Sensitivity of the wound edge gene signature 'WD14' in responding to clinical change; a longitudinal cohort study

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Short Title: Genetic assessment of chronic wounds

Key words: chronic wounds, non-healing wounds, genetic test, gene transcript quantitation, stratified therapies

Abstract

Introduction

Genetic prognostication of chronic wounds is one recognised method of early identification of clinical wound healing status in order to target rigorous and advanced treatment regimens to hard-to-heal wounds. The WounD14 (WD14) gene signature is a recently developed scoring tool, derived from genetic interrogation of wound edge biopsies. It has been shown to predict the propensity of chronic venous leg ulcers to heal. However, it is unknown how WD14 responds with time and to changes in clinical wound healing status. The aim of this pilot study was therefore to evaluate if changes in the clinical healing status of wounds were identified by WD14 gene signature changes.

Methods

WD14 was developed through a process of gene screening, refining and subsequent validation in three separate patient cohorts. Validation was undertaken in 85 consecutive patients referred to a tertiary wound healing unit with chronic venous leg ulcers, who underwent a wound edge biopsy to interrogate for a 'healing' or 'non-healing' genotype. A smaller cohort of patients (18%) underwent a second biopsy, which comprises this pilot cohort reported herein. 12 weeks after the biopsy wounds were clinically assessed for healing status and compared to WD14 genotype.

Results

Sequential biopsies and WD14 scores were obtained from 16 patients. WD14 gene signature predicted clinical wound healing status among this cohort at either visit (total analysis of 32 wound edge biopsies) with a positive predictive value (PPV) of 85.2% (95% CI 74.1% to 92.0%) and negative predictive value (NPV) of 80.0% (95% CI 34.2% to 96.9%). Six wounds

altered their clinical status between the two visits; in this cohort WD14 has a PPV of 66.7% (95% CI 47.3% to 81.7%) and NPV of 100%.

Conclusion

Although the WD14 gene signature did change with wound healing status, further and larger studies are required to clarify precisely the role of this gene signature and its ability to prognosticate accurately over time with wounds of differing clinical status.

Introduction

Chronic wounds pose a significant global challenge to patients and healthcare professionals with associated morbidity, reduced quality of life and significant financial cost.[1] The annual cost of wound management within the UK National Health Service (NHS) is estimated to be between £4.5 and 5.1 billion.[2] Leg ulcers have been identified as the most common of all chronic wounds by The Health Improvement Network (THIN) database of over 11 million UK patients nationwide.[2] In the Western world, 1% to 2% of the population suffer from chronic leg ulcers with a peak prevalence between 60 and 80 years of age.[3] A cohort study of NHS patients from the THIN database highlighted a 71% increase in annual prevalence of wounds and 48% increase in patient management cost in 2018 compared to 2013.[4] The negative impact on patients' quality of life can be attributed to pain, sleep disturbance, social isolation, loss of time from work and depression.[5]

Chronic wounds are caused by a range of underlying pathologies and there are many factors that can influence healing.[1] Historically, prediction of wound healing potential relied heavily upon relatively simplistic parameters such as ulcer size and duration, with a noticeable lack of sensitive prognostic tests.[6-8] This has made individual wound healing prognostication difficult, and limiting clinician's ability to pre-emptively offer targeted, aggressive and/or expensive therapies to hard-to-heal wounds in order to accelerate wound closure.[9] Currently, recruitment of patients to clinical studies and providing a structured assessment and diagnosis of healing potential in clinical practice are very difficult. Until treatment approaches based on precision medicine principles are adopted, care for patients with chronic wounds will remain reliant on simplistic prognostic tools only.

