such, testing wound fluid for bacterial protease activity could be a useful method to detect pathogenic bacteria that are capable of causing infection prior to the appearance of clinical signs.

What is the WOUNDCHEK™ Bacterial Status test?

WOUNDCHEK™ Bacterial Status is an innovative lateral flow test for the qualitative assessment of bacterial protease activity directly from a swab sample from a chronic wound. WOUNDCHEK™ Bacterial Status is intended for diagnostic use, at the point of care, as an aid in the healthcare professional's assessment of whether a wound may become non-healing due to bacterial pathogenesis as indicated by the presence of bacterial protease activity.

The WOUNDCHEK™ Bacterial Status test uses chronic wound fluid from the wound, collected using a method known as the Serena Technique®, whereby the cleansed wound is moistened with saline and the surface is swabbed with a rolling action until the entire swab foam tip is coated (Serena, 2014). A positive result of the test indicates the presence of BPA in the wound. This detection may allow for earlier clinical intervention to prevent the wound from continuing along the infection continuum to more serious clinical infection or sepsis.

What do results of the BPA test mean in practice?

Since presence of BPA is indicative of impending or active infection, its detection could allow recognition of bacteria behaving pathologically, even without clinical signs of infection, prompting treatment to reduce bacterial burden.

A positive result on the BPA test alerts the clinician to the presence of bacteria in a wound behaving pathogenically, allowing them to determine in an objective manner if bacterial burden needs to addressed when clinical signs may not be immediately apparent, and intervene prior to the development of frank infection.

As seen in the pathway for use of the point-of-care bacterial protease test provided in Figure 3, the test result allows the clinician to improve care of the patient, by:

- **Implementing measures to reduce the wound's bacterial burden, such as optimising host response, preventing further wound contamination, debridement, increasing the frequency of dressing changes or using topical antiseptics like silver or PHMB**
- **Monitoring and reassessing the wound regularly**
- **Continuing standard care, as appropriate.**

If healing is delayed but the result is negative and clinical signs of infection are absent, the clinician is able to choose an alternative approach to care, such as testing for elevated protease activity or considering protease-modulating interventions.

Bacteria in biofilms can secrete proteases and have been shown to produce them at greater levels than when in the planktonic state (Evans et al, 1994). Wounds that test positive on the BPA test may also contain a biofilm on at least some of wound bed (Attinger et al, 2015). Although it is not known whether the BPA test could be used to indicate biofilm presence, many of the treatments used for a BPA positive wound are also used for biofilm management, such as barrier dressings, debridement and antiseptics (Howell-Jones et al, 2005; Phillips et al, 2010; Rhoads et al, 2008).

As an adjunct to existing wound assessment techniques, which are generally subjective in nature, use of this test could have a number of benefits, both clinical and economic. In an era of increasing antibiotic resistance, solutions that could reduce their overuse and support the targeted use of antibiotics are valuable to the wider healthcare system (Lauchli et al, 2015). Indeed, where diagnostic tests are able to successfully guide clinician decisions, this could lead to reduction in long-term complications (for example, loss of limb) and improvements in patient quality of life (such as reduction in pain).

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USE OF BACTERIAL PROTEASE ACTIVITY: CASE STUDY

BACKGROUND

This patient had a diabetic foot ulcer to the right plantar aspect. The wound had no signs of clinical infection when reviewed at Week 1 and Week 4, but tested positive on the test for BPA on both occasions. By Week 5, the patient had a number of signs of infection and had been referred to surgery for amputation.

Week 0:

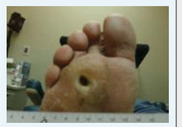
- No clinical signs of infection
- However, tested positive for BPA
- Treatment provided: Mepilex & Mepitel

Week 4:

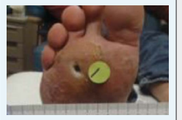
- No clinical signs of infection
- However, tested positive for BPA
- Treatment provided: Aquacel

Week 5:

- Patient was bedridden with chills and pains
- Foot was swollen and there was increased odour
- Third digit purple with a 9cm x 8cm area of redness on dorsal foot
- Patient referred to surgery for amputation



Week 0



Week 4

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Summary

The presence of bacteria on the surface of a wound may not necessary indicate infection or healing impairment. Indeed, in many instances healing still occurs despite the existence of bacteria. However, when bacteria start to behave pathogenically complications can occur, including local or systemic infection. Recent studies have demonstrated that BPA can be used as an objective marker for bacterial pathogenicity, even when typical clinical indicators of infection are not apparent. The innovative WOUNDCHEK™ Bacterial Status lateral flow test provides qualitative assessment of BPA directly from a chronic wound swab sample. This detection may allow for earlier clinical intervention to prevent wounds from continuing along the infection continuum to more elevated infection status.

