BACTERIAL PROTEASES: A MARKER FOR A 'STATE OF PATHOGENESIS' IN CHRONIC WOUNDS

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Introduction

Chronic wounds are open to the environment and are susceptible to contamination by bacteria, potentially leading to infection. Some of the consequences of a chronic wound infection are tissue breakdown, pain, additional impedance of the healing ability of the wound, amputation (e.g. in a diabetic foot ulcer) and systemic infection, which can be life-threatening[1,2]. Identifying infection in chronic wounds is challenging because current clinical practice employs using clinical signs and symptoms ('NERDS'[3]) which are not necessarily distinct from other conditions, such as chronic inflammation[4]. Bacteria and their proteases can stimulate a pro-inflammatory host response[5] and, eventually, clinical signs due to this inflammatory response and tissue damage may be seen (figure 1).[6]

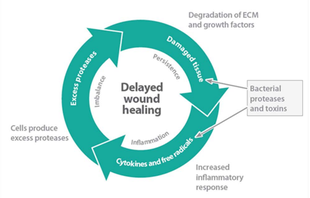


Figure 1. Cycle of chronic wounds showing involvement of bacterial proteases (ECM = extracellular matrix)

The host response often includes elevated inflammatory markers, e.g. cytokines including tumour necrosis factor alpha (TNFα) and interleukin-1 beta (IL-β)[5]. Unfortunately, clinical signs may not be apparent if the inflammatory response is impaired or defective (e.g. when other co-morbidities are present, such as diabetes or immunosuppressive conditions), thereby increasing the risk of infection[2,7]. Bacteria are in a pathogenic state when they are either in the process of, or they are capable of, causing disease, i.e. infection. One indication of pathogenicity is the production of enzymatic virulence factors or bacterial proteases[8]. The detection of bacterial protease activity (BPA) in a chronic wound would be indicative of the presence of bacterial pathogenesis which is a precursor to clinical signs and symptoms of infection[1,9]. Bacterial pathogenesis is undesirable since, at this stage, the wound is in a part of the wound infection continuum that typically requires intervention (figure 9).

Methods

Study 1: Duplicate swabs were taken from 186 chronic wounds (including LU, DFU, PU & other non-healing wounds) and comingled to ensure homogeneity. One swab was extracted for testing in a laboratory protease assay using casein as substrate, including an inhibitor of human neutrophil elastase ('iCasein' assay) and for analysis for cytokines IL-1β and TNF-α and for culture for quantitative microbiology. The second swab was tested on a prototype rapid lateral flow point of care bacterial protease test. The BPA is expressed as line intensity on the lateral flow test line evaluated using a visual analogue scale (VAS). Appearance of any test line (i.e. ≥0.25 VAS) on the bacterial protease test is indicative of presence of BPA.

Study 2: Wound fluid swabs were taken from 366 chronic wounds (including LU, DFU, PU & other non-healing wounds) were extracted for testing in the iCasein assay and assessment by the clinician for the number of clinical signs of infection ('NERDS') present. Wounds were classified as BPA positive if an iCasein activity threshold (125 mUnits/swab) was exceeded. An additional swab was taken for culture and quantitative microbiology.

Results

Results from Study 1 are summarised in figures 2, 3 & 4. The mean levels of pro-inflammatory cytokines IL1-β (figure 2) and TNF-α (figure 3) are significantly higher in wounds that are BPA positive versus wounds that are BPA negative.

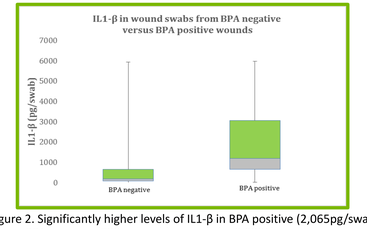


Figure 2. Significantly higher levels of IL1-β in BPA positive (2,065pg/swab) versus BPA negative (722pg/swab) wounds (p<0.0001)

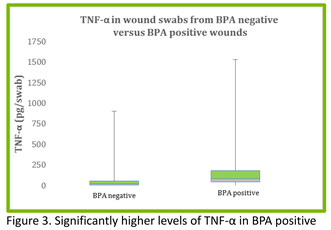


Figure 3. Significantly higher levels of TNF-α in BPA positive (167pg/swab) versus BPA negative (56pg/swab) wounds (p=0.0002)

There is a marked increase in the number of wounds yielding positive results on a rapid point of care bacterial protease test when the total bioburden or exceeds 10^5 CFU/ml (figure 4).

