

Hemoglobin spray as adjunct therapy in complex wounds: Meta-analysis versus standard care alone in pooled data by wound type across three retrospective cohort controlled evaluations

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Abstract

Objectives: Low tissue oxygenation is a predictor of healing outcomes in complex wounds. Adjunct hemoglobin to aid oxygen diffusion has been demonstrated to achieve superior healing outcomes; however, the relative healing benefit across different wound types and evaluations has not yet been estimated. This article does this for the first time.

Methods: Data were pooled from previously published real world controlled evaluations, three retrospective cohort controlled studies of a variety of wounds within standard care across hospital and primary care in the North of England in patients with diabetic foot ulcers (n=40), chronic wounds (n=100), and sloughy wounds (n=200). Wounds were equally distributed between adjunct hemoglobin and control and the hemoglobin spray was used as per instructions for use and applied twice weekly, mostly for the duration of the wounds in all three evaluations. Wound healing over 26 weeks was the primary outcome in each of the evaluations, with additional healing and quality of life indicators including pain, wound size, slough coverage, wound exudate levels, adverse events, and dressing regimen used, over time. Each wound type with 10 or more patients in both the standard care alone and adjunctive hemoglobin groups (n=257/73% of patients) was evaluated.

Results: Cox proportional hazards log-rank regressions demonstrated significantly higher weekly chance of healing in each wound type (β , 95% range, sample, p): trauma 1.55 (1.23–1.96, n=110, p<0.001), diabetic foot ulcers 2.39 (1.52–3.75, n=60, p=0.01), venous leg ulcers 4.98 (1.69–14.7, n=33, p=0.04), burns 1.82 (1.11–2.99, n=30, p=0.02), and post-surgical wounds 2.75 (1.53–4.96, n=24, p=0.001). Results on additional healing indicators were consistent with the main findings. Notably, controlling for ischemia in diabetic foot ulcers resulted in an increased β of 5.68 (2.33–13.86, n=29, p<0.001).

Conclusion: Adjunct hemoglobin spray, when implemented within standard care, is likely to achieve substantial healing benefits to patients, in particular for diabetic foot ulcers, venous leg ulcers, and post-surgical wounds.

Keywords

Dermatology, nursing, surgery, hemoglobin spray, complex wounds, oxygen

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Introduction and background

Complex wounds, wounds that heal slowly or have healing complications such as slough, contribute to loss of quality of life through pain, malodor, risk of exudate strike-through, and restrictions on daily activity. Low level of tissue oxygenation is a predominant risk factor and a predictor of healing outcomes, notably in the context of peripheral vascular disease, increased oxygen demand of healing tissue, and the generation of reactive oxygen species.¹ Facilitated diffusion using hemoglobin was demonstrated more than 50 years ago, with

oxygen diffusion rates improving by several hundred percent in vitro,² but has only recently been recognized as a promising approach to increase oxygen availability within the

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wound bed of complex wounds.³ Recent research in a small sample of five wounds on the impact of oxygen levels within the wound bed suggests significant and quickly achieved improvements in oxygen diffusion with increases of 2%–50% (mean of 21%) within 5 min of application and 15%–58% (mean of 41%) within 20 min.⁴ Hemoglobin solution requires regular reapplication, with instructions for use in the CE-mark labeling suggesting reapplication at least twice weekly (Granulox®, Hälsa Pharma GmbH). Substantial healing benefits more than double those of standard care are expected in venous leg ulcers (VLUs) based on randomized controlled trial (RCT) data in 72 patients demonstrating a mean reduction in wound size of 53% versus a 21% mean increase in the control group over 13 weeks.^{5,6} Additional positive RCT data were also reported in a study over 6 months in 28 patients with lower leg ulcers where 1/14 legs healed with a standard care regimen versus 13/14 using a hemoglobin regimen, but where the dressings were also varied and some of the benefit may have been a combination of different dressings and the hemoglobin spray.⁷ Trial benefits have translated into substantial real world benefits, with more than double the healing rates demonstrated in diabetic foot ulcers (DFUs),⁸ chronic wounds,⁹ and sloughy wounds¹⁰ when implemented within standard care according to proposed consensus treatment guidelines.³ However, so far, no attempt has been made to assess the relative benefit achieved for the specific wound types normally seen within a standard care setting within a representative wound caseload population. This article sets out to do so by pooling all available comparative real world data to date and assessing the benefit for each observed wound type.

