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The use of point of care protease diagnostics to improve wound healing: A prospective case-controlled study

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Abstract

Forty patients with chronic ulcers (Intervention Group), treated at four Italian wound clinics were enrolled and treated based on the TIMEH principles. Therapy decisions were assisted by two qualitative lateral flow point of care (PoC) tests, WOUNDCHEK™ Bacterial Status and WOUNDCHEK™ Protease Status, that detect bacterial protease activity (BPA) and elevated host inflammatory protease activity (EPA) respectively. Outcomes were monitored for 30 days. The outcomes and treatments were compared with a Control Group that were not tested. The Control Group had significantly more trauma wounds, so all patients and those with trauma wounds removed were analysed separately. Using test results to inform treatment decisions was strongly associated with increased rates of healing and improvement, odds ratio 13.00, 95% CI 4.16-40.66, p<0.0001 (all data) or odds ratio 10.40, 95% CI 3.13-34.53, p=0.0001 (trauma wounds removed). Significantly more surgical debridement (all data p=0.0089, trauma wound removed p=0.0025), systemic antibiotics (all data p=0.0036, trauma wounds removed p=0.0005) and significantly less antiseptics (all data p=0.0001, trauma wounds removed p=0.0001) were used in the Intervention Group. The percentage of wounds positive for BPA or having EPA reduced over time and was associated with improving mean TIMEH scores in the Intervention Group, suggesting treatment modifications stimulated by testing had improved outcomes. The expeditious test results were useful in determining treatment choice, monitoring effectiveness of treatment and especially helpful in judicious use of advanced therapies and invasive surgical interventions. This study confirms using PoC tests for BPA and EPA can assist treatment decisions and improve healing.

Keywords: Wounds; Diagnostics; Protease; Infection; Inflammation; Healing

1. Introduction

Non-healing cutaneous wounds are a significant socio-economic burden on health care systems.[1] The problem is escalating primarily due to ageing populations and concomitant increase in prevalence of venous disease, metabolic syndrome, diabetes and peripheral arterial disease (PAD).[2] Diabetic foot ulceration is the most common cause of non-traumatic lower limb amputation (LLA) and has poor prognosis with a 5-year mortality post LLA of 50-80%, worse than many carcinomas.[3] Despite advances in treatments and wound management protocols, healing rates remain sub-optimal. Although gold standard therapy such as graded compression is widely available, only around half of venous leg ulcers are healed within twelve months.[4] Wound age is a significant risk factor for non-healing, so it is imperative to establish early the underlying causes of ulcers and target therapeutic approaches accordingly. Often, more efficacious but expensive wound therapies e.g., skin substitutes, are unsuccessful as they are applied after other more conservative strategies have failed and when the wound healing potential has reduced. Identifying the causes of delayed healing early with expeditious application of appropriate therapies could be beneficial in terms of promoting healing.

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WOUNDCHEK™ Bacterial Status and WOUNDCHEK™ Protease Status (Woundchek Laboratories, Gargrave, UK) are point of care (PoC) tests that detect the presence of bacterial protease activity (BPA) and host neutrophil derived inflammatory protease activity including matrix metalloproteases (MMPs) and elastase (known as elevated protease activity or EPA), respectively using wound fluid swabs. Both WOUNDCHEK™ Bacterial Status (WCBS) and WOUNDCHEK™ Protease Status (WCPS) are qualitative lateral flow PoC tests that yield results in 15 minutes and indicate whether pathogenic bioburden or host inflammation respectively are potential causes of non-healing. BPA is a common virulence factor secreted by pathogenic bioburden[5,6] and has been linked to non-healing.[7,8,9,10,11] The presence of elevated levels of neutrophil derived inflammatory proteases (e.g., MMP-2, MMP-8, MMP-9 and elastase) are widely accepted to be associated with wound chronicity.[12,13,14]

The aim of this study is to prospectively evaluate the beneficial effect of using both WCBS & WCPS tests to guide therapy decisions in patients being scored using TIMEH[15] methodology (i.e. combining scores for no-viable tissue (T), infection (I), exudate (M) and retardation of epithelialisation (E)) and comparing TIMEH outcomes with a cohort of patients that were not treated based on test results.

