Assessment of clinical effectiveness of haemoglobin spray as adjunctive therapy in the treatment of sloughy wounds

Objective: To assess use of an adjunctive topical haemoglobin spray in the treatment of sloughy wounds.
Method: In addition to a standard wound care regimen, consecutive patients with sloughy wounds self-administered haemoglobin spray treatment twice a week until the wound was healed. All patients were followed-up for 26 weeks. Results were compared with a retrospective cohort of 100 consecutive patients, treated during the same period the previous year with standard wound care alone. Data were collected on wound characteristics including percentage of slough, exudate levels, wound pain, and wound size.
Results: After 26 weeks, 94/100 patients (94%) treated with haemoglobin spray were completely healed compared with 63/100 control patients (63%). Positive results were evident as early as week one with 52% mean wound size reduction using the heamoglobin spray versus 11% in the retrospective control ($p<0.001$). At baseline, mean slough coverage was higher in the haemoglobin group, 58% versus 44% in the control group ($p<0.001$). By week four, mean slough coverage was 1% in the haemoglobin versus 29% in the control group ($p<0.001$). Reductions in exudate and pain levels ($p<0.001$) were also observed.
Conclusion: Overall, results of this evaluation showed the addition of adjunctive haemoglobin spray to standard wound care treatment achieved positive clinical outcomes for patients self-managing complicated sloughy wounds, by supporting reduction of wound exudate and slough within the complex multifaceted process of wound healing.
Declaration of interest: The authors provide advisory and speaking services to pharmaceutical and other health-care organisations including, but not limited to, infirst Healthcare Ltd. Infirst Healthcare provided the haemoglobin spray free of charge to the evaluation centre but did not have any influence on the design of the evaluation or the collection of the data. Infirst Healthcare also provided independent support for data management, statistical analysis and medical writing to help the authors publish the results of this evaluation.

debridement • haemoglobin • sloughy wounds • topical oxygen therapy • wound healing

Oxygen is a fundamental requirement for wound healing and plays a role in the majority, if not all, of the biological processes within a wound. In chronic wounds, low levels of oxygen, due to damaged blood vessels, are considered an underlying factor in reduced healing rates, as the hypoxic environment of the chronic wound affects many wound healing requirements.[1]

Clinical studies have demonstrated that enhanced wound tissue oxygenation improves wound healing, reduces microbial colonisation and decreases slough formation.[2,3] Continuous oxygen supply to the tissue through microcirculation is vital for the healing process, and for resistance to infection since wounds cannot heal without an adequate oxygen supply.[1,4,5] However, this is a multifactorial process and the body's tissues have no capacity for retaining oxygen molecules. Devascularised tissue in wounds makes it more difficult for oxygen to penetrate the wound bed, but it is essential that consistent oxygen delivery is maintained, along with essential elements, such as glucose, collagen, proteins, and metabolites, if healing is to occur effectively.[6]

Slough is a known barrier to wound healing[7] and is routinely observed in most chronic wounds, either as thick confluent layers or as patches on the surface of wounds.[8] It ranges in colour from white, suggestive of low microbial counts, to yellow or green, suggesting higher microbial counts.[9,10] Although slough formation is common during the inflammatory stage of the healing process,[11] its presence supports microbial growth and biofilm formation, infection and increased exudate which, when combined, inhibit healing and increase maceration in the periwound area.[5,6] Therefore, de-sloughing represents a critical process in wound bed preparation, and helps to support and enhance healing.[9]

Few technologies have proved effective at delivering improved oxygenation. However, a recently approved technology for use in chronic wounds, a topical haemoglobin spray comprising purified haemoglobin, aims to address this challenge by facilitating diffusion of oxygen into the wound bed.[12] Upon application, the haemoglobin spray disperses and covers the wound evenly, and *in vitro*, the addition of haemoglobin has been demonstrated to increase oxygen diffusion by

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Table 1. Baseline characteristics and wound aetiology

	Standard therapy group (n=100)	Haemoglobin spray group (n=100)	p-value
Age (years)			
Mean	42.5	39.3	0.34*
(Minimum, maximum)	(5, 92)	(5, 90)	
Sex			
Males, n (%)	48 (48%)	49 (49%)	
Females, n (%)	52 (52%)	51 (51%)	
Wound duration (months)			
Mean	1.5	1.6	0.75*
(Minimum, maximum)	(0.5, 15)	(0.7, 18)	
Wound size (cm^2)			
Mean	26.9	17.0	0.08*
Median	8.1	7.1	0.06**
(Minimum, maximum)	(0.4, 353.4)	(0.8, 169.6)	
Wound aetiology			
Trauma	35	43	
Venous leg ulcer	10	11	
Burn	10	9	
Abscess	5	10	
Insect bite	5	9	
Post-surgical wound	7	5	
Injection site wounds	7	5	
Diabetic foot ulcer	9	0	
Other (e.g. tattoo, inflammatory, skin graft, pressure ulcer, arterial leg ulcer)	12	8	

*Statistics reported are t-tests unless otherwise specified; **Mann-Whitney U-test p=0.062

over >400% in a low oxygen environment.[13] This enhanced oxygenation associated with topical haemoglobin spray has been demonstrated *in vivo* by Petri et al. who used non-invasive photoacoustic tomography to measure the oxygen saturation of five chronic leg ulcers, following the application of a topical haemoglobin spray.[14] Results showed that the average oxygen saturation increased significantly from 56.4% before application to 69% (p=0.042) after five minutes, and 78.8% (p=0.043) 20 minutes after the topical haemoglobin application, demonstrating its effectiveness in increasing oxygenation in hard-to-heal wounds.[14] Adding haemoglobin to water can increase the oxygen carrying capacity by a factor of 70 in comparison with water alone,[15] and so adding haemoglobin to wound exudate is expected to have a similar impact.