Standard care for patients with non-healing chronic wounds frequently includes wound edge biopsies in order to exclude occult neoplasm or autoimmune-mediated pathology. It can also be undertaken by microbiological examination. Biopsies generally heal rapidly without prolonging overall ulcer healing time.[10] The WounD14 (WD14) gene signature was recently developed for use in predicting hard-to-heal chronic venous leg ulcers (VLU).[11] Wound edge biopsies were interrogated, and differences in expression of 14 genes were combined to a single score, which was able to predict wound healing outcome at three months with a sensitivity of 61.4% and a specificity of 85.4%.[11] The development of this tool only looked at a single biopsy, taken from a patient at a single time point, with follow up at 12 weeks, and it is not known if and how WD14 gene signature responds to wound healing changes over time. The aim of this pilot study is therefore to evaluate if changes in the clinical healing status of patients' wounds are identified by the WD14 gene signature.

Methods

The WD14 gene signature was created using three cohorts of patients: screening, validation and study cohorts (ethical approval numbers: 04/WSE02/10; SJT/C617/08; 09/WSE02/51).[11] A longitudinal cohort study was performed comprising of 85 consecutive patients referred to a tertiary wound healing unit, as previously described by Bosanquet et al in 2012 and therefore the methods will be only briefly reviewed herein.[12] Patients with chronic wounds consistent with underlying venous disease were diagnosed by a senior wound healing physician at a tertiary wound healing centre.

The inclusion criteria were VLU present for a minimum of three months despite best medical care, age 18 years or above and wound size greater than $2cm^2$ and less than $100cm^2$. Exclusion criteria were the presence of overt signs of infection, evidence of peripheral arterial disease (ABPI < 0.8), patients with wounds which appeared non-benign, autoimmune, or of uncertain aetiology, and patients receiving systemic immunosuppression or chemotherapy. Wound care was prescribed and delivered *as per* the TIME (Tissue, Infection/Inflammation, Moisture, Edge) wound bed preparation guidelines and the wound area was assessed at each visit by a specialist wound care nurse.[13,14]

Wound edge biopsies were performed after obtaining informed consent. Individual patient's bleeding risk was assessed pre-procedure and anticoagulation temporarily suspended if necessary. The biopsy site was cleaned and anesthetised with local anaesthesia (1% lignocaine) and a 6mm core biopsy capturing both wound base and the leading keratinocyte edge was obtained. Haemostatic dressings were applied and removed at the next dressing change. Antibiotics were not routinely prescribed. All biopsies underwent concurrent

histological examination to exclude occult neoplastic or autoimmune disease. Biopsies were placed in dry ice immediately and then transferred to a -80^{o}C freezer. The frozen specimens were stored until blinded batch analysis was performed. For histological analysis, up to 10 frozen sections (7µm thickness) were placed on glass slides whilst a further 50-75 sections were combined and homogenised for RNA analysis. The methods used in genetic analysis have been described in detail previously.[11]

Patients in the study cohort had their VLU wound edge biopsy WD14 gene signature score calculated and dichotomised into a 'healing' or 'non-healing' genotype at baseline. After 12 weeks, wounds were clinically assessed and classified as 'healing' if there was a reduction in wound size or 'non-healing' if they were static or deteriorating. Of the 84 patients, WD14 predicted outcome with a sensitivity of 61.4% and a specificity of 85.4%.

Sixteen patients from the study cohort (18%) consented to a further biopsy of their VLU, which comprises the study cohort reported herein. This biopsy was performed at varying times after the initial clinical healing status was determined. The sequential biopsies were again interrogated for a 'healing' or 'non-healing' genotype using the WD14 gene signature score, and again followed up after 12 weeks to assess their clinical wound healing status. This study aimed to assess whether WD14 gene signature changed with time and whether it could identify changes in clinical wound healing status. Data from both the first and second biopsy were compared to assess if the WD14 gene signature was sensitive to changes in the clinical course of wound healing.

Statistical analysis

Data analysis was performed using SPSS® version 27 (IBM Armonk, New York, USA). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for WD14 gene signature were calculated as percentages with their 95 per cent confidence intervals for different subgroups of patients.

Results

This longitudinal cohort study assessed the effect of changes in the WD14 gene signature on clinical wound healing over time and determined its sensitivity, specificity, PPV and NPV in predicting clinical wound healing changes using a sequential wound edge biopsy from VLUs.