2. Material and methods

2.1. Patients

Patients with small (<50cm^3) to medium sized (50-100cm^3) chronic wounds at least 2 months old of various aetiologies (venous, arterial, inflammatory, pressure, metabolic & traumatic); from November 2020 to December 2020 were recruited from four institutions (Asti, Bari, Pieve del Cairo & Turin). Ten wounds from each centre were tested with WCBS & WCPS tests initially (Stage 0), after approximately fifteen days (Stage 1) and after approximately 30 days (Stage 2) (Intervention Group). Not all wounds were tested with both tests at every stage and could not be tested if healed. TIMEH scores (described below) were recorded at Stage 0, 1 & 2 to monitor progress of the wounds. TIMEH scores over a thirty-day period of forty random patients from the same clinics (ten per site) that were not tested with WCBS & WCPS were used as comparative controls (Control Group). Wounds in both groups were categorised at the end of the study as either a) 'Healed' if complete epithelialisation had occurred, b) 'Improved' if the TIMEH score had decreased between Stage 1 and Stage 2, c) 'Static' if the TIMEH score was the same at Stage 1 and Stage 2 or d) 'Worsening' if the TIMEH score increased between Stage 1 and Stage 2. The study was approved by the local scientific committee.

2.2. TIMEH methodology

Table 1. summarises the scoring system used for the TIMEH methodology. Individual scores for T (Non-viable tissue), I (Infection), M (Exudate) and E (Retardation of epithelialisation) are added together for a total TIMEH score.

Table 1 TIMEH scoring system

	Score	**0**	**1**	**2**	**3**	**4**
T	Non-Viable Tissue	Absent	Present 25%	Present 50%	Present 75%	Present 100%
I	Infection	Absent	Present 25% Contaminated	Present 50% Colonised	Present 75% Critically Colonised	Present 100% Infected
M	Exudate	Absent	Present 25% Scarce	Present 50% Abundant	Present 75% Abundant Coloured	Present 100% Abundant Coloured Odour
E	Retardation of epithelialisation	Absent	Present 25%	Present 50%	Present 75%	Present 100%

2.3. Wound treatments

All wounds in both groups were treated as per the standard care for the wound clinic following the TIMEH principles. In addition, physicians treating the Intervention cohort could use the WCBS & WCPS test results to help guide therapy decisions. Wound treatments were recorded at Stages 0, 1 & 2 and collated into the following categories; sharp/mechanical debridement, soft debridement (including enzymatic and autolytic), systemic antibiotics, local antibiotics, antiseptics (including antimicrobial dressings), topical negative pressure therapy, MMP inhibitors (including oxidised regenerated cellulose), moist wound healing (including hydrocolloid, hydrofiber & alginates), biophotonic therapy, hyperbaric, and plastic surgery (including skin grafts and flaps).

2.4. Statistical analysis

Patient demographics and wound data were compared using t-test for continuous variables or two-sided chi squared with Yates correction for categorical values (www.graphpad.com). After thirty days the outcome categories of healed, improved, static and or worsening were combined to binary healed/improved or static/worsening. The odds ratio was calculated (www.medcalc.org) of being either healed/improved versus static/worsening for Intervention Group compared to the Control Group. A p-value of <0.05 was considered significant

3. Results

Patient demographics and initial TIMEH score are detailed in Table 2.

Table 2 Patient demographics and initial TIMEH scores

Parameter	Intervention	Control	p
n	40	40	
Mean Age (SD)	72.4 (17.58)	73.3 (13.31)	0.797
Male (%)	34	57	0.0728
Female (%)	66	43	-
Inflammatory (%)	10	12.5	0.7235
Venous (%)	15	12.5	0.7454
Metabolic (%)	7.5	15	0.4792
Trauma (%)	32.5	7.5	0.0119
Arterial (%)	0	7.5	0.2392
Pressure (%)	35	45	0.4936
Mean area (SD)	30.3 (50.0)	40.9 (48.3)	0.3379
Mean initial TIMEH (SD)	10.9 (2.5)	8.4 (3.7)	0.0008

Post-traumatic wounds were significantly more numerous in the Intervention Group, so the dataset was also analysed with trauma wounds removed (table 3.). The mean initial (Stage 0) TIMEH score was significantly higher for the Intervention Group wounds for both the complete dataset (table 2.) and the subset where trauma wounds were removed (table 3.).