Studies show that haemoglobin spray is effective in promoting healing, as well as reducing inflammation, exudate and slough.[5,12,16] Its effects on slough reduction particularly warranted further investigation. Bateman conducted a preliminary evaluation, in the community setting, to determine whether the use of the haemoglobin spray expedited wound healing in 25 patients presenting with sloughy wounds.[6] Results after four weeks demonstrated that all wounds met the positive endpoints of slough elimination and wound size reduction.[6] An expansion of this evaluation to 100 patients treated with haemoglobin spray was then conducted, also showing that, after four weeks' treatment, all wounds demonstrated positive endpoints of slough elimination and wound size reduction.[7] This evaluation presents further data on these 100 patients treated with haemoglobin spray over a longer time period (up to 26 weeks), and compares the data with a historical control cohort of 100 patients from the same clinic, from the same period, treated during the previous year with standard wound care alone. The aims of this evaluation were to investigate the effects of facilitated oxygen delivery in assisting the patient with enhanced wound healing and reduced pain, as well as determining the specific effects of oxygen/haemoglobin spray on slough and exudate levels.

Methods

This evaluation was conducted in a community care setting of a general practice/walk-in centre (GP/WIC). Patients were recruited if presenting with any wound that showed evidence of slough but who did not require immediate hospitalisation. Patients were recruited consecutively and received haemoglobin spray (Granulox, infirst Healthcare Ltd, London, UK) in addition to their individualised standard wound care regimen. The active ingredient of the spray is 10% carbonylated haemoglobin, other ingredients include 0.7% phenoxyethanol, 0.9% sodium chloride, 0.05% N-acetylcysteine and water to 100%.

Control patients were identified retrospectively, in consecutive order, from patient notes from the same period the previous year. The control patients, which were not pre-selected in any way and were included using the same inclusion and exclusion criteria as the haemoglobin spray group, received individualised standard wound care only. Thus, patients in both groups were treated as per the normal standard practices of the GP/WIC and based on individual medical/nursing needs, with the only difference being the use of adjunctive haemoglobin spray in one group.

This evaluation was not conducted as a formal clinical study, but rather a 'real life' assessment of the use of haemoglobin spray for the treatment of sloughy wounds. Ethics Committee approval was not required in line with the UK National Health Service (NHS) policy at the time on clinical evaluations of CE-marked products used within their licensed indications without randomisation. The evaluation was conducted according to the principles of the Declaration of Helsinki. Patients treated with haemoglobin spray were required to give verbal consent following explanation of the evaluation, review of the product, and an information leaflet was provided to patients before receiving treatment. This procedure was documented by the clinician in the patients' medical notes. Patient inclusion criteria comprised those patients who

presented with a healing or non-healing wound with evidence of at least 10% sloughy or necrotic tissue, (as calculated by measurement of the wound dimensions and the area covered by sloughy tissue using a sterile paper ruler), wounds that were not diagnosed as infected and requiring treatment with antibiotics at baseline, and patients and/or their carers who consented to haemoglobin spray being used and applied as part of their standard care arrangements. Exclusion criteria included patients who were pregnant (in accordance with the labelling of topical haemoglobin spray at the time the evaluation was conducted), patients diagnosed with an infected wound at baseline, or those who declined entry into the evaluation.

Patients who met the inclusion criteria and consented to treatment had their wounds assessed in line with consensus recommendations.[17] Patients were trained on how to apply the haemoglobin spray to the wound bed in conjunction with their normal cleansing and dressing regimen. They were instructed to deliver the haemoglobin spray by spraying the wound for 1–2 seconds, with the nozzle held approximately 5–10cm away from the skin and pointed towards the wound, until it was covered by a thin film. Patients applied the haemoglobin spray twice a week until the wound was healed. All patients were followed-up for 26 weeks so that the time-to-healing and any subsequent relapse (if applicable) could be monitored. They were assessed on a regular basis (patients were reviewed as part of their normal clinical review as wound care and progress dictated) for wound assessments to be carried out. Any invasive interventions, such as debridement, were carried out by qualified clinicians as necessary.

Wound data were collected whenever the patients were seen and wound evaluation carried out and recorded in the patient notes using a designated evaluation form, based on the Applied Wound Management assessment form,[18] the standard wound care documentation used by the health-care provider. The data were transferred to anonymised case report forms for data entry and analysis by the authors. Data collection included wound diagnostics, such as:

- Wound infection status
- Wound dimensions (width and length)
- Wound exudate level (high, moderate or low).
- Wound characteristics: Percentage of the wound characterised as granulation tissue, slough, necrotic tissue, and epithelial tissue
- Whether bone or tendons were visible within the wound bed.