BPA made easy

Wounds International November 2016 www.woundsinternational.com

Introduction

The mere presence of bacteria in chronic wounds may not indicate infection or impaired wound healing. Contaminated wounds contain bacteria but there is no human/host reaction, while in colonised wounds there may be a host reaction without healing being affected. However, there is a point at which bacteria do begin to inhibit healing, resulting in local and, eventually, systemic infection if left untreated. This point of pathogenicity can be associated with when the bacteria begin to secrete proteases. This Made Easy discusses bacterial protease activity (BPA) as an objective marker of pathogenicity and a means of identifying wounds progressing towards infection.

Authors: Dr Paul Chadwick. Prof. Franck Duteille, Prof. Marco Romanelli, Tomas Serena, Dr Severin Laeuchli, Dr. José Luis Lázaro Martínez.

Box 1 Host proteases: a review

Increasingly, it is being acknowledged that host and bacterial cells may work synergistically to cause tissue breakdown in the wound bed (McCarty et al, 2011).

The major protease groups involved in wound healing are matrix metalloproteinases and serine proteases (e.g. human neutrophil elastase). During normal wound healing, proteases break down damaged extracellular matrix proteins and foreign materials, allowing for wound closure (Wounds International, 2011).

Evidence shows that in some chronic wounds proteases are markedly elevated; these excess proteases – produced by the host cells – damage the extracellular matrix, and degrade growth factors and their receptors (Wounds International, 2011).

Box 2 Useful definitions (Lauchli et al, 2015)

- **Pathogen:** a microorganism that causes or is able to cause disease
- **Pathogenicity:** the ability of a microorganism to cause disease
- **Quorum sensing:** interbacterial communication that regulates gene expression according to the population density of bacteria
- **Virulence:** a quantitative measure of the likelihood that a pathogen will cause disease
- **Virulence factor:** a molecule produced by bacteria to facilitate colonisation, replication and spread within a host

What are bacterial proteases?

Proteases are enzymes that act on protein molecules, breaking them down into peptides and amino acids. Bacterial proteases are virulence factors known to be secreted by a number of bacteria commonly seen in chronic wounds, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis* and *Enterococcus faecalis* (Koziel and Potempa, 2013).

How do bacterial proteases influence the development of infection?

Bacterial proteases degrade host tissue proteins, impair host immune defenses and aid the local and systemic spread of bacteria. Bacterial proteases hinder immune cell function by suppressing chemotaxis, preventing phagocytosis and impeding immune cell communication (Figure 1) (Finlay and Falkow, 1997; Wilson et al, 2002; Koziel and Potempa, 2013). In addition, bacterial proteases can stimulate the production of human/host proteases via immune system activation (Box 1).

What is the wound infection continuum?

While many wounds heal successfully despite the presence of bacteria, for other wounds, it can cause various complications, such as:

- **Tissue breakdown**
- **Pain**
- **Impedance of the wound's healing ability and delayed healing**
- **Life-threatening complications, such as systemic infection.**

The influence of bacteria in a wound can be described on a continuum of clinical importance (Figure 2), with increasing levels of vigilance or intervention required where the wound is in a state of pathogenicity, local infection or systemic infection. The different stages of clinical importance are determined by bacterial pathogenicity, as well as the host response and signs of inflammation or tissue damage.

What challenges could be faced when assessing wounds affected by bacteria?

Lack of clinical signs:

The host response to bacteria and their proteases often includes inflammatory markers such as interleukin-1 beta (IL-1β) or tumour necrosis factor-alpha (TNF-α). However, the typical clinical signs of infection may not always be present if the patient's inflammatory response is weakened, such as where comorbidities like diabetes or immunosuppressive conditions are present (Serena et al, 2015).