At Stage 0, 51% (20/39) of wounds were positive for BPA and 74% (20/27) had elevated host inflammatory protease activity (EPA). By Stage 1, the prevalence of positive BPA and EPA had declined to 11% (3/35) and 45% (17/37) respectively. By Stage 3, 13% (3/23) wounds were positive for BPA and 25% (6/24) had EPA. Over all three Stages, 28% (27/97) of wounds were positive for BPA and 49% (43/88) of wounds had EPA. The prevalence of BPA positive and EPA wounds at the three stages are graphically depicted in Figure 1 and was compared with the mean TIMEH scores for all data in the Intervention Group (Table 4). The decrease in BPA positive prevalence at Stage 1 and the prevalence of EPA at Stages 1 & 2 was matched by concomitant decreases in TIMEH score i.e. as protease levels decreased, healing improved. Where both tests were performed (n=81), a total of 10 (12%) were positive for BPA and EPA.

Mean TIMEH scores at Stage 0 for all wounds were 10.88 (SD 2.46) and 8.43 (SD 3.69) for the Intervention and Control Groups respectively. The mean TIMEH score for all wounds in the Intervention Group decreased significantly to 7.73 (SD 2.24, p<0.0001) at Stage 1 and to 5.70 (SD 2.67, p<0.0001 vs Stage 1 and p=0.0010 vs Stage 0) by Stage 2. The mean TIMEH score for all wounds in the Control Group did not significantly change at Stage 1 (7.45 SD 3.40, p=0.2204) or at Stage 2 (8.00 SD 4.34, p=0.6464 vs Stage 1 and p=0.5432 vs Stage 0). The mean TIMEH score after trauma wounds removed in the Intervention Group also decreased significantly from 11.59 (SD 2.30) at Stage 0 to 8.11 (SD 2.27, p<0.0001) at Stage 1 and to 6.00 (SD 2.74, p<0.0001 vs Stage 1 and p=0.0054 vs Stage 0) by Stage 2. The mean TIMEH score after trauma wounds removed in the Control Group did not significantly change from 8.78 (SD 3.60) at Stage 0 to 7.84 (SD 3.23, p=0.2410) at Stage 1 and to 8.00 (SD 4.34, p=0.4118 vs Stage 1 and p=0.8601 vs Stage 0) at Stage 2. The

mean TIMEH scores and statistics are summarised in Table 4. The TIMEH scores with trauma wounds excluded for Intervention Group and Control Group are schematically compared in Figure 2.

Table 3 Patient demographics and initial TIMEH scores with trauma wounds removed

Parameter	**Intervention**	**Control**	**p**
n	27	37	
Mean Age (SD)	75.4 (13.4)	74.4 (13.2)	0.7803
Male (%)	36	57	0.1916
Female (%)	64	43	
Inflammatory (%)	15	14	0.9172
Venous (%)	22	14	0.5642
Metabolic (%)	11	16	0.8289
Arterial (%)	0	8	0.3592
Pressure (%)	63	49	0.3779
Mean area (SD)	37.6 (58.9)	43.0 (49.6)	0.6896
Mean initial TIMEH (SD)	11.6 (2.3)	8.8 (3.6)	0.0007

Table 4 TIMEH scores at Stages 0, 1 & 2 for all wounds and subset with trauma wounds removed

	Mean TIMEH (SD) Stage 0	**Mean TIMEH (SD) Stage 1**	**P Stage 1 vs Stage 0**	**Mean TIMEH (SD) Stage 2**	**P Stage 2 vs Stage 0**	**P Stage 2 vs Stage 1**
Intervention All data	10.88 (2.46)	7.73 (2.24)	<0.0001	5.70 (2.67)	<0.0001	0.0010
Control All data	8.43 (3.69)	7.45 (3.40)	0.2204	8.00 (4.34)	0.6464	0.5432
Intervention Without trauma	11.59 (2.30)	8.11(2.27)	<0.0001	6.00 (2.74)	<0.0001	0.0054
Control Without trauma	8.78 (3.60)	7.84 (3.23)	0.2410	8.00 (4.34)	0.4118	0.8601