Method

Known real world evaluations with retrospective controls were sourced and a literature review using PubMed was conducted (not registered), using a broad range of keywords including wounds or ulcer and hemoglobin or haemoglobin to identify additional comparative real world data evaluations without any restrictions for years, language, or source. No additional evaluations other than evaluations conducted by the current authors were identified. Wound types and healing outcomes were extracted from data on file from three previously reported and similar 6-month controlled evaluations of hemoglobin spray compared with standard care alone from before hemoglobin was introduced, in $n=2 \times 20$ patients with DFU with a SINBAD score of less than 3 in a tertiary care hospital wound clinic,⁸ in $n=2 \times 50$ wounds of any etiology that had failed to achieve >40% wound size reduction over the preceding 4 weeks,⁹ and in $n=2 \times 100$ wounds of any etiology with >10% slough coverage at baseline,¹⁰ both of the latter in primary care clinics.

In all three evaluations, the hemoglobin spray was used as per instructions for use and applied with dressing changes twice weekly in line with standard of care in the reporting

clinics, mostly for the duration of the wounds. No other changes than hemoglobin spray was made at baseline. Antibiotics, amputation, debridement, and any other aspects of standard care, including adjustments of dressing size or frequency, were allowed as per standard care as wounds improved and size decreased, or as it deteriorated. Primary endpoint in all three evaluations was wound healing to 6 months. In each of the evaluations, a control cohort was selected from the same clinics from the same time of year during the prior year, with the same patient inclusion and exclusion criteria that would have qualified for and received the hemoglobin solution the following year. The same hemoglobin solution spray was used in each of the evaluations reported (Granulox®, Hälsa Pharma GmbH). Risk of bias for each of the evaluations according to the Cochrane method was indicated from the real world nature of the data collected, without any form of blinding of treatments. Other risk of bias was largely mitigated for the same reason, the use of data collected from real world cohorts of patients within regular care with minimal exclusion criteria. Each evaluation was approved by the respective local institutional review boards. All patients in the hemoglobin treatment evaluation groups provided verbal informed consent. No additional institutional review board approval was sought for the current meta-analysis of the previously reported evaluations.

Several differences were reported at baseline between the treatment and the control groups, although each was deemed unlikely to significantly favor the hemoglobin-treated groups. In the DFU evaluation, no significant differences were reported at baseline. In the chronic wound evaluation and the slough evaluation, clinical practice had changed over the previous year in that nursing staff would no longer do all dressing changes, and patients and their regular care provider/family were encouraged to complete planned dressing changes. In addition, the mean reported pain scores were higher at baseline in the hemoglobin groups in both the chronic wound and sloughy wound evaluations and the control group had more large and longer persistent wounds in the chronic cohort evaluation. In the sloughy wound evaluation, there were more patients with neuropathy and limb ischemia in the control group. See Table 1 for details of the three data sources and observed differences at baseline and reported adverse events. Risk of bias was controlled for via Cox proportional hazards regression for known wound healing covariates. See “Results” section.

Five wound sub-types were identified from the case records of wounds included across the three evaluations with 10 or more patients in both groups: trauma ($n=110$), DFU ($n=60$), VLU ($n=33$), burn ($n=30$), post-surgery ($n=24$). The proportion of wounds receiving hemoglobin treatment versus standard care alone was similar (38%–55%) across wound types. The wounds identified as VLU did not exclude patients with comorbid peripheral arterial disease. Other wound types with less than 10 wounds in either group

Table 1. Overview of data sets.