This cohort comprised of 16 patients who consented for a sequential biopsy and subsequently underwent a second biopsy of their VLU wound after their initial 3 month clinical follow-up. Patients comprised of mainly women (66.6%) with a median age of 70 years (IQR 64.5 – 80; see table 1). Co-morbidities and medial history included immunosuppressive therapy usage (35.7%), rheumatoid arthritis (7.1%), active cancer (7.1%) and malnutrition (10%). Wound duration was recorded as greater than 2 years in over half of the cohort.

Following the initial 3 month clinical follow-up, 16 patients who consented for a sequential biopsy underwent a second biopsy of their VLU wound. The second biopsies were obtained after a median of 3.5 months (IQR 3.0 months) following the initial biopsy. 10 wounds showed evidence of healing at follow up after the first biopsy, and 14 showed evidence of healing after the second. The WD14 genotype correctly predicted the healing status for 13 of 16 wounds at first biopsy and 14 of the 16 wounds at second biopsy. (Table 1, Figure 1). The WD14 gene signature predicted clinical wound healing status among this cohort at either visit (total analysis of 32 wound edge biopsies) with a PPV of 85.2% (95% CI 74.1% to 92.0%) and NPV of 80.0% (95% CI 34.2% to 96.9%).

1. **Patients with no change in clinical status between first and second visit**

Among the 9 wounds which were clinically assessed as consistently 'healing' on both 1st and 2nd visits, 8 of the 9 wound edge biopsies had correctly predicted a 'healing' WD14 genotype on the 1st biopsy and 9 of the 9 wound edge biopsies had correctly predicted a 'healing' WD14 genotype on the 2nd biopsy. (Table 1)

One wound was 'non-healing' on both 1st and 2nd visits. The wound edge biopsy had correctly predicted a 'non-healing' WD14 genotype on 1st biopsy but incorrectly predicted to 'healing' on the 2nd biopsy. (Table 1). In this cohort of patients experiencing no change in their clinical wound healing status between the 1st and 2nd visit, WD14 gene signature demonstrated a PPV of 94.4% (95% CI 80.9% to 98.6%) and NPV of 50.0% (95% CI 8.7% to 91.3%).

2. Patients with clinical change between first and second visit

One wound was clinically assessed as 'healing' on the 1st visit followed by 'non-healing' on the 2nd visit. The wound edge biopsy had correctly predicted a 'healing' WD14 genotype on the 1st biopsy but incorrectly predicted 'healing' on the 2nd biopsy (Table 1). Five wounds were clinically assessed as 'non-healing' on the 1st visit followed by 'healing' on the 2nd visit. Three of the 5 wound edge biopsies had correctly predicted a 'non-healing' WD14 genotype on the 1st biopsy and 5 of the 5 wound edge biopsies had correctly predicted a 'healing' WD14 genotype on the 2nd biopsy. (Table 1). In this cohort of patients experiencing a change in their clinical wound healing status between the 1st and 2nd visit, WD14 gene signature demonstrated a PPV of 66.7% (95% CI 47.3% to 81.7%) and NPV of 100%.

Discussion

This study describes the results of sequential WD14 gene signatures with time and compares them to clinical wound healing status. Within this cohort of 16 patients (32 wound-edge biopsies) WD14 gene signature fared well overall with a PPV of 85.2% and NPV of 80.0%. For patients with no change in clinical wound healing status between first and second visit, the test demonstrated a PPV of 94.4% and NPV of 50.0%**.** For patients with a change in clinical wound healing status between first and second visit, the test demonstrated a PPV of 66.7% and NPV of 100.0%**.**