In addition to wound characteristics, any adverse events (AEs) and wound pain (using a 10cm visual analogue scale (VAS)), assessed pre-dressing change, were recorded in both groups, as well as the current treatment regimen, including any debridement procedures, off-loading, wound rinse, dressings used, and the frequency of dressing changes. The same data were also collected from the retrospective cohort of patients who were treated with the standard wound care regimen (i.e. before introduction of haemoglobin spray) by the author, using the same inclusion and exclusion criteria, during the same period the previous year, and using the same data collection form to ensure minimisation of sampling bias.

Statistical analysis

Statistics were reported using a chi-squared test for group level (nominal) data and an unpaired two-tailed t-test for numeric (parametric) values. Proportions were evaluated using z-tests. Statistical significance was defined as $p<0.05$. No adjustment for multiple statistical analysis was made, and multivariate or regression statistics were not conducted. The primary outcome was defined as wound healing over 26 weeks.

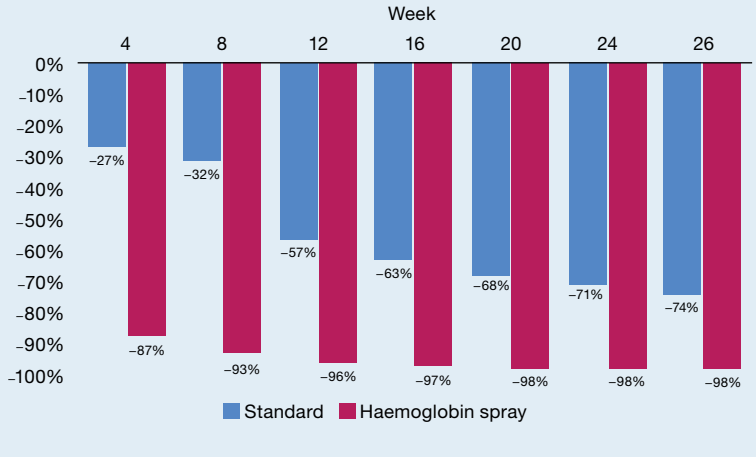
Results

Patient Disposition

A total of 200 patients were included in this evaluation: 100 patients in the haemoglobin spray group (49% males, 51% females, mean age: 39.3 years), and 100 patients in the retrospective control group (48% males, 52% females, mean age: 42.5 years). Patients presented with a diversity of sloughy wound aetiologies, the most common of which were trauma (n=78), venous leg ulcer (VLU) (n=21) and burns (n=19) (Table 1). Patients were followed-up for 26 weeks, however, five patients in the control group died during the evaluation period. No patients died or were lost to follow-up in the haemoglobin spray group.

There were no significant differences between the groups in the baseline characteristics of age, gender, wound type distribution, baseline wound size or pre-baseline wound persistency. Wounds had been present for an average of 1.6 months in the haemoglobin

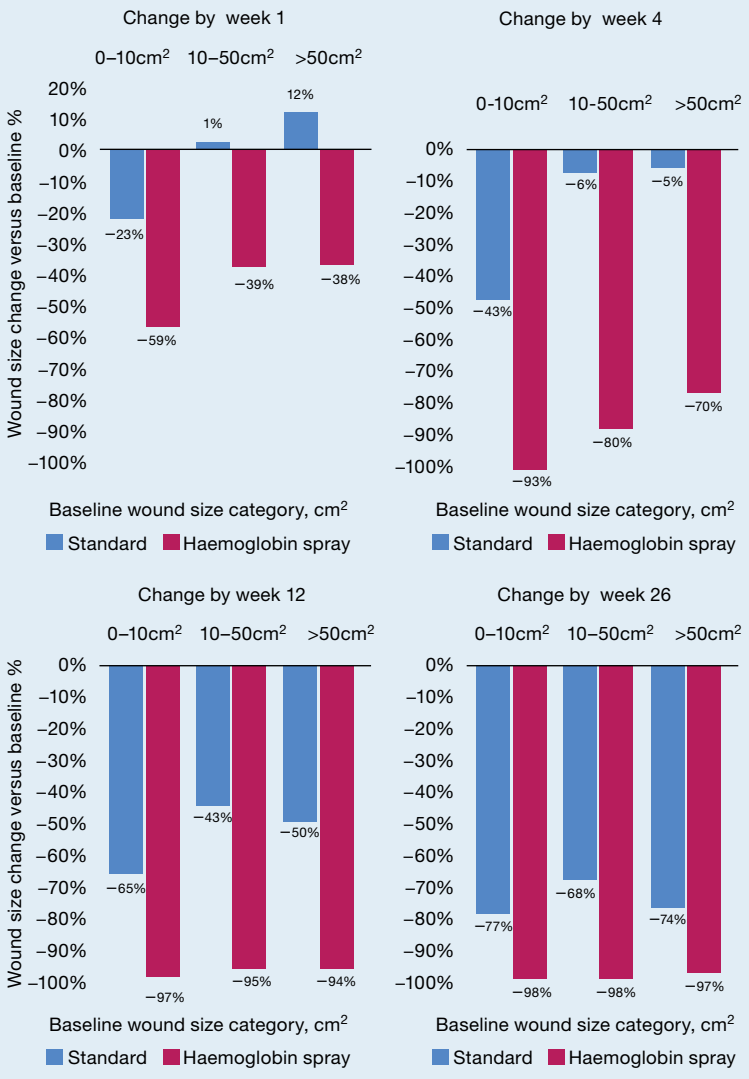
Fig 1. Mean change in wound size versus baseline by week (weeks 4 to 26)



