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Elevated wound fluid pH correlates with increased risk of wound infection

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ABSTRACT

Introduction: There is no definitive method to determine infection status in non-healing wounds. Measurement of wound pH might be a promising indicator of infection as it is relatively easy to perform, provides objective results within a few seconds, and is inexpensive. The aim of this investigation was to determine if wound pH could be a potential indicator of early or established infection in non-healing wounds.
Methods: We explored the relationship between wound pH and two indicators of wound infection: expert clinical judgement and elevated neutrophil-derived enzyme activity. Data was used from 120 wound samples previously collected at Medisch Spectrum Twente hospital.
Results: With increasing wound pH, there was also an increase in the proportion of infected wounds as determined by expert clinical judgement. In addition, increases in the activities of myeloperoxidase, elastase and lysozyme were also associated with elevated pH.
Conclusions: The strength of the relationship between wound pH and clinical judgement or enzyme activities observed in this study is not sufficient to promote the use of elevated pH alone as an indicator for wound infection. However, the use of pH in combination with other indicators for wound infection, such as elevated neutrophil enzyme activity, warrants further research.

1. Introduction

Wound healing is a complex process involving both tissue degradation and regeneration. Many factors can influence wound healing, one of which is pH [1]. The pH of normal intact skin is between 4.5–5.0 (i.e., acidic), and this range is known to be optimal for skin health in terms of moisture content, barrier function, scaling, and maintenance of resident commensal microflora [2]. When the skin is breached, normal acute wound healing has been reported to progress optimally at a slightly acidic environment [3], which is conducive to fibroblast proliferation, epithelialisation, angiogenesis, and microbial control [4,5]. In contrast, wounds that fail to heal in an orderly manner and deteriorate to a chronic condition, are generally characterised by an alkaline pH (> 7.0) [5]. The cause of alkalinity is not well understood, however, microbial metabolism, especially anaerobic metabolism, is prone to release ammonia and polyamines which may increase external pH, especially if not cleared quickly by the immune system. For example, *Proteus mirabilis* converts urea to ammonia which causes local skin irritation. Alkaline pH has also been associated with wound biofilm development, which is a precursor to clinical infection [6]. Moreover, some classes of antibiotics function optimally at alkaline pH (e.g., fluoroquinolones, aminoglycosides, macrolides), while other classes, such as the beta-lactams, function most effectively at acidic pH [7]. This is an important factor to bear in mind when considering antibiotic therapy in chronic, non-healing wounds.

An increase in wound pH was shown to be an early indicator of infection (i.e., prior to clinical signs manifesting) in a study of 26 subjects with second degree burns [8]. Additionally, certain tissue-degrading enzymes such as elastase, plasmin and matrix-metalloproteinase-2 have high turnover rates at pH 8.0 [1]. This indicates that an alkaline wound environment may be associated with both tissue destruction and increased risk of infection. Although an increase in wound

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pH has been observed prior to clinical signs of infection in burn wounds [8], to our knowledge, there is limited clinical evidence that directly associates elevated pH with clinical signs of infection in other acute and chronic wounds.

The aim of this investigation was to determine the relationship between wound pH and infection in acute and chronic wounds using data collected in a previously published clinical study [9]. As there is currently no definitive method to determine the presence of infection in complex, non-healing, wounds, we explored the relationship between wound pH and two indicators for wound infection status: expert clinical judgement and elevated neutrophil-derived enzyme activity (myeloperoxidase [MPO], human neutrophil elastase [HNE], lysozyme [LYZ]). These enzymes are upregulated and secreted by the first responding cells of the host innate immune system, the neutrophils, in response to the microbial invasion and insult [10–12], and have previously been demonstrated to be promising indicators of wound infection [9].

2. Methods

This investigation describes further exploratory analyses conducted on data from wound samples collected in a previously reported study conducted at the Department of Surgery of Medisch Spectrum Twente (MST) Hospital, Enschede, Netherlands [9], which was approved by the MST review board and conformed with the 1975 Declaration of Helsinki. In this analysis, we included measurements from 120 wounds, including wounds from 81 patients previously reported, plus an additional 39 wounds from patients that had been excluded from the primary analysis due to missing microbiological or total protein content assessments. After informed consent was provided, wound infection status ("infected" or "not infected") was determined by the clinical judgement of expert clinicians comprising experienced doctors, nurse practitioners, wound care nurses and podiatrists at the Department of Surgery of MST [9]. Judgement was based on their extensive clinical experience, including published clinical signs and symptoms such as moist-wet wound bed, serous or sanguineous exudate, partial wound necrosis, wound malodour, purulent discharge, and sanguineous and purulent discharge [13–16]. Prior to debridement and cleansing of the wound, wound fluid was collected from a non-necrotic part of the wound bed using a sterile swab for measurement of pH and enzyme activities. Wound fluid from the swabs was transferred to tubes containing 10 ml of 0.9% NaCl solution and stored in a monitored refrigerator at 4 °C until transportation to Qualizyme Laboratories, Graz, Austria for analysis.

2.1. pH measurement

To initiate a pH-dependent colour change, 100 µl of 1.6 mM Bromothymol Blue indicator solution was added to 100 µl of the wound fluid sample (diluted in 10 ml of 0.9% NaCl). The pH of the sample was visually determined by comparing the resulting colour against reference samples with known pH values.

2.2. Neutrophil enzyme assays

The methodologies for assaying wound fluid for MPO, HNE and LYZ activities from the 120 samples included in the analyses in this study are described by Blokhuis-Arkes et al. [9]. To summarise, enzyme activities were determined using absorbance assays in a Tecan Infinite M200 platereader (Tecan, Maennedorf, Switzerland). The activity of MPO was determined using a guaiacol-based assay as described in Hasmann et al. [12]. *N*-Methoxysuccinyl-Ala-Ala-Pro-Val *p*-nitroanilide was used as the substrate to determine HNE activity [10] and a turbidity assay with lyophilised *Micrococcus lysodeikticus* cells was used to determine LYZ activity [11]. All enzyme assays were carried out in buffered systems to avoid any influence of the initial wound pH on the measured enzyme activity.

Table 1
Characteristics of the study population (n = 120).

		Frequency (%)	Median (range)
Sex	Male	71 (59)	
	Female	49 (41)	
Age (years)			68.4 (14.4–90.5)
Wound diagnosis	Arterial ulcer	14 (12)	
	Venous ulcer	7 (6)	
	Mixed arterial/ venous ulcer	3 (3)	
	Diabetic foot ulcer	33 (28)	
	Traumatic wound	20 (17)	
	Oncologic ulcer	3 (3)	
	Pressure ulcer	14 (12)	
	Amputation wound	12 (10)	
	Other	14 (12)	
Wound duration (days)			60 (1–4,745)

2.3. Data analysis

To investigate the relationship between wound pH and possible indicators of wound infection, measured wound pH values were plotted against expert clinical judgement ("infected" or" not infected") and measured activity of the enzymes MPO, HNE and LYZ. Enzyme activity was defined as the rate at which the enzyme can convert a specific substrate (in units per ml). Histograms were used to present the relationship between wound pH and number of wounds judged as infected by expert clinicians. The relationship between measured enzyme activity and wound pH was described by using boxplots, as these plots provide insight into the distribution of enzyme activity levels (measured on a continuous scale).

3. Results

The characteristics of the 120 patients included in the analyses in this article are shown in Table 1. The majority of patients were male, and median age was 68 years. The most frequent wound type was diabetic foot ulcers, followed by traumatic wounds, which is representative for the wound clinic at MST hospital.

The distribution of pH measured from the 120 wounds sampled is shown in Fig. 1. The pH of the wound samples ranged from pH 5 to pH 9; the majority (n = 73) of wounds had a measured pH of 6. A low pH (defined for this analysis as pH < 7), was measured in 77 wounds (64%), while in the remaining 43 wounds (36%) a high pH (defined as pH ≥ 7) was measured.

3.1. pH versus clinical judgement

Clinical signs of infection were (in order of frequency observed): moist-wet wound bed (84%), serous exudate (70%), partial wound necrosis (26%), sanguineous exudate (17%), wound malodour (16%), purulent discharge (9%), and sanguineous and purulent discharge (4%). Based on expert clinical judgement, 30 wounds (25%) were judged as infected and 90 wounds (75%) were judged as not-infected. The mean pH value of wounds judged as infected was pH 7.2, versus a mean of pH 6.5 in wounds judged as not infected. There was an increase in the proportion of wounds judged infected with increasing pH, as shown in Fig. 2.

3.2. pH versus MPO, HNE and LYZ enzyme activities

Box plots of pH versus MPO, HNE and LYZ activities are shown in Fig. 3A–C, respectively. There was a general trend for enzyme activity to increase with an increase in wound pH. However, the variability in enzyme activity for all enzymes was high, particularly in wound

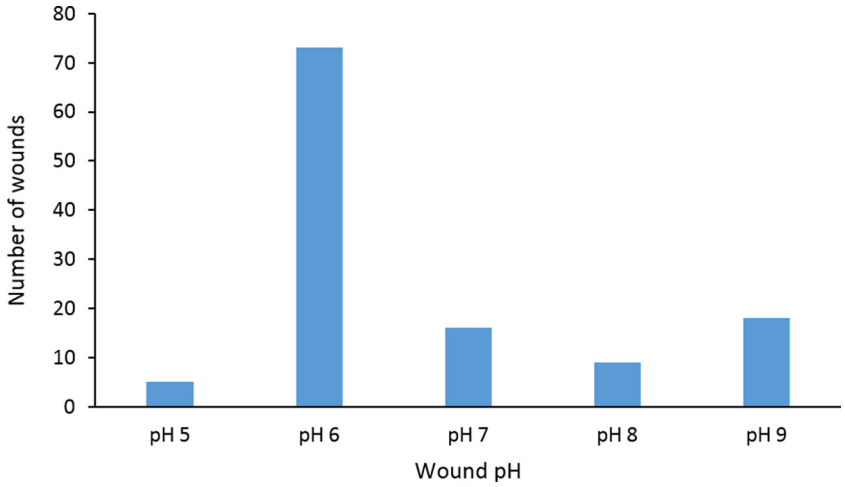

Fig. 1. pH of wound samples (n = 120).

samples of higher pH (i.e., pH 7–9).

4. Discussion

The analyses in this investigation have indicated a relationship between elevated wound pH and increased risk of wound infection. With increasing wound pH, there was an increase in proportion of infected wounds as determined by expert clinical judgement. In addition, increases in the activity of neutrophil-derived enzymes, MPO, HNE and LYZ, were also associated with increased wound pH.

pH is the concentration of hydrogen ions (H^+) in solution, and is measured as a negative logarithm, ranging from 1 (strongly acidic, high H^+ concentration) to 14 (strongly alkaline, low H^+ concentration). The mean pH value in wounds judged as infected was pH 7.2, compared with a mean pH value of 6.5 in wounds judged as not-infected. While this difference in wound pH may appear to be relatively small, the difference in H^+ concentration, given the logarithmic scale of pH, is 5-fold higher in not-infected wounds (3.16×10^{-07} M) compared with infected wounds (6.31×10^{-08} M). Thus, a small change in pH value indicates large changes in H^+ concentration in the wound.

The results from this investigation do not provide sufficient evidence to demonstrate an absolute association of elevated pH alone with wound infection. A possible explanation for this weak correlation is that there is currently no definitive method to identify the presence or absence of infection in complex, non-healing wounds. Current methods mainly rely on clinical judgement, using clinical signs and symptoms of wound infection, and microbiological analysis of wound samples. Microbiological analysis of wound swab or biopsy samples can identify the presence of readily-culturable microorganisms, but presence of certain organisms alone, or combinations thereof, is not a reliable indicator of wound infection [17], nor are microbial numbers [18]. Since wound microbiology is often complex, and no specific organism (or groups of) are necessarily indicative of infection, qualitative microbiological analysis of wound swabs are of questionable value [19]. In addition, it takes several days before culture results are available, whilst in clinical practice a decision about whether to initiate antimicrobial treatment has to be made within a patient's appointment (or assessment period). As a result, expert clinical judgement may currently be considered as a 'quasi-gold' standard for determining the presence of infection. However, the accuracy of expert clinical judgement is unknown as this method is subjective and dependent on level of expertise, leading to variable results. In addition, it is possible for early stage infection to be present in wounds before clinical signs are apparent (i.e., sub-clinical signs) [8].

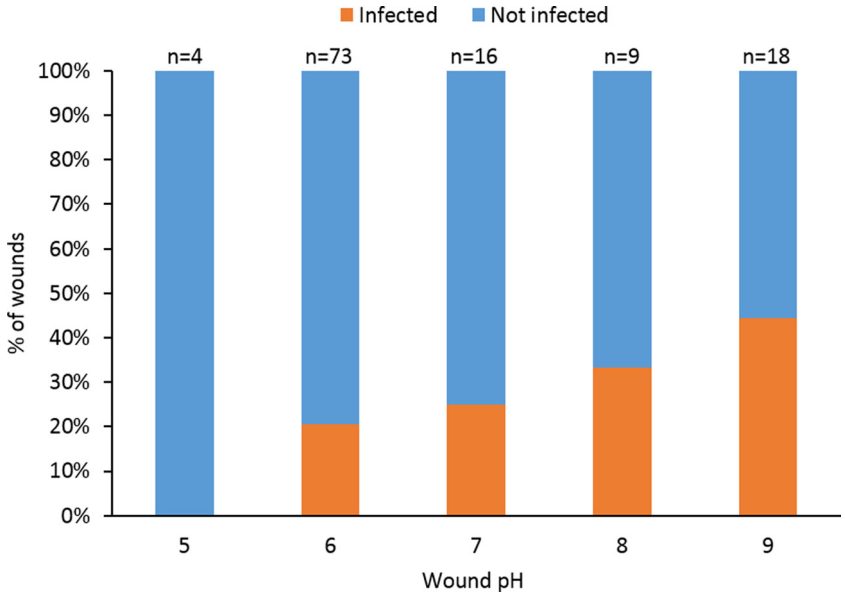

Fig. 2. The proportion (%) of wounds clinically judged as infected or not infected at each measured pH.

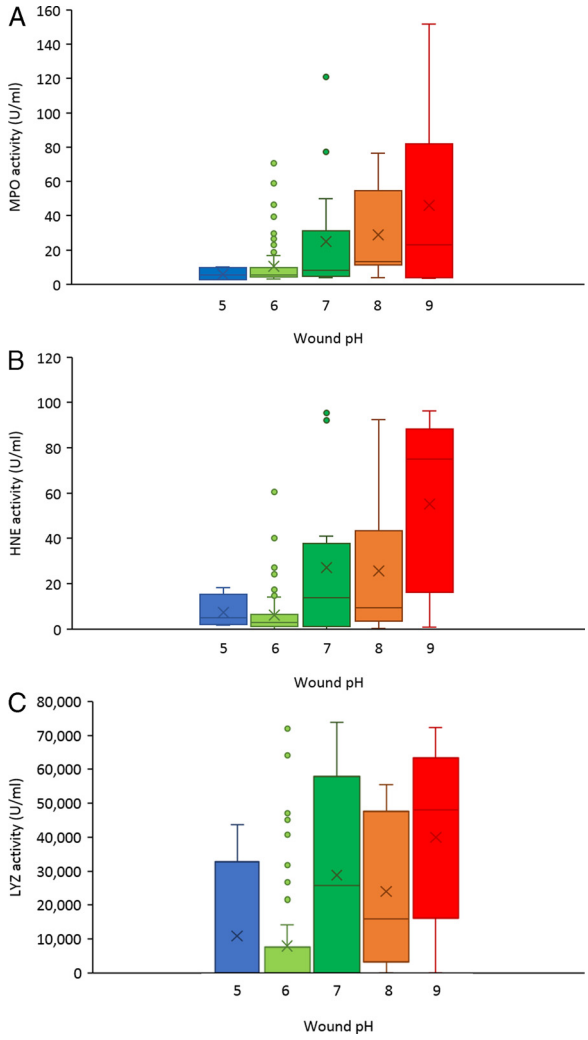

Fig. 3. Enzyme activity versus wound pH for (A) MPO, (B) HNE, (C) LYZ. X = mean; line = median.

When exploring the relationship of wound pH with presence of infection as determined by expert clinical judgement, no wounds were judged as being infected at pH 5 (although a small sample, n = 4), whereas at pH 9, 55% of wounds were judged to be infected. Of the 45% of wounds at pH 9 that were judged as not infected, it cannot be ascertained whether they were, in fact, at an early stage of infection such that clinical signs of infection were not clear, or whether other factors were responsible for the elevated pH in the absence of infection (e.g., wound dressings, topical antimicrobials, metabolic factors). Therefore, we also used an alternative approach to detect wound infection: measurement of the activity of neutrophil-derived enzymes (MPO, HNE and LYZ) in wound fluid. These enzymes are produced by the host's immune system in response to the presence of pathogens. Increased enzyme activity measured from wound fluid has been demonstrated to be an encouraging indicator of wound infection [9]. Our analysis has revealed a general trend for neutrophil-derived enzyme activity to increase with the increase in wound pH. However, variability in enzyme activities was high, particularly at a higher pH. The trends are consistent with the findings from another recent clinical study, which also found correlations between wound pH and expert clinical judgement [20].

Based on the results from our exploratory analysis, measurement of pH might be a promising indicator of infection as it is easy and inexpensive to perform, and provides immediate objective results. Early detection of infection, or confirmation of established wound infection, may enable prompt administration of appropriate antimicrobial therapies (e.g., systemic antibiotics, topical antiseptics) with the aim to avoid spreading infection and potentially serious sequalae such as sepsis, limb loss, and ultimately death [17,21]. However, overuse of antibiotics has resulted in the emergence of multi-drug-resistant strains of bacteria, which poses a serious global threat to the effectiveness of antibiotics and their future use [22]. Antibiotic stewardship is therefore essential in the treatment of acute and chronic wounds to ensure appropriate and timely use of antibiotics whilst avoiding overuse. Early and accurate detection of emerging wound infection is one means of contributing to antibiotic stewardship by enabling the application of appropriate local and topical antimicrobial strategies and avoiding the need for systemic antibiotics.

Given the clinical, spatial and biochemical heterogeneity of wounds, measurement of pH alone might not suffice as an independent marker of emerging or established infection. However, combining this relatively easy and fast method with other promising markers such as measured neutrophil-derived enzyme activity in one diagnostic tool might fulfil the need for accurate and fast detection of wound infection. We therefore encourage further research into combining markers for wound infection to ensure that the most effective treatment is provided to patients in a timely manner to prevent onset of severe signs, and potentially help to prevent overuse of antibiotics and support antibiotic stewardship initiatives.

Ethics

This investigation describes further exploratory analyses conducted on data from wound samples collected in a previously reported study conducted at the Department of Surgery of Medisch Spectrum Twente (MST) Hospital, Enschede, Netherlands, which was approved by the MST review board and conformed with the 1975 Declaration of Helsinki. Informed consent was obtained from all patients included in the study.

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Declaration of Competing Interest

D. Metcalf and P. Bowler are employees at ConvaTec, a Medtech company; M. Burnet, A. Heinzle and E. Sigl are co-founders of Qualizyme Diagnostics GmbH & Co KG and C. Gamerith is an employee of this company. M. Haalboom has nothing to declare.

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