The number of wound treatments employed over the three clinic visits (Stage 0, 1 & 2) were combined and are summarised in Table 5, with and without trauma wounds. The Intervention Group received significantly more surgical debridement and systemic antibiotics than the Control Group, whereas the Control Group received significantly more antiseptics. No other treatment differences reached statistical significance. The significant differences noted remained significant when trauma wounds were removed from the analysis (Table 5). Wounds testing positive for BPA received significantly more surgical debridement ($p<0.0001$), topical negative pressure ($p=0.0015$) and systemic antibiotics ($p<0.0001$) and significantly less moist wound healing dressings ($p=0.0029$) than wounds that were negative. Individually the use of topical antibiotics and antiseptics for BPA positive wounds were not significantly higher compared to BPA negative wounds but when combined they were ($p=0.0025$). Wounds with EPA had significantly more surgical debridement ($p=0.0010$), soft debridement ($p=0.0057$), topical negative pressure ($p=0.0039$), systemic antibiotics ($p=0.0298$), topical antibiotics ($p=0.0020$) and significantly less use of moist healing dressings ($p=0.0030$) and MMP inhibitors ($p=0.0061$) than wounds with low host inflammatory protease activity (LPA).

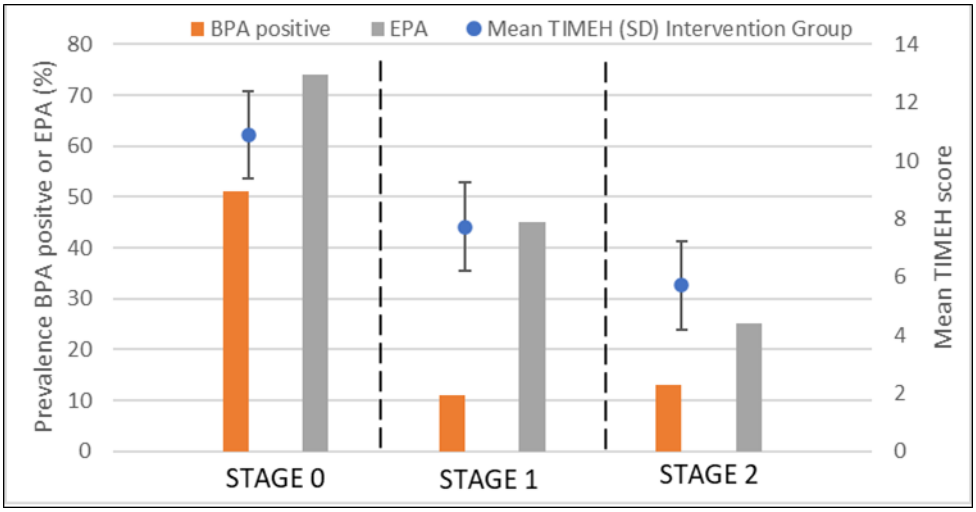

Figure 1 Prevalence of BPA positive and EPA wounds and mean TIMEH scores for the Intervention Group (all data included)