	Diabetic foot ulcers ⁸	Chronic wounds ⁹	Sloughy wounds ¹⁰
Design	20 consecutive patients, with 20 consecutive retrospective cohort controls from same setting and same period year prior using same protocol	50 consecutive patients, with 50 retrospective consecutive cohort controls from same setting and same period year prior using same protocol	100 consecutive patients, with 100 consecutive retrospective cohort controls from same setting and same period year prior using same protocol
Care setting	Tertiary care hospital clinic	Primary care clinic	Primary care clinic
Inclusion criteria	Diabetic foot ulcer below ankle, SINBAD 2 or below, age > 18, and persistent for minimum of 12 weeks	<40% wound size reduction in last 4 weeks despite standard care	Min 10% wound slough coverage at baseline
Exclusion criteria	As per label at the time; pregnancy, clinical infection requiring antibiotics at baseline	As per label at the time; pregnancy, clinical infection requiring antibiotics at baseline	As per label at the time; pregnancy, clinical infection requiring antibiotics at baseline
Primary endpoint	Wound healing	Wound healing	Wound healing
Secondary endpoints	Wound size, slough coverage, exudate, pain, satisfaction ^a , ease of use ^a	Wound size, slough coverage, exudate, pain, satisfaction ^a , ease of use ^a	Wound size, slough coverage, exudate, pain, satisfaction ^a , ease of use ^a
Follow-up	28 weeks (data to week 26 considered for consistency)	26 weeks	26 weeks
Patients lost to follow-up	3 patients lost to follow-up or died. Two deaths in the control group at weeks 6 and 7, one death in the hemoglobin group at week 10	11 patients lost to follow-up or died. One in the hemoglobin group at week 12 and 10 in the control cohort, at weeks 2, 8, 9, 10	5 patients lost to follow-up, all in the control group
Observed systemic differences at baseline versus controls	No systematic differences other than hemoglobin application reported. No statistically significant differences between groups at baseline for any variable. Note: off-loading was offered to all suitable patients and there was no difference in off-loading use between control and the hemoglobin cohorts	Dressing changes exclusively done by professional nursing staff in the control group, while majority of dressing changes in the hemoglobin group were done without nurse support. Larger wound sizes and wound persistency in the control and higher mean pain scores in the hemoglobin group	Dressing changes exclusively done by professional nursing staff in the control group, while majority of dressing changes in the hemoglobin group were done without nurse support. More patients with neuropathy and limb ischemia in the control group and higher mean pain scores in the hemoglobin group
Adverse events reported over the 28/26 week follow-up	Two deaths, one amputation, six sharp debridement treatments, and five courses of antibiotics, in four patients, in the control group. One death (and no surgeries or antibiotics required) in the hemoglobin group	Six deaths, eight cases of surgical debridement, and eight courses of antibiotics (in eight patients) required in the control group. One death (and no surgery or antibiotics) in the hemoglobin group	Five deaths, 14 cases of surgical debridement, and three cases of infections requiring antibiotics treatment in the control cohort. One case of surgical debridement, and one case requiring antibiotics treatment in the hemoglobin group (no deaths)

^aTreatment group only.

Table 2. Number of wounds by type.

Wound types identified	Hemoglobin spray	Standard care alone
Trauma	61	49
Diabetic foot ulcer (DFU)	23	37
Burn	14	16
Venous leg ulcer (VLU)	16	17
Post-surgery	10	14
All other types	46	37
	170	170

included injection site wounds (IV drug users), arterial leg ulcers, abscesses, insect bites, pressure sores, shingles

blisters, moisture lesions, a tattoo wound, a skin-graft, an extravasation wound, and an inflammatory wound (n=83) (see Table 2 for distribution of wound types in the consolidated data set).

To assess the relative benefit realized for each wound type, a Cox proportional hazards regression was selected for primary endpoint analysis. Cox proportional hazards regression was chosen over a simpler Kaplan–Meier analysis approach in order to better account for the variability at baseline typical for all-comer real world data sets and to enable an estimate of the specific impact of the adjunct hemoglobin spray. Additional analysis was completed for reported secondary outcomes for which data were available: (1) “Slough,” associated with delayed healing, increased risk of wound