Bacterial Proteases made easy

Wounds International

PRODUCTS FOR PRACTICE

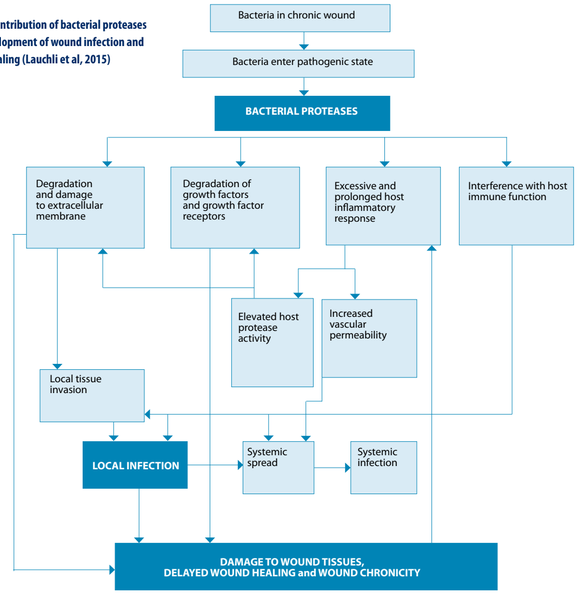


Figure 1: Contribution of bacterial proteases to the development of wound infection and delayed healing (Lauchli et al, 2015)

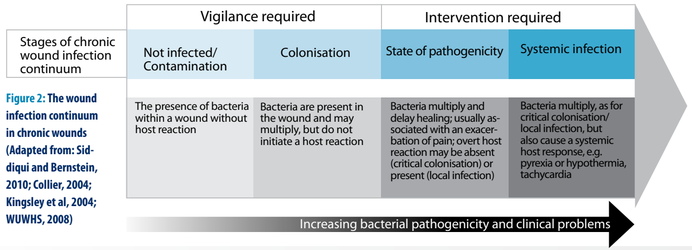


Figure 2: The wound infection continuum in chronic wounds (Adapted from: Siddiqui and Bernstein, 2010; Collier, 2004; Kingsley et al, 2004; WUWHS, 2008)

Study 1 (Serena et al, 2015) – evaluated the relationship between total bioburden and BPA levels in wounds.

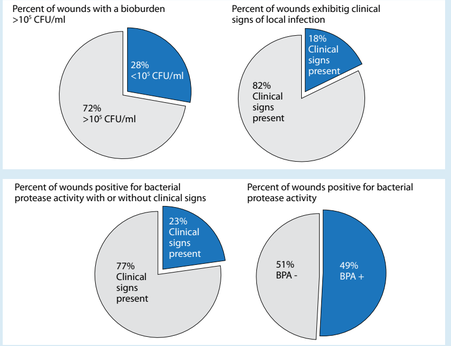
Duplicate swabs were taken from 186 chronic wounds. One swab was tested on a prototype rapid lateral flow point-of-care bacterial protease test, while the other was extracted for testing in a laboratory protease assay (using casein as substrate, including an inhibitor of HNE ['iCasein' assay]), for analysis of cytokines IL-1β and TNF-α, and for culture for quantitative microbiology.

Results showed that mean levels of pro-inflammatory cytokines IL-1β and TNF-α are significantly higher in wounds with a positive BPA test, compared with wounds that tested BPA-negative (p<0.0001 and p=0.0002, respectively). Moreover, when BPA activity was tested against bioburden, a markedly higher number of wounds yielded positive results on a rapid point-of-care BPA test when the total bioburden exceeded 105 CFU/ml.

Study 2 (Serena et al, 2015) – evaluated the relationship between BPA levels, clinical signs of infection and wound bioburden.

Wound fluid swabs were taken from 366 chronic wounds (including pressure ulcers, diabetic foot ulcers, and other non-healing wounds). These were extracted for testing in the 'iCasein' assay and clinician assessment for clinical signs of infection, based on the 'NERDS' acronym (Non-healing, Exudative, Red and bleeding, Debris, Smell - presence of three of more 'NERDS' is considered indicative of local infection (Sibbald et al, 2003). Where an iCasein activity threshold of 125 mUnits/swab was exceeded, the wound was classified as BPA-positive. An additional swab was taken for culture and quantitative microbiology.