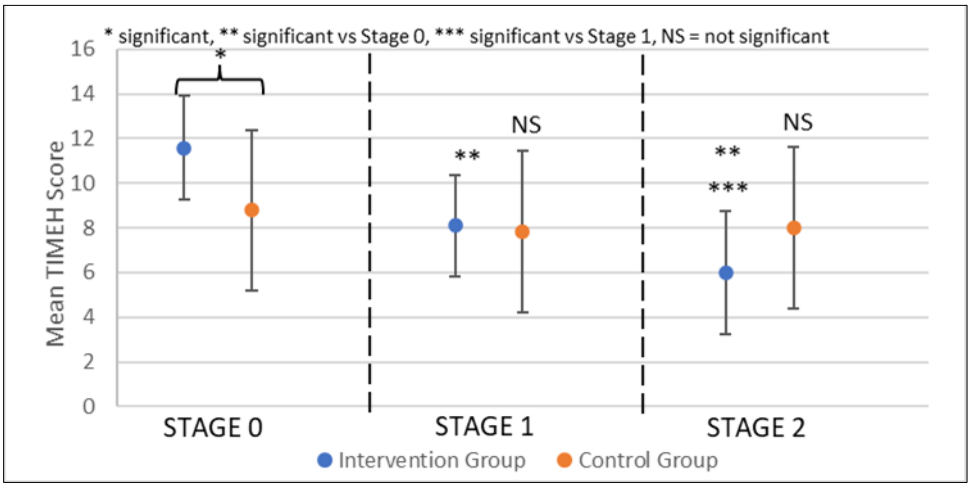

Figure 2 Mean TIMEH scores for Intervention Group and Control Group with trauma wounds excluded

In the Intervention Group, fifteen wounds were healed by Stage 2 and twenty were improved compared to two healed and twelve improved in the Control Group. The Intervention Group had two static wounds and three worsening compared to thirteen static wounds and thirteen worsening wounds in the Control Group. The odds ratio for being healing or improved for the Intervention Group compared to the Control Group was 13.00 (95% CI 4.16-40.66), p<0.0001. In the subset with trauma wounds removed, nine Intervention wounds were healed by Stage 2 and thirteen were improved compared to one healed and ten improved in the Control Group. The Intervention Group had two static wounds and three worsening compared to thirteen static wounds and thirteen worsening wounds in the Control Group. The odds ratio in the subset without trauma wounds for being healing or improved for the Intervention Group compared to the Control Group was 10.40 (95% CI 3.13-34.53), p=0.0001.

Table 5 Number of wound treatments in total (Stages 0, 1 & 2 combined) with and without trauma wounds included

Treatment	**Intervention (n= 40 wounds x 3 visits)**	**Control (n=40 wounds x 3 visits)**	**p**	**Intervention No trauma (n=27 wounds x 3 visits)**	**Control No trauma (n=37 wounds x 3 visits)**	**p**
Surgical debridement	21	7	0.0089	18	7	0.0025
Soft debridement	32	29	0.7668	23	21	0.1710

Topical negative pressure	11	7	0.4622	10	7	0.2311
Systemic antibiotics	10	0	0.0036	10	0	0.0005
Topical antibiotics	19	12	0.2482	15	12	0.1912
Antiseptics	8	38	0.0001	6	38	0.0001
Biophotonic therapy	7	2	0.1741	6	2	0.1202
Moist wound healing	39	32	0.3961	25	32	0.8848
Hyperbaric	3	0	0.2452	3	0	0.1458
Plastic surgery	7	5	0.7671	4	2	0.4159
MMP inhibitor	10	8	0.8064	5	2	0.2278

4. Discussion

Currently wound management is based largely on clinical signs and holistic evaluation of the patient and relies heavily on clinician experience. Laboratory test results are not usually available for a number of hours or even days, which compromises their utility, particularly in primary care. There is a paucity of tests available that give insight into aberrations in the wound microenvironment and yield results at the point of care. The point of care tests for BPA and EPA were easy to perform and integrate into the clinic consultation. The test results were obtained in fifteen minutes, which was essential to assist with the treatment modification made during the patient clinic visit. Knowledge of the BPA & EPA status of the wound was particularly useful in directing judicious use of advanced therapies and invasive surgical intervention such as sharp debridement and skin grafts. Repeat testing was also important to understand if protease levels are lowering or increasing i.e., more or less problems associated with bioburden or inflammation.