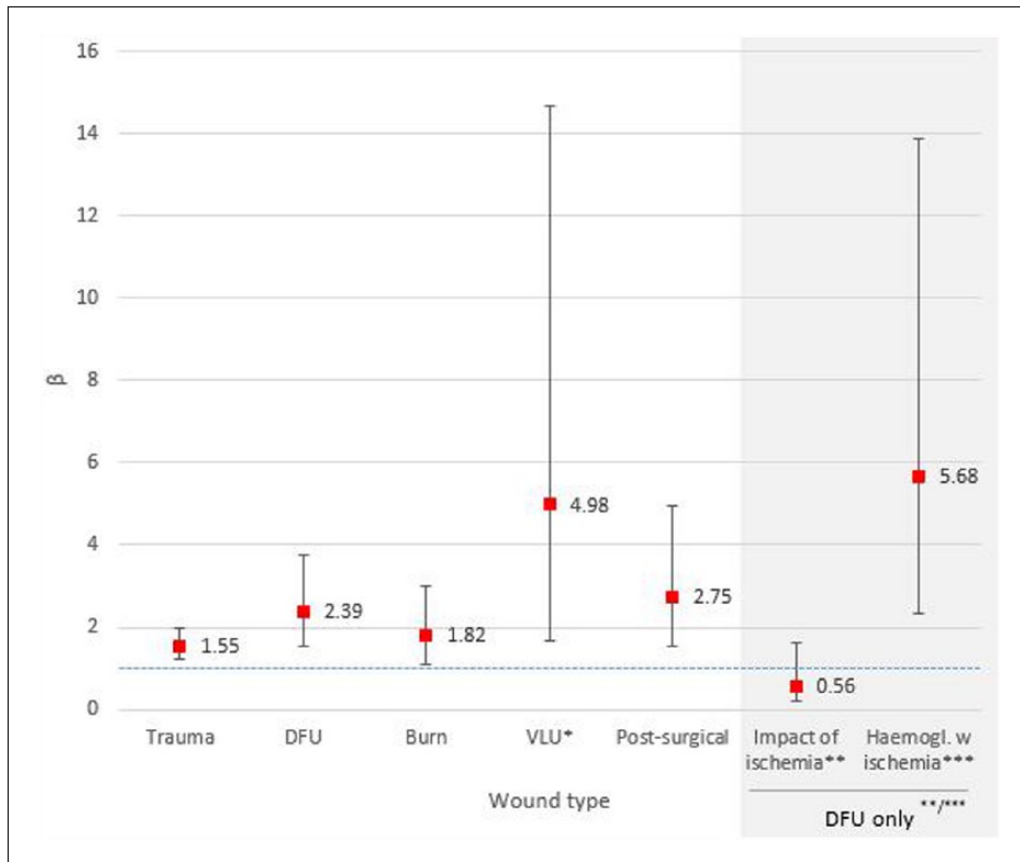


Figure 1. Weekly (increased) probability of healing, β , over 26 weeks from Cox regression of time to wound healing by wound type. *Sensitivity case with one added wound healing event in the control group for VLUs to enable comparison. **Impact of ischemia within DFU sample. ***Impact of adjunct hemoglobin while controlling for effect of ischemia. Whisker plots show 95% confidence interval.

infection, as well as increased wound malodor and exudates,¹¹ with its reduction set as an imperative for achieving effective wound healing;¹² (2) “Pain.” Average reported pain scores (10 cm visual analogue scale (VAS)), a dominant quality of life indicator in chronic wound patients;¹³ and (3) wounds size reduction, a reliable healing progress indicator, with wound size reduction over 4 weeks often suggested as the best single measure of wound healing progression.^{14–16} Statistical analysis using t-tests was completed for each of the secondary endpoints. Results were considered significant if $p < 0.05$. No adjustments for multiple analysis were made for secondary endpoints.

Results

A significant benefit from adjunct hemoglobin spray was observed for wound healing using Cox proportional hazards log-rank regressions for healing by week with patients dead or lost to follow-up censored at the time of death or loss of follow-up, with control for known baseline covariates across data sets (baseline wound size, baseline wound persistency, and patient age). A

significantly higher weekly chance of healing was observed in each wound type with adjunct hemoglobin spray (β , 95% range, sample, p); trauma 1.55 (1.23–1.96, $n = 110$, $p < 0.001$), DFU 2.39 (1.52–3.75, $n = 60$, $p < 0.01$), burns 1.82 (1.11–2.99, $n = 30$, $p = 0.02$), and post-surgical wounds 2.75 (1.53–4.96, $n = 24$, $p = 0.001$). Cox proportional hazards regression was not possible to run for the VLU group as none of the 17 VLUs in the control group had healed at the 26-week follow-up (vs 12 of 16 in the adjunct hemoglobin group). Kaplan–Meier analysis for VLUs suggested a statistically significant benefit, $p < 0.001$ ($n = 33$) but a hazard ratio was not possible to calculate as no healing event was observed in the standard care group. Sensitivity analysis where one of the wounds in the control group was assigned as healed at week 26 was additionally conducted in order to enable Cox regression. The wound with the greatest average size reduction versus baseline over time, patient “C5,” was set as healed at week 26. Results suggested a β of 4.98 (1.69–14.7, $n = 33$, $p = 0.04$) but due to the addition of the healing event in the control group this will correspondingly underestimate the effect size. See item with * in Figure 1 for