Chronic wound management remains a major concern within the UK National Health Service as it accounts for up to 6% of the annual budget while causing significant pain, social isolation, depression and loss of time from work for patients.[5,15-17] Wound prognostication is therefore extremely crucial to enable healthcare professionals to target advanced care strategies towards chronic wound management, implement personalised wound care and enhance patient satisfaction by keeping them fully informed on treatment outcomes.[9] Genetic prognostication of chronic wounds is one recognised method of early identification of 'healing' and 'non-healing' wounds in order to target rigorous and advanced treatment regimens to hard-to-heal wounds. Accurate prediction of 'healing' could provide reassurance to both clinicians and patients and therefore, these wounds can be treated using standard regimens. The incurred financial savings can then be utilised in specialist novel therapies for non-healing wounds such as dermal substitutes, allogenic cultured skin equivalents and hyperbaric oxygen therapy.[18]

Bosanquet et al demonstrated a sensitivity of 63.6 per cent and a specificity of 85.4 per cent of WD14 gene signature in predicting clinical healing in chronic VLUs.[11] These results are broadly similar to results presented here (PPV of 85.2% and NPV of 80.0%). The initial WD14 gene signature is able to provide good prediction of wound outcomes, and interrogation does not significantly alter the standard assessment of chronic wounds as chronic ulcers usually undergo a wound edge biopsy to exclude occult neoplasm or autoimmune disease.

However further biopsies are generally not warranted in usual clinical practice, unless there is uncertainty regarding the biopsy results, or the wound significantly changes appearance. Subsequent wound biopsies would therefore need to be shown to be of significant benefit to warrant routine clinical use of WD14 gene signature. Despite an excellent PPV for wounds showing both no change in healing status between first and second visits (94.4%), and NPV for wounds with change in clinical status (100%), WD14 still incorrectly classified some wounds. Furthermore, most patients were healing at both first and second visit, and few demonstrated clinical change between visits. We therefore have scant data as to the responsiveness of WD14 to clinical change, despite some promising results. We have demonstrated that WD14 does change with time; it is not a 'fixed'. However, the benefits of sequential WD14 biopsies in predicting clinical wound healing in chronic wounds remain uncertain.

Other tools have been evaluated for predicting wound healing outcomes. The pathophysiology of wound healing is multifactorial with variable influence from proteolytic activity within the extracellular matrix, wound pH, circulating cytokine and protease levels, gene expression and tissue bacterial levels.[19-25] Power et al published a systematic review in

2017 assessing the potential for pH, exudate composition and temperature of wounds in predicting wound healing.[26] Findings suggested that wound pH changes from alkaline to acidic indicated a trend towards improving wound healing. Higher levels of MMP-9 (Matrix metalloproteinase-9) in wound exudate were observed to be elevated in acute or non-healing wounds while lower levels were observed in healing wounds.[27-30] Higher temperature measurements were found in acute, non-healing wounds and lower temperatures in healing wounds.[26] However, the external validity of these results was significantly limited by the low quality of included studies, small sample sizes and heterogeneity of study methodology.[31,32]

While our study is useful to describe changes in the WD14 genotype with time and clinical wound healing status, several limitations must be noted. The small sample size of 16 patients with venous leg ulcers from a tertiary wound healing unit may affect the generalisability of the study findings. All wounds were considered 'hard-to-heal' and WD14 may be more sensitive to change in an unselected cohort of VLUS. There were no 'non-healing' WD14 gene signatures on 2nd biopsies and hence the NPV in this cohort was incalculable. Few patients demonstrated a change in healing status, so we are unable to assess WD14 utility in predicting change for most patients.

It must be considered whether there is a biological basis for healing or a practical basis as many clinicians have observed that some patient's wounds convert from non-healing to healing when appropriate standard of care is provided. In addition, clinical observation would also suggest that when wounds become infected they can convert from healing to non-healing. As such, these observations need further investigation and verification. In

order to account for these confounding factors and better understand the role of WD14 gene signature in sequential wound edge biopsies, larger studies including more patients with changes in their clinical state are warranted to provide more reliable and externally valid results prior to usage in routine clinical practice.

Sequential biopsies might also be of value to help determine if a particular intervention is achieving the desired effect of transforming a clinically non-healing into a healing wound. Whilst a gene signature result is clearly only a surrogate outcome, reliable data existed to support prediction of clinical healing outcomes, then treatments could be trialled and assessed rapidly. However, we were unable to fully assess WD14 gene signature score due to lack of precision in identifying change in clinical status in this smaller cohort.

Conclusion

Although this study highlighted that the WD14 gene signature changed with time and with clinical wound healing status, further and larger studies are required to clarify precisely the role of this gene signature and its ability to change over time. Once WD14 gene signature prediction is further investigated and validated, there may be a potential role for use in clinical trials to rapidly predict the effect of therapeutic agents on wound healing.

Table 1. Summary of baseline characteristics

Characteristic	n (%)	Missing data
Age, median (IQR)	70 (64.5-80)	
Sex		1
Women	10 (66.6)	
Men	5 (33.3)	
Comorbidities		
Rheumatoid arthritis	1 (7.1)	2
Connective tissue disease	0 (0.0)	2
Active cancer	1 (7.1)	2
Immunosuppression therapy	5 (35.7)	2
Malnutrition	1 (10)	4
Alcoholism	0 (0.0)	5
Ex-smoking	2 (25)	6
Duration of ulcer		1
<6 months	2 (13.3)	
6-12 months	2 (13.3)	
12-24 months	2 (13.3)	
2-5 years	5 (33.3)	
>5 years	4 (26.7)	

Table 2: Correlation between WD14 gene signature and clinical wound healing status at first and sequential biopsies. A WD14 gene signature score of 1 indicated a predicted "healing" prognosis, whereas a score of 0 indicated a predicted "non-healing" prognosis.

Generic case number	Initial biopsy (WD - ID)	Signature score at first biopsy	Clinical Status of first biopsy at 3/12 FU	Sequential biopsy (WD - ID)	Signature score of sequential biopsy	Clinical Status of sequential biopsy at 3/12 FU
1	119	1	Non-healing	206	1	Healing
2	134	1	Healing	148	1	Healing
3	136	0	Non-healing	149	1	Healing
4	105	0	Non-healing	137	1	Non-healing
5	132	1	Healing	155	1	Healing
6	147	1	Healing	170	1	Healing
7	150	1	Healing	175	1	Healing
8	165	1	Healing	184	1	Healing
9	172	1	Healing	189	1	Healing
10	176	0	Healing	193	1	Healing
11	161	1	Non-healing	194	1	Healing
13	181	1	Healing	210	1	Healing
14	120	0	Non-healing	213	1	Healing
15	202	0	Non-healing	215	1	Healing
16	117	1	Healing	205	1	Non-healing
17	201	1	Healing	223	1	Healing

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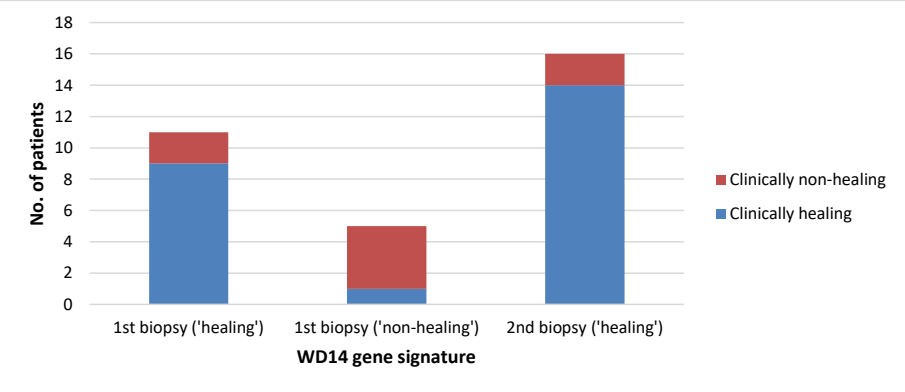
Figure 1. WD14 gene signature changes with time and with clinical wound healing status



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