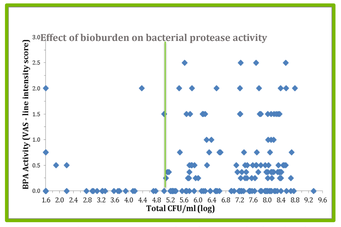


Figure 4. Bacterial protease activity (BPA) against bioburden (CFU/ml)

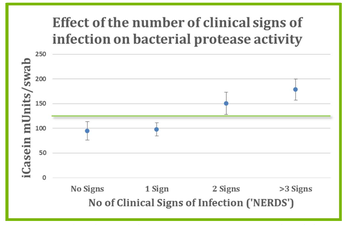
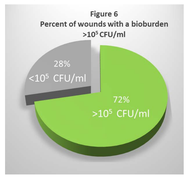
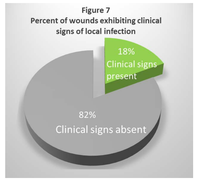
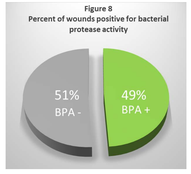


Figure 5. Median bacterial protease activity in wounds exhibiting differing numbers of clinical signs of infection

Study 2 evaluated the relationship between the BPA , clinical signs of infection ('NERDS') and bioburden levels in wounds. Figure 5 demonstrates the median BPA in wound fluid increases when two signs or more signs are present.







A high proportion of wounds (72%) had bacterial counts $>10^5$ (figure 6) but only a relative small proportion (18%) exhibited 3 or more clinical signs of infection (figure 7). In contrast, nearly half of the wounds (49%) tested positive for BPA (figure 8).

Discussion

Increased production of pro-inflammatory cytokines (e.g. IL1-β & TNF-α) is known to be one of the host responses to infection by pathogenic bacteria[5]. The results in figures 2 & 3 show that increased levels of IL1-β & TNF-α are detected when wounds test positive for BPA. However the data shown in figure 5 reveals that increased BPA can be detected even when the wound is asymptomatic for infection (i.e. only 2 'NERDS' present). Therefore, the presence of bacterial proteases above a threshold level of activity can indicate to a clinician that the wound is progressing to the point when the host is mounting a biochemical response to the insult and the wound is developing towards infection (figure 9) that requires intervention even though overt signs are not obvious to the clinician.

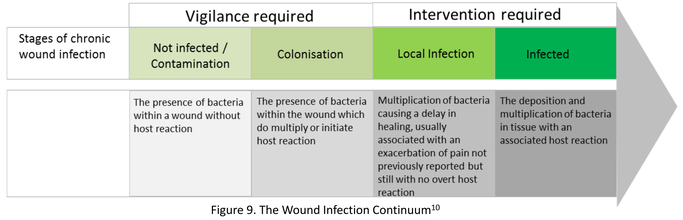


Figure 9. The Wound Infection Continuum[10]

It is unsurprising that figure 4 demonstrates that BPA is detected much more frequently when the bioburden is $>10^5$. The link between bacterial load, infection and wound healing has previously been well documented in the literature, particularly in acute wound healing[11]. However, it is interesting that some wounds with a bioburden $>10^5$ are BPA negative, whilst a few wounds with a bioburden $<10^5$ are BPA positive. This observation is consistent with literature reports that wounds can heal when colonised with relatively high levels of bacteria[12] whilst others succumb to infection when colonised by relative few of a highly virulent species of bacteria [11]. As reported in the larger study of 366 wounds, a high proportion (72%) had bacterial counts $>10^5$ but only 18% of this cohort of patients exhibited three or more clinical signs of infection. By contrast, around half of the wounds (49%) tested positive for BPA indicating that they are in a 'state of pathogenesis' i.e. the bacteria colonising the wound are displaying pathogenic behaviour, with around three quarters of these patients being asymptomatic for infection.

Conclusions

Presence of pathogenic bacteria in a wound can cause a 'state of pathogenicity' that leads to local infection and impairs healing. Clinical examination can wrongly diagnose infections in chronic wounds. Some chronic wounds fail to exhibit the classic signs of infection and inflammation in wounds can be misinterpreted as infection[2]. Culture techniques have limited reliability on their own, frequently leading to the over diagnosis of infection[7].

Testing wound fluid for BPA using a rapid point of care test may be a useful method for detecting the presence of pathogenic bacteria, at a clinically significant stage in the infection continuum, even before the signs of infection are apparent. Integrating a point of care test for BPA as part of routine wound assessment could be a valuable tool in treatment pathways to inform clinicians that the wound is in a 'state of pathogenesis' which could lead to overt infection and be a possible contributor to wound chronicity and have a negative effect on morbidity and mortality of the patient.

References

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The prototype rapid lateral flow point of care bacterial protease test described in this poster is not FDA cleared for sale in the US or any other part of the world.

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