurse) under the direction and orders of a physician. If a protease test POC is as easy to use as a home pregnancy test, the panel also agreed that, at some point in time, with appropriate education, and under physicians orders, the patient or a family member conceivably could be taught to perform the test and report results.

**Long-term care.** Long-term care facilities, such as long-term acute care, skilled nursing, assisted living, inpatient rehabilitation, and independent living could reap the benefits of a POC protease test. The panel agreed that skilled nursing is very cost-sensitive; therefore, decisions to stop expensive long-term treatments that are no longer working (ie, advanced wound dressings) based on POC protease tests would be of direct benefit to patients because they reduce unnecessary care and of economic benefit because they would reduce costs, risk of complications, and number of physician visits.

**Economic impact.** The panel unanimously agreed that the cost of treating wounds that fail to heal is enormous. Any test that can indicate that a particular treatment is appropriate or inappropriate will not only save money, but also could enable the wound to heal faster with attendant quality-of-life improvements for the patient, as well as lower the risk of further complications.

### Diagnostic Protease Tools to Change Practice: Adoption and Validation

Ultimately, to be widely accepted and used outside of the laboratory as a POC test, a protease test must be Clinical Laboratory Improvement Amendments- (CLIA) waived — ie, a simple laboratory examination or procedure cleared by the Food and Drug Administration (FDA) for home use; employs simple and accurate methodologies that make the likelihood of erroneous results negligible; or poses no reasonable risk of harm to the patient if the test is performed incorrectly.<sup>123</sup>

The panel discussed the fact that validation of a test in various settings could start by using a survey in one clinical center to gain proof-of-concept experience, followed by the development of a registry in which the outcomes of thousands of tested wounds associated with testing could be subject to more robust inquiry vis-à-vis time to healing, hospitalization, amputation, and other associated comorbidities.

### Level of Evidence

The level of evidence of studies and their descriptions to support the consensus points in this document are shown in Table 3,<sup>13,26,27,29,32-34,36,38-56,58,59,62-64,66,69-73,79-81,86,87,89,91,99,101,106,108,111,113,114,118,119,121</sup> available online with this article at [www.o-wm.com](http://www.o-wm.com). Evidence-based medicine classification followed Table 1 of the

review by Carter.<sup>124</sup> References to reviews, commentaries, workshops, and clinical practice guidelines are not included in Table 3.

### Conclusion

The panel was unanimous that wound care lacks specific diagnostic or theranostic tests that can be utilized to direct specific therapies. Many chronic wounds become stalled or stuck in the inflammatory phase of healing despite good basic wound care and lack of objective clinical signs. This lack of clinical objectivity may result in increased time to healing, risk for increased complications, and subsequent resource utilization. This increased ambiguity, complexity, and subsequent morbidity carries both patient-specific and economic considerations. A POC protease test used judiciously could mitigate such risks, improve the economics of wound care, and improve the patient's quality of life by identifying those wounds in which protease levels are elevated.

A POC protease test that is as easy to use as a home pregnancy test holds the potential for a significant change in the care pathway because less guesswork would be involved. The panel suggests that next phases at that point would involve proof-of-concept trials and registry development, followed by trials to determine diagnostic parameters under controlled conditions in various settings and wounds of different etiologies.

The panel unanimously agreed that successful adoption of a POC diagnostic test that measures inflammatory proteases would be a revolutionary step toward improving the treatment of stalled wounds and personalizing wound healing.

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