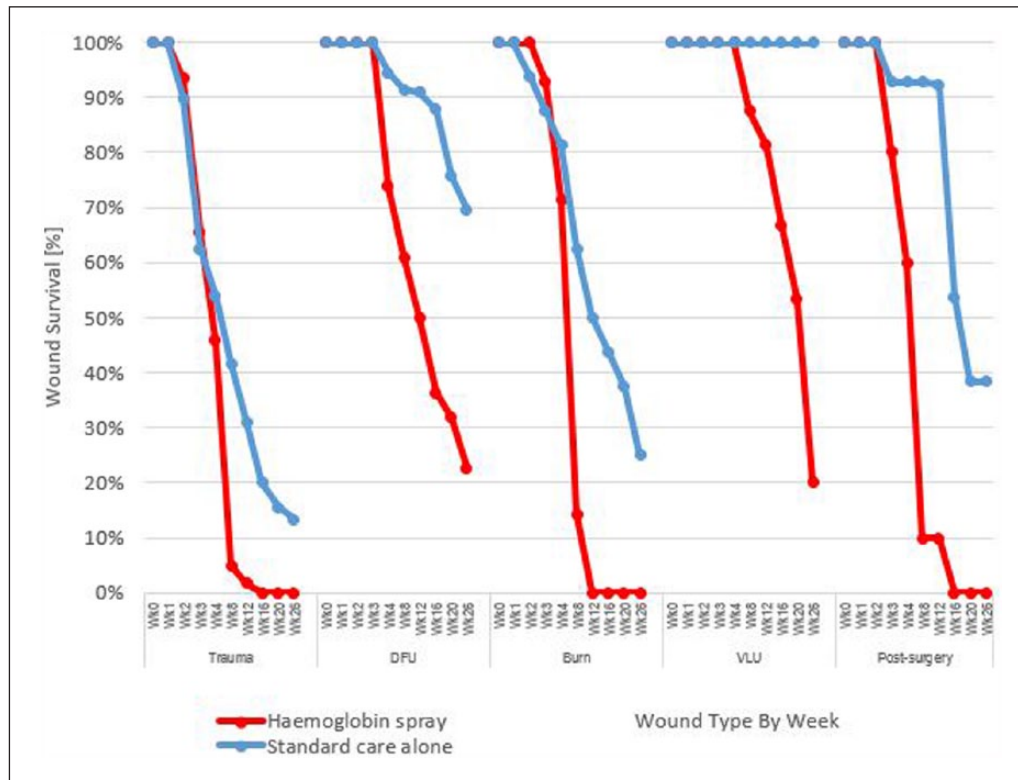


Figure 2. Wound survival over time, graphed for all wounds with complete follow-up.

observed β by wound type and 95% confidence intervals. For the DFU wounds, additional information on limb ischemia was available for all wounds. The total number of ischemic DFU wounds, or number of healing events, was too small to yield a significant model for ischemic DFU wounds on their own (overall model score $p=0.18$), with $n=10$ wounds in the hemoglobin group (43% of DFU limbs) of which five healed and $n=19$ (51% of DFU limbs) in the control group of which three healed ($\beta=1.94$, $0.86-4.39$, $n=29$, $p=0.11$). However, ischemia could be added to a significant model for all DFU wounds, generating a β for ischemia of 0.56, which was insufficiently powered on its own to reject the null-hypothesis ($\beta=0.56$, $0.19-1.61$, $n=29$, $p=0.28$), but results were significant for adjunct hemoglobin while controlling for ischemia ($\beta=5.68$, $2.33-13.86$, $n=29$, $p<0.001$). See items marked ** and *** in the grey section of Figure 1 for observed β by wound type and 95% confidence intervals.

Across the five selected wound types, the overall β (95% range, sample, and p -value) was 1.86 (1.58–2.19, $n=257$, $p<0.001$), suggesting a typical 86% greater expected chance of healing per week and a 95% chance of more than 58% increased weekly probability of healing with adjunct hemoglobin spray versus standard care alone across the evaluated wound types (see Figure 2 for wound survival at major time-points for patients with complete follow-up for each of the five wound types).

As an additional sensitivity analysis, the Cox regression was also run across all wounds in the three evaluations, with wound type as an additional covariate, with results suggesting a similar effect size across all observed wounds across wound types $\beta=1.84$ (1.58–2.15, $n=340$, $p<0.001$). As expected, patient age, baseline wound size, and wound persistency were consistently associated with reduced weekly chance of healing, that is, age, per year, $\beta=0.98$, $p<0.001$, baseline wound size, per additional cm^2 , $\beta=0.99$, $p<0.01$, wound persistency, per additional month, $\beta=0.92$, $p=0.06$.

Secondary endpoints, wound healing, and quality of life indicators

Supportive results for the positive effects from adjunct hemoglobin spray were also observed for additional wound healing indicators, wound slough coverage, wound pain as a quality of life indicator, and percent wound size reduction, across each wound type.

Slough

As early as by week 2, the mean wound slough coverage was significantly lower in all five reported wound types versus standard care alone (53%–88% greater reduction, all $p<0.001$, analyzed via students t -test): trauma–53%, $t=8.05$, $p<0.001$; DFU–87%, $t=5.95$, $p<0.001$; burn–75%, $t=5.37$, $p<0.001$; VLU–66%, $t=6.28$, $p<0.001$; post-surgery–88%, $t=4.39$,