Week	4	8	12	16	20	24	26
t-test p-values	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p=0.002

All data used up to death or loss of follow-up by week

Fig 2. Wound size change versus baseline wound size (cm^2), weeks 1 to 26



Sample size at baseline:
0-10cm^2: n=65 (haemoglobin spray); n=55 (control)
>10-50cm^2: n=25 (haemoglobin spray); n=30 (control)
>50cm^2: n=10 (haemoglobin spray); n=15 (control)
Includes all patients remaining in the evaluation, all patients who died at any point up to time of analysis are excluded

spray group (maximum: 18 months), compared with 1.5 months in the control group (maximum: 15 months) (p=0.75). The average baseline wound size was larger in the control group (26.9cm^2, range: 0.4–353.4cm^2) compared with the haemoglobin spray group (17.0cm^2, range: 0.8–169.6cm^2), but this difference was not statistically significant (p=0.08). Median wound size was similar at 8.1cm^2 and 7.1cm^2, respectively (Table 1). However, both baseline pain scores and percentage slough coverage were significantly higher in the haemoglobin group than in the control group. Mean pain scores were 6.2 in the haemoglobin spray group and 3.4 in the control group, with 30 more wounds generating pain in the haemoglobin spray group compared with the control group (p<0.001). Mean slough coverage, defined as sloughy or necrotic tissue, was 58% at baseline in the haemoglobin spray group, and 44% in the control group (p<0.001).

Wound healing

By the end of the 26-week evaluation period, 94/100 patients (94%) treated with haemoglobin spray were completely healed, compared with 63/100 control patients (63%). Rapid wound size reduction was observed in the haemoglobin spray treatment group, and by week one there was already a significant difference between the two groups in terms of mean wound size reduction compared with baseline: 52% mean wound size reduction in the haemoglobin spray group versus 11% in the control group (p<0.001). By week four, the mean reduction in wound size was 87% for patients in the haemoglobin spray group compared with baseline, versus 27% in the control group (p<0.001). By week eight, this had increased to a mean wound size reduction of 93% versus 32% (p<0.001), and at week 12 this had further increased to 96% versus 57% (p<0.001) in favour of haemoglobin spray. By completion of the evaluation at 26 weeks, patients in the haemoglobin spray group had a mean wound size reduction of 98% compared with 74% of control patients (p=0.002) (Fig 1).

The greater reduction in mean wound size seen in the haemoglobin spray group, compared with control, occurred regardless of baseline wound size. For the smallest wound sizes at baseline (0–10cm^2), a wound size reduction of 59% was seen in the haemoglobin group versus 23% in the control group at week one. For larger wounds (>10–50cm^2) a wound size reduction of 39% was seen in the haemoglobin spray group versus an increase in mean wound size of 1% in the control group at week one. For the largest wounds (>50cm^2), a wound size reduction of 38% was seen in the haemoglobin spray group versus an increase of 12% in the control group, at week one. This pattern of superior wound size reduction in favour of haemoglobin spray could also be seen at all subsequent weeks, up to week 26 (Fig 2).

In terms of the number of wounds that had not healed by completion of the evaluation (i.e. had not achieved full epithelialisation by week 26), there was a marked difference between the two groups in favour of haemoglobin spray. By week eight, the proportion of wounds remaining unhealed was 25% in the haemoglobin spray group compared with 55% in the control group. The maximum difference was seen at week 10, with 34 percentage points difference between the groups (19% of haemoglobin spray-treated patients had non-healed wounds compared with 53% of control patients). By completion of the evaluation at week 26, there was a 28 percentage point difference between the two groups, with only six patients (6%) in the haemoglobin spray group still having wounds that had

not healed completely, compared with 32 patients (34%) in the control group (Fig 3).

Slough levels

At baseline, mean slough coverage was higher in the haemoglobin spray group at 58% compared with 44% in the control group ($p<0.001$). Despite this, as early as week one, there was a significant difference in mean reduction in wound slough coverage of 67% compared with only 8% reduction in the control group. By week four, there was a mean slough reduction of 99% versus 33% in favour of haemoglobin spray. There was a slight re-emergence in mean slough levels from week 20 up to week 26 in the haemoglobin spray group which was due to four patients stopping using the haemoglobin spray before their wounds were fully healed, and who subsequently suffered a worsening of their wound. The difference in mean slough coverage between the groups was statistically significant in favour of haemoglobin spray at all time points between weeks one and 26 ($p<0.05$) (Fig 4).