Of the tested wounds, 72% had bacterial counts that exceeded 105 CFU/ml, indicating elevated bioburden. Only a small proportion exhibited three or more clinical signs of infection (18%). In contrast to bioburden and clinical signs, more than half of the wounds swabbed positive for BPA (51%); over three quarters of these wounds (77%) did not exhibit local signs of infection. It is suspected that they were in a pathogenic state, which had not yet progressed to overt infection.



Difficulty interpreting microbiological investigations:
While a bacterial load of >1x105 CFU/g is typically used for diagnosing wound infection, this threshold may not be appropriate for all wounds; for example, healing may be delayed below this threshold in patients with impaired immune defences or if particularly virulent bacteria are involved, whereas other wounds with bacterial bioburden above this threshold may heal without intervention. Moreover, in some instances microbiological examinations can be difficult to interpret, especially when multiple bacteria are present. As such, culture results should not replace clinical judgement, but can be used to guide choice of antimicrobial therapy, where appropriate (Sibbald et al, 2003; Healy et al, 2006).

Chronic inflammation versus infection:
Wounds may be stuck in a perpetual cycle of inflammation only partially attibutable to bacteria; this damages the extracellular matrix and degrades growth factors, which stimulate release of inflammatory mediators to cause a heightened inflammatory response, further damaging tissue and delaying healing. As such, it is important to differentiate between inflammation and infection (Gardner et al, 2001; Dissemond et al, 2013).

Could bacterial protease activity be a useful marker for pathogenicity?

Bacteria in chronic wounds can be non-pathogenic or pathogenic in nature. One indication of the pathogenicity of bacteria is expression of enzymatic virulence factors, such as bacterial proteases, which may stimulate excessive inflammation in the host and, eventually, damage wound tissue and hinder immunologic response.

The results of two recent studies conducted by Serena et al (2015) demonstrate that bacterial protease activity (BPA) can be used as a marker for a 'period of pathogenesis' in chronic wounds (Study 1 and Study 2). As

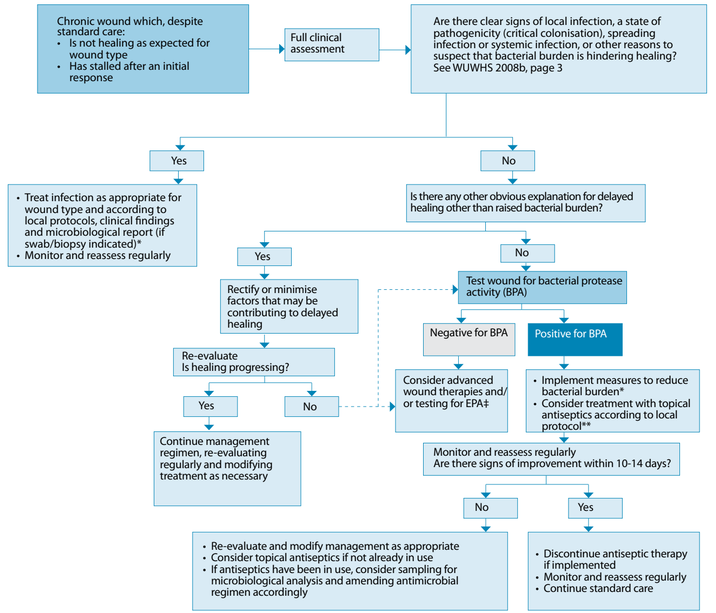


Figure 3: Pathway for the use of point-of-care test for bacterial protease activity (Adapted from: Lauchli et al, 2015)

Incorporate into management plan:
- Optimisation of host response: nutrition, hydration, glycaemic control, tissue perfusion
- Reduction of bacterial load: prevent further contamination or cross-contamination, facilitate wound drainage, debride wound, increase dressing change frequency, cleanse wound at every dressing change, manage excess exudate, manage malodour, topical antiseptic +/- systemic antibiotic(s)
- General measures such as management of symptoms, patient and carer education, optimise patient cooperation, ensure psychosocial support [WUWHS, 2008b]

**Systemic antibiotics are usually reserved for patients with spreading or systemic infection; avoid use of topical antibiotics [WUWHS, 2008b]
‡ If positive for elevated protease activity (EPA), consider incorporating protease-modulating interventions into management [International Consensus, 2011; Dissemond et al, 2013]
NOTE: Some patients with a DFU and bacterial bioburden may not show clinical signs of infection due to neurological, immunological or vascular conditions. It may be beneficial to test earlier than 15 days in such cases.