The results of the present study show a clear association of decreasing TIMEH scores over time with decreasing prevalence of BPA positive wounds and EPA (Figure 1) in the Intervention Group. TIMEH scores did not change significantly over the study in the Control Group, although the PoC tests were not used on this cohort, so the protease levels were not known. The use of the test results to inform treatment decisions was strongly associated with increased rates of healing and improvement (all data odds ratio 13.00, 95% CI 4.16-40.66, $p<0.0001$, odds ratio with trauma wounds removed 10.40, 95% CI 3.13-34.53, $p=0.0001$). This is not surprising when considering both the presence of BPA and EPA have been shown previously to correlate with poor healing outcomes. A pivotal trial[9] of WCBS at seven US wound clinics involving 266 wounds demonstrated BPA as an independent risk factor for non-healing with a hazard ratio of 0.56 (95% CI 0.36-0.87, $p=0.009$). A post hoc meta-analysis[16] of this pivotal data with a pilot cohort (total 314 wounds) indicated BPA was a risk factor for lower limb amputation (relative risk 5.3, $p=0.03$). EPA has been shown to be highly predictive of skin substitute graft survival with 100% graft success for LPA wounds versus 36% success in EPA wounds, $p<0.0001$.[17] Moreover, in a test and treat study[18] with a retrospective comparator cohort, MMP inhibitor treatment (oxidised regenerated cellulose) directed by the WCPS result improved healing outcomes (44% increase in healing/improved in 107 test and treated wounds versus 87 in the matched control cohort, $p=0.033$). A large (n=414 chronic wounds) prospective US trial of WCPS in twelve wound clinics revealed EPA as an independent risk factor for non-healing with an odds ratio of 0.43, $p=0.0016$ (unpublished data). The improved outcomes for the Intervention Group in this study were observed despite the mean initial TIMEH score being significantly higher for Intervention wounds compared to the Control wounds for both the complete dataset and the subset with trauma wounds removed. Decreasing mean TIMEH scores for the Intervention Group from Stage 0 through to Stage 2 were temporally associated with decreases in both BPA positive and EPA prevalence (Figure 1) suggesting that treatment modifications stimulated by the testing has contributed to the improved outcomes.

The increased targeted use of surgical debridement and systemic antibiotics in the Intervention Group compared to the Control Group and surgical debridement, systemic antibiotics, and topical negative pressure in the BPA positive versus BPA negative cohort presumably contributed to the improved outcomes. Surgical debridement is one of the most effective strategies at removing biofilm followed by use of systemic or local antibiotics and topical negative pressure can help reduce host inflammatory response. The prevalence at enrolment of BPA positive wounds (51%) was similar to that found in previous studies. The pivotal US trial of WCBS had a BPA prevalence of 39%,[9] whereas a randomised controlled trial in primary care in Pennine, UK was higher at 70% [19]. The EPA prevalence of 74% at Stage 0 in this trial was higher than previous published studies but declined markedly following treatment at Stage 1 and Stage 2. A previous study reported EPA rates of 18%,[20] and the large prospective US trial had an EPA prevalence of 23% (unpublished data), although there was a high degree of heterogeneity between clinics dependant on patient

demographics. The prevalence of wounds positive for BPA decreased more rapidly over time (51%, 11% & 13% at Stages 0, 1 & 2 respectively) than wounds with EPA (74%, 45% & 25% at Stages 1 & 2 respectively) possibly due to the treatments having a more rapid effect on bioburden and infection than host inflammatory response. The reduced use of MMP inhibitors in EPA wounds is counter intuitive but could be due to the relatively low usage of these products in general or an initial focus on treatments to eliminate infection.

Limitations

The study had some limitations. Enrolment in study Groups was not randomised and the Control Group cohort had significantly less trauma wounds and they were not tested for BPA or EPA so their protease status throughout the study period was not known. Removing trauma wounds from the analysis made the Intervention and Control Groups uneven sizes (27 & 37 respectively). Wounds were only followed for 30 days so longer-term outcomes were not known. Treatments weren't standardised and protocols following positive WCBS & elevated WCPS weren't mandated. Additionally, test results and treatments could not be blinded leading to a risk of bias.

5. Conclusion

This pragmatic comparative study has confirmed that decreasing protease levels is associated with improved healing trajectory and that using PoC tests for BPA and EPA can be extremely useful in directing therapy and monitoring progress to promote improved healing outcomes in chronic skin lesions. The expeditious test results were particularly useful in assisting judicious use of advanced therapies and invasive surgical interventions. A larger randomized clinical trial is warranted in order to establish the full benefit of adopting PoC testing in standard wound care protocols.

Compliance with ethical standards

Disclosure of conflict of interest

Funding for the study was provided by Woundchek Laboratories, Inc. No other conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

Declaration

Woundchek Laboratories donated WOUNDCHEK™ Bacterial Status and WOUNDCHEK™ Protease Status tests for the study and provided funding

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