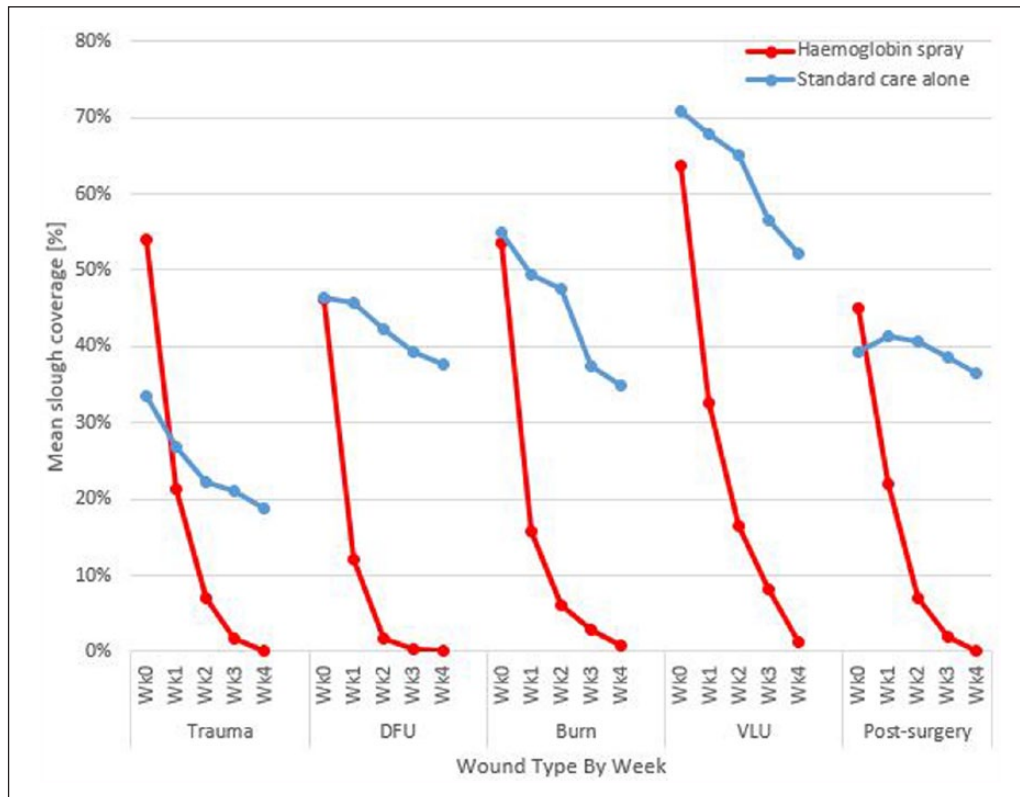


Figure 3. Mean wound slough coverage, %, by week, by wound type.

$p < 0.001$ (see Figure 3 for mean wound slough coverage over the first 4 weeks).

Pain

Average reported pain scores were significantly lower within 2 weeks for the hemoglobin groups versus controls (all $p < 0.01$, t -test), with 49%–78% greater baseline-adjusted pain score reductions versus standard care alone (trauma, 49% greater reduction, $t = 6.49$, $p < 0.001$; DFU 73%, $t = 5.24$, $p < 0.001$; burn 59%, $t = 4.55$, $p < 0.001$; VLU 69%, $t = 3.42$, $p < 0.001$; post-surgery 78%, $t = 7.50$, $p < 0.001$). These reductions were observed despite higher mean reported pain scores at baseline in the hemoglobin groups relative to standard care controls in each of the wound types (see Figure 4 for mean pain scores).

Wound size reduction

Wound size reduction difference between groups by 4 weeks showed a mean 49%–102% greater wound size reduction in the hemoglobin group (mean wound size increased in the control group for post-surgical wounds due to deteriorating wounds). The statistics at week 4 were all significant in favor of hemoglobin at $p = 0.02$ or lower (trauma, -55% , $t = 3.26$, $p = 0.02$; DFU, -49% , $t = 3.27$, $p = 0.002$; burn, -52% , $t = 2.94$, $p = 0.01$; VLU, -56% , $t = 8.34$, $p < 0.001$; post-surgery,

-102% , $t = 4.13$, $p < 0.001$). While healing outcomes were significantly improved at the benchmark 4-week indicator, a statistically significant difference was evident already within the first week of initiation of adjunct hemoglobin, with significantly greater wound size reduction across all wound types (trauma, -29% , $t = 3.22$, $p = 0.02$; DFU, -34% , $t = 5.54$, $p < 0.001$; burn, -47% , $t = 4.43$, $p < 0.01$; VLU, -28% , $t = 6.12$, $p < 0.01$; post-surgery, -45% , $t = 2.71$, $p = 0.01$). See Figure 5 for mean wound size change versus baseline by week.