Exudate levels

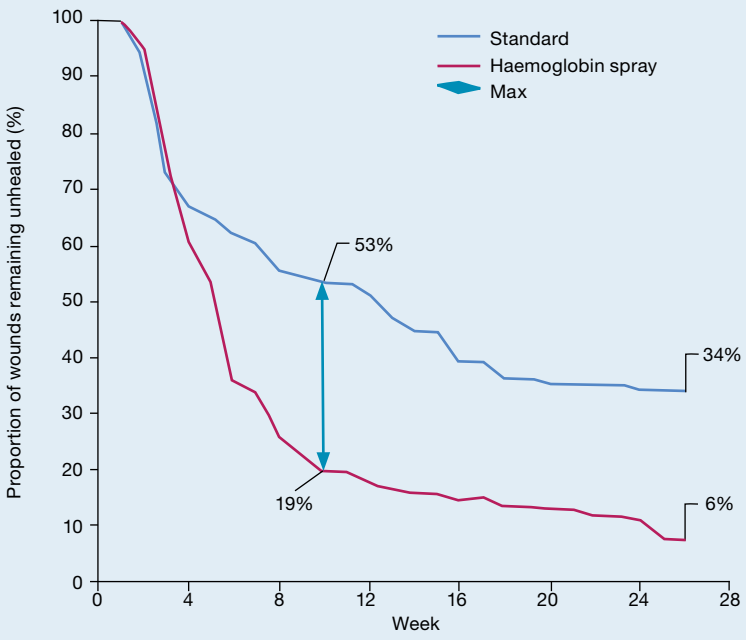
At baseline, both the haemoglobin spray and control groups had a similar number of patients with high or moderate levels of exudate (97 patients and 94 patients, respectively), but by week four, only five patients in the haemoglobin group had high or moderate levels of exudate compared with 55 patients in the control group. By week 26, 94% of haemoglobin spray-treated patients had no high or moderate exudate compared with 67% of control patients (Fig 5). There were two patients who saw a re-emergence of high levels of exudate during the evaluation, in both cases the haemoglobin spray treatment had been prematurely stopped.

Pain assessment

Pain levels were assessed pre-dressing change for those patients who reported pain at baseline (89/100 haemoglobin spray patients, 59/100 control patients). Significant improvements in mean reported pain scores at week one for haemoglobin spray treated patients, with a 60% reduction in pain scores in the haemoglobin spray group compared with only 15% reduction in the control group ($p<0.001$). By week four, there was a 93% reduction in pain scores in the haemoglobin group compared with a 49% reduction in the control group ($n=59$) ($p<0.001$). Average pain scores reflected this trend (Fig 6), with average baseline pain scores of 6.2 and 3.4 for haemoglobin spray and control groups, respectively. This reduced to 0.6 and 1.9 for haemoglobin spray versus control, respectively, at week four ($p<0.001$). By week eight, average pain scores were 0.3 and 0.9 for haemoglobin spray patients versus controls ($p<0.05$). At the end of the evaluation period at week 26, mean pain scores in the two groups were similar, being 0.2 and 0.1 for haemoglobin spray versus control (not significant). There were four patients in the haemoglobin spray group with persisting wound pain after 26 weeks, with these being patients who had stopped the haemoglobin spray treatment prematurely (at weeks nine, 10, 16 and 21) and, subsequently, suffered increased pain.

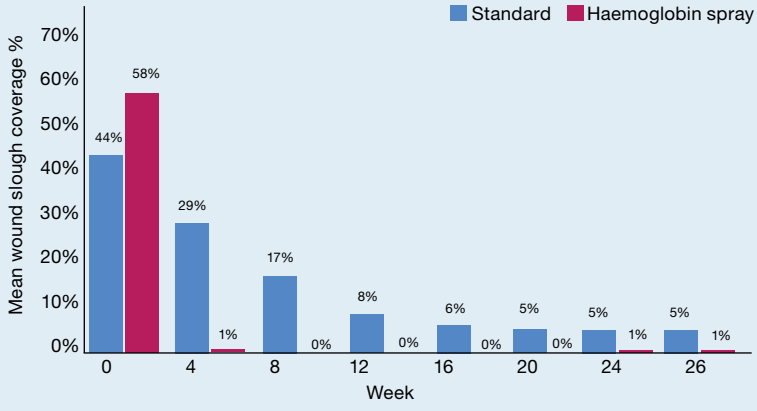
Thus, although average pain scores reduced to a similar level for both groups from weeks 16 until completion of the evaluation, haemoglobin spray was associated with a more rapid pain score reduction

Fig 3. Wounds remaining unhealed (baseline to week 26)



Excludes five patients who died in the control group and did not complete the evalutaion (haemoglobin spray, n=100; control, n=95). $p<0.01$ for periods from week six onwards

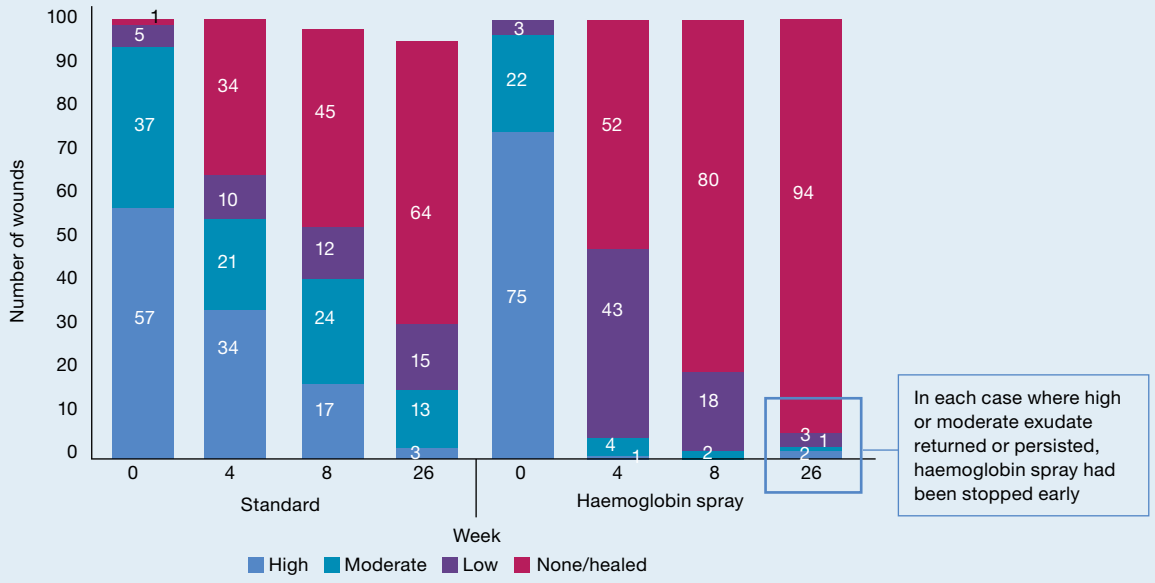
Fig 4. Mean wound slough coverage (baseline to week 26)



Week	0	4	8	12	16	20	24	26
t-test p-values	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	0.01	0.04

Average wound coverage of sloughy or necrotic tissue. Healed wounds considered as slough-free. Patients included until lost to follow-up. Five patients died in the control group; there were no deaths in the haemoglobin spray group, n=100/100 at baseline. 95/100 at week 26

Fig 5. Distribution of exudate levels by week (baseline to week 26)



*Including all wounds up to date of death or loss of follow-up. Four out of five patients who died in the control group had moderate to high exudate at baseline. There were no deaths in the haemoglobin spray group

compared to the control. This reduction was most notable over the first four weeks of treatment.

Safety

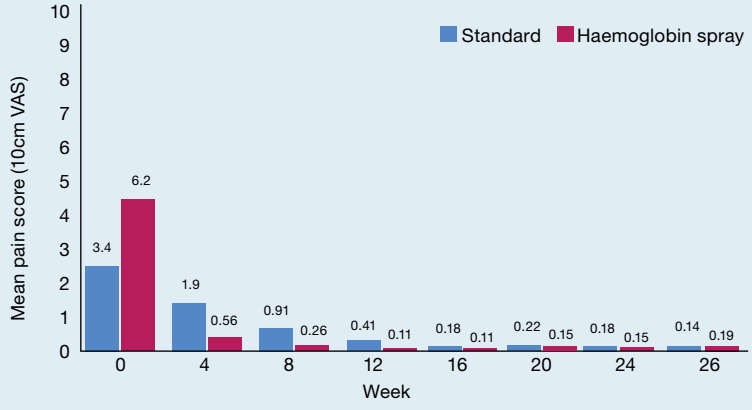
Deaths

There were five deaths in the control group and none in the haemoglobin spray group. None of the deaths was related to the patients' wounds.

Additional interventions

There were 17 interventions in the control group compared to only two in the haemoglobin spray group. In the control group, 14 patients required unplanned surgery for wound debridement and three patients had an infection requiring antibiotics. In the haemoglobin spray group, one patient had unplanned surgery for wound debridement and one patient had an infection requiring antibiotic treatment.

Fig 6. Average reported pain score (baseline to week 26)



Week	0	4	8	12	16	20	24	26
t-test p-values	<0.001	<0.001	0.009	0.02	0.48	0.57	0.81	0.65

*Healed wounds considered pain-free unless reported as painful following healing during follow-up (none). All wounds included up to week of death or loss of follow-up. All four patients in the haemoglobin spray group with remaining wound pain had stopped haemoglobin spray prematurely, in weeks 9, 10, 16 and 21, and subsequently suffered increased pain

Discussion

The aims of this evaluation were to investigate the effects of facilitated oxygen delivery in assisting the patient with enhanced wound healing and reduced pain, as well as determining the specific effects of oxygen/haemoglobin spray on slough and exudate levels.

For many stalled, hypoxic chronic wounds, it is evident that lack of oxygen represents a rate-limiting factor to healing.[19,20] The reason for this could be that damaged tissue has increased oxygen demands, and so chronic wounds require additional oxygen for effective and optimal healing.[1,21] Many clinical studies have shown positive clinical outcomes following the administration of oxygen to wounds.[19,20,22–25] It is well accepted that oxygen has an important role in several processes, including fibroblast migration and replication,[26] collagen production,[27] stimulation of angiogenesis[26] and antimicrobial activity of leukocytes and macrophages,[28] endothelial cell proliferation,[29] neovascularisation,[30] epithelialisation [29] and wound healing. However, the body has no capacity to store oxygen and is dependent on a continuous supply to facilitate optimum tissue regeneration.[6]

Traditional strategies for increasing oxygen supply have shown limited success, and non-interventional oxygen therapies, such as topical or hyperbaric oxygen therapies (HBOT), have only shown modest results. Furthermore, in the case of HBOT, it also subjects the patient to significant risks.[31,32] Topical oxygen therapy has been limited, in terms of success, due to diffusion constraints and its ability to penetrate through wound exudate. Indeed, Piantodasi found that topically delivered oxygen was only able to diffuse 50–100 microns.[33] Furthermore, necrotic tissue, slough, debris, devitalised tissue and biofilms can all restrict oxygen availability into the wound bed.[34]

Slough is a well-known barrier to wound healing[7] and its reduction and removal is important for effective and timely wound healing,[35] particularly in problematic and hard-to-heal wounds. Sloughy wounds present a challenge as they are time-consuming to manage, often require specialist input for slough removal, and negatively impact on a patient's quality of life (QoL).[7] Slough is composed of fibrin, proteins, serous exudate, leukocytes and microorganisms that can build up rapidly on a wound surface.[31] Although, the presence of slough within the wound bed is not necessarily a sign that a wound is not healing, its presence supports microbial growth and biofilm formation, infection and increased exudate which, when combined, inhibit healing and increase maceration in the periwound area.[5,6] Although over 90% of chronic wounds show evidence of bacteria present within a biofilm, persistent macroscopic slough may be an indicator for the presence of microscopic biofilms.[9,35] In wounds where slough re-forms quickly after successful wound cleansing, there is a greater likelihood that biofilms are present.[9] It has also been suggested that chronic wounds trapped in the inflammatory healing phase have an increased incidence of slough formation, often relating to increased levels of exudate.[36] As the formation of slough is considered a result of numerous elements linked to inflammation, immune hyperactivity, and biofilms, improved oxygenation may prove beneficial for all these issues.

De-sloughing represents a critical process in wound bed preparation and helps to support and enhance the wound healing process.[9] For effective wound healing, debridement (i.e. removal of non-viable and necrotic tissue) and de-sloughing (i.e. removal of slough) is essential.[9] It is imperative that new technologies, with low or no risk to the patient, are developed that can prevent or reduce the development of slough and its recurrence. Adjunctive topical haemoglobin wound spray is one novel approach to aids slow-healing wounds by acting as an oxygen transporter, facilitating diffusion from surrounding air and improving oxygen availability in the wound bed.[5,37] A pilot study by Petri showed that average oxygen saturation increased from 56.4% to 78.8% after application of haemoglobin spray.[14]

Results from this evaluation showed a clear, rapid improvement in wound healing when haemoglobin spray was added to the standard wound care regimen, with haemoglobin spray being associated with a rapid reduction in slough levels from week one onwards. However, it is important to note that haemoglobin spray is used as an adjunct to standard care, so wound bed preparation and care needs to be maintained as per usual medical practice before the use of the haemoglobin spray, so the wound bed is ready to achieve the best outcome. It is also recommended that haemoglobin spray treatment is maintained until complete wound closure. The effects of premature treatment discontinuation were seen during this evaluation where four patients discontinued haemoglobin spray treatment early, before complete wound closure, and there was a re-emergence of slough and pain

Reductions were also seen in exudate and pain levels, all of which contribute to significant improvements for patients in the haemoglobin spray group. These benefits were realised without any apparent increase in risks, with no indications of increased interventions in the haemoglobin spray group. The fact that haemoglobin spray treatment was associated with fewer interventions (i.e. surgical debridement and infections) indicates patient benefit could be realised earlier in the treatment pathway than current recommendations suggest, and wound care costs may also be reduced.

These benefits of haemoglobin spray are likely to be due to its ability to facilitate the delivery of oxygen faster and deeper into the wound bed compared with the natural diffusion of atmospheric oxygen.[14] However, further studies are needed to clearly establish its mode of action, which could include links to inflammation, and microorganisms/biofilms. For example, oxygen delivered by the haemoglobin spray may influence macrophage promotion and the chemotaxis of other immune cells. In chronic wounds, natural killer cells and natural killer T-cells increase the expression of pro-inflammatory cytokines and reduce anti-inflammatory cytokines. It is possible, therefore, that oxygen may play a role in suppressing the over-production of pro-inflammatory cytokines.[38] Furthermore, haemoglobin spray may impact on the development of reactive oxygen species (ROS) which act as intracellular messengers in the cell cycle, helping to drive endothelial cell signalling, enhancing angiogenesis and supporting cell migration and proliferation, as well as being involved in triggering vascular epidermal growth factor (VEFG-F) expression from both macrophages and keratinocytes.[39] The additional oxygen delivered by the haemoglobin spray could also have immunomodulatory effects by providing neutrophils with the large quantities of oxygen necessary to allow them to destroy microbes via the respiratory burst process.[40] It may also enhance nitric oxide production, which is important in wound

healing through its effects of reducing inflammation, increasing angiogenesis, enhancing cellular proliferation, collagen deposition, matrix development and wound remodelling.[41]

There is a substantial and growing body of evidence that improvements in oxygen availability in wounds have a positive effect on healing, in particular for wounds with compromised macro- or microvascular blood supply.[14] Oxygen is vital for various processes in the healing of wounds including collagen deposition, epithelialisation, fibroplasia, angiogenesis, and resistance to infection. Impeded delivery of oxygen-rich blood to the wound tissue, therefore, impedes physiologic healing with a resultant hypoxic wound microenvironment characterised by insufficient nutrient and oxygen delivery to the regenerating tissue. Tissue confronted with an acute, mild-to-moderate hypoxic challenge typically adapts, whereas, conversely, tissue subjected to chronic, severe hypoxia does not survive. Similarly, for elevated wound tissue oxygen partial pressures, where a moderate hyperoxic challenge can stimulate the production of growth factors and the formation of new blood vessels, extreme hyperoxia can induce mitochondrial apoptosis, growth arrest, and oxidative stress via the formation of ROS. Thus, the inherent complexity of the healing process requires the precise combination of hypoxic cellular signalling and antioxidant defence mechanisms with adequate tissue oxygenation'.[42]

The positive clinical results in terms of wound healing, slough, exudate and rapid pain reduction were also achieved with substantially reduced resource usage, since patients could self-manage their sloughy wounds at home, rather than by health professionals in a hospital or community setting. Improved healing leads to reductions in costly and time-consuming interventions, such as debridement, and allows patients to self-manage their wounds. Clinicians must be proactive in their approach to wound care by adopting new and advanced technologies that improve healing rates, thereby enabling patients to self-manage and reduce their reliance on high cost care.[43]

Data suggest haemoglobin spray treatment delivers positive outcomes in a wide variety of wound groups, including sloughy and non-healing, in a diversity of clinical settings (i.e. acute and community care)[5,6] so it has the potential to benefit a large patient population presenting with wound problems. Haemoglobin spray has demonstrated short-term effects on slough and exudate levels, as well as long-term benefits of wound closure. These findings challenge the way clinicians currently use debridement and other de-sloughing techniques. Surgical debridement carries a risk to the patient and a significant cost. Thus, it could be argued that revision of current clinical pathways is warranted, with haemoglobin spray recommended for use as a first-line debridement technique instead of surgical debridement and other currently used de-sloughing methods.[44]

Reflective questions

- Is there a role for haemoglobin spray in treating sloughy wounds?
- Does using haemoglobin spray to treat sloughy wounds have benefits for health professionals such as reducing nursing time and use of dressings?
- Are there any additional benefits other than faster slough clearance in using haemoglobin spray compared with standard treatment in terms of recovery time and pain levels?

Limitations of the evaluation

It should be noted that this evaluation was not conducted as a formal randomised clinical study, but rather as a 'real world' evaluation in the primary care setting. However, the outcomes of this, and the previous smaller scale evaluations in sloughy wounds,[6,7] may be used to inform future formal randomised clinical studies in this field.

The patients recruited to this evaluation were relatively young, with a mean age of approximately 40 years old, and suffered sloughy wounds due to a variety of aetiologies ranging in severity from insect bites to trauma. Although nine of the included patients presented with a diabetic foot ulcer (all allocated to haemoglobin spray treatment), there was no analysis of any potential underlying conditions in these patients that may have had a significant impact on wound healing, such as diabetes or arterial/vascular disorders. In addition, it should be noted that the two groups were not fully balanced at baseline, with pain scores and percentage slough coverage being significantly higher in the haemoglobin group than in the control group, thus indicating that the control group may have less severe wounds. However, the haemoglobin group still showed significant improvement despite this, reinforcing its beneficial effects.

It must be acknowledged that measuring maximal length and perpendicular width to estimate the wound area can prove inaccurate, and does not account for changes in wound shape. The precision and reliability of digital planimetry over more conventional methods may be considered for future studies.

Finally, this evaluation did not include objective measurement of oxygen levels in the treated wounds. Direct non-invasive measurement of tissue oxygen tension in chronic wounds is technically difficult but novel techniques are being developed, such as photoacoustic imaging,[14] which it may be possible to include in future studies.

Conclusions

In conclusion, this 26 week evaluation shows that the addition of haemoglobin spray as an adjunct to a standard wound care regimen results in positive patient clinical outcomes by promoting effective wound healing, as well as reducing wound slough and exudate levels, and rapidly decreasing wound pain. Furthermore, it may be used to

help prompt a stalled and unresponsive wound to start healing which is thought to occur by reducing inflammation through the modulation of immune cells which, in turn, leads to a reduction in slough and exudate levels. Further well designed, controlled clinical studies are required to support these clinical findings and to establish the mechanism by which haemoglobin spray reduces inflammation, slough and exudate. If similar results emerge, then the addition of haemoglobin spray to standard wound care regimens should be considered in line with proposed consensus guidelines.[17] JWC

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