Discussion

The reported data did not exclude any patients other than as per the product labeling and were evaluated compared with retrospective standard care cohorts from the same clinics. These are key strengths of the data and analysis in representing the improvements to expect when implementing adjunct hemoglobin spray within standard care for treatment of wound with healing complications in general, and for trauma wounds, DFUs, burns, VLUs, or post-surgical wounds specifically.

While on one hand, a strength, the real world intervention data are also the key weakness. The lack of blinding and prospective randomization leaves it possible that some of the benefits observed were due to a new treatment in itself, a *Hawthorne effect*, after the now infamous studies showing that observation alone could lead to improved productivity. This effect of observation was also demonstrated in clinical

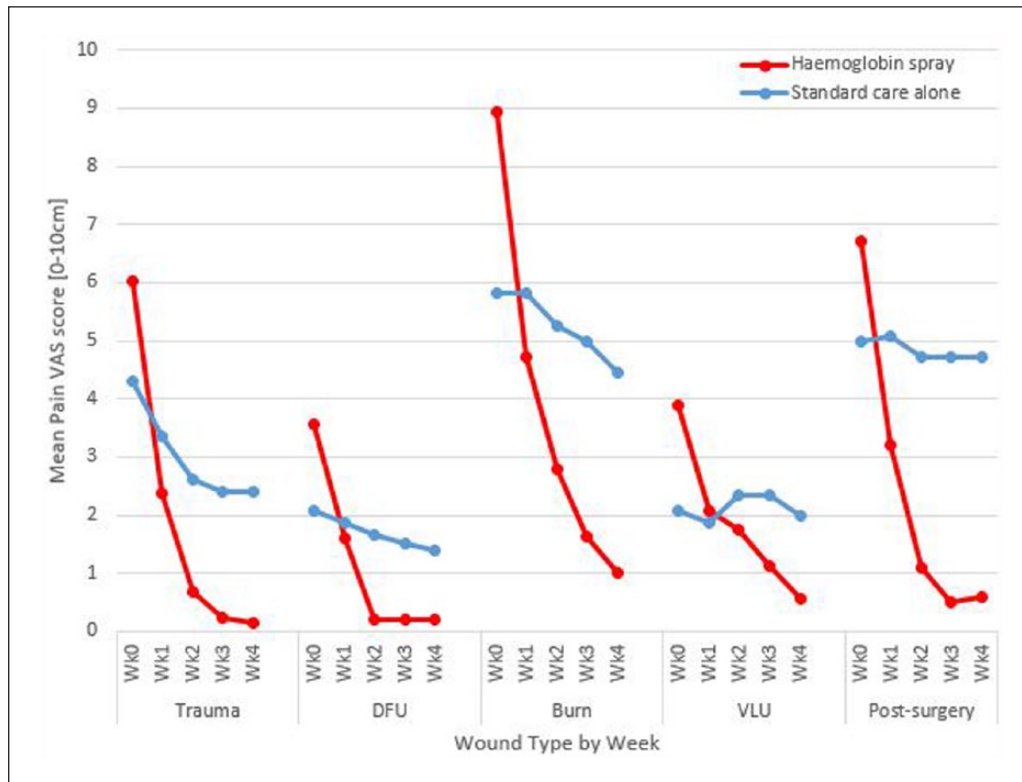


Figure 4. Mean pain VAS score by wound type by week.
VAS: visual analogue scale.

research where just more intense outcomes monitoring with no change in treatments was found to improve outcomes.¹⁷ In the case of the evaluations used in this study, the actual monitoring and measures of healing outcomes were the same but the perceived monitoring by patients may have been higher given the novel treatment. In addition, self-care had been introduced as an element in the care pathway in the two larger evaluations, although this is unlikely to have resulted in a benefit over dressing changes by a professional nurse.

The benefit observed in this review of real world outcomes when implemented within standard care compares favorably with the large healing benefits observed in the pivotal RCT in non-healing VLU,⁶ which demonstrated a 74% difference in mean wound size change versus baseline at 13 weeks, versus a 68% difference at week 12 in the current evaluation in VLUs (data not shown). Therefore, while imperfect in terms of control of possible benefits from a change to a novel regimen and perceived increased observation per se, the evaluation outlined above provides a big step forward in terms of developing an estimate of the relative healing benefit by wound type that can be expected when implementing adjunct hemoglobin therapy into the care pathway. The results clearly demonstrate substantial and rapid healing benefits over standard care alone to patients across all of the most frequently encountered wound types, suggesting clinicians and policymakers should support the

adoption of adjunct hemoglobin spray in line with consensus guidelines published previously.³

To note is that some ischemic DFU wounds may have been excluded due to the SINBAD > 2 exclusion criteria used in the DFU evaluation (40 of the 60 DFU wounds were captured from the DFU evaluation study) and an evaluation that included wounds regardless of complication profile could have generated a higher, or lower, healing benefit than the 5.7 times greater weekly chances of healing observed for DFUs.

Further research should clarify the value of adjunct hemoglobin in terms of cost-effectiveness, as a means to speed the development of normative guidelines for appropriate adoption and funding of the use of adjunct hemoglobin use via NICE, IQWiG, CDER, HAS, PBAC, CADTH, and similar agencies that increasingly determine whether patients will get access to new treatments.

Conclusion

Adoption of hemoglobin spray as adjunct to standard of care, while allowing for self-administration, in the regular treatment of trauma, DFU, burn, VLU, or post-surgical wounds with delayed or complicated healing is expected to achieve substantial healing benefits to patients. Significant benefits were observed very early after the initiation of adjunct

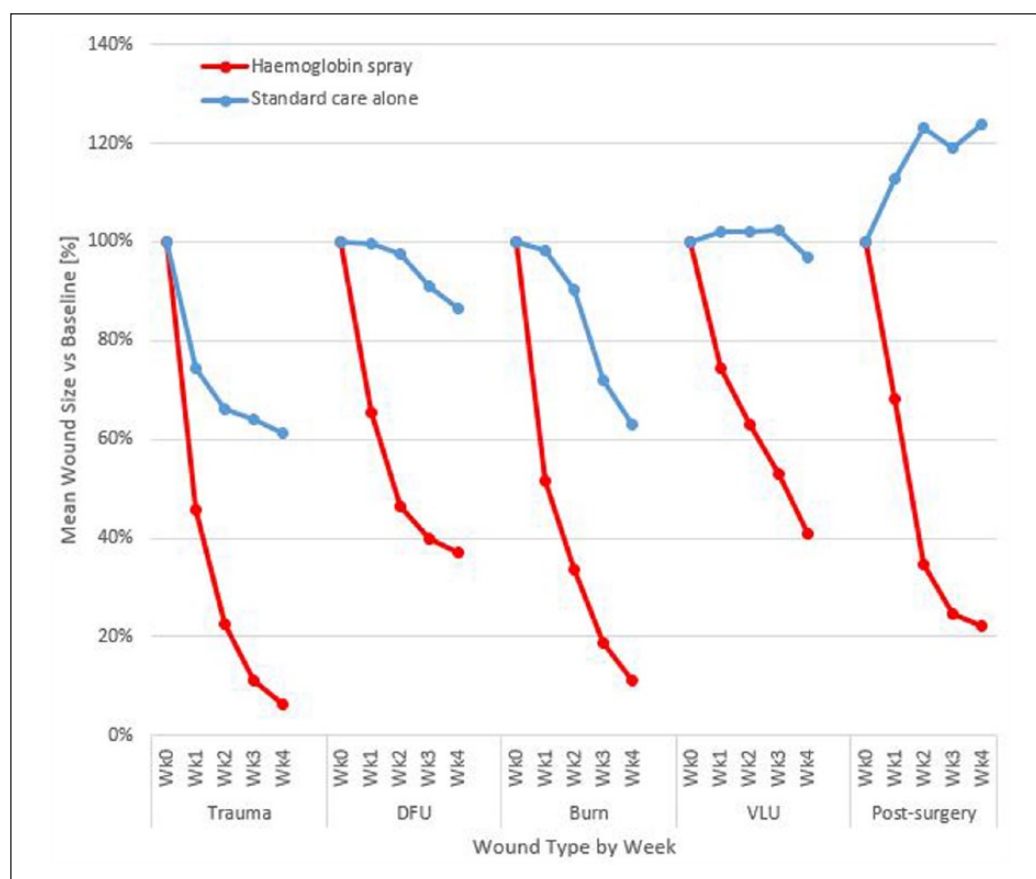


Figure 5. Mean wound size versus baseline, by wound type by week.

hemoglobin spray, with statistically significant benefits over the retrospective standard care alone control cohort observed already within 1 week from the first application across wound types. While a significant benefit was observed across wound types, the highest relative benefit was observed for VLU patients, with an expected more than five times greater healing rate, followed by post-surgical wounds at 2.8 times greater rate of healing, and with DFU, burn, and trauma with healing improvement rates at 2.4, 1.8, and 1.6 times greater rate of healing over 26 weeks when controlled for covariance from known healing predictors at baseline available across all wound types; baseline wound size, wound persistency at baseline and patient age. In the case of DFUs, when additionally including diagnosis of limb ischemia, the healing benefit associated with hemoglobin treatment was increased to 5.7 times greater weekly chance of healing.

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S.H. collected the data and F.E. performed the statistical analysis, and the manuscript was written jointly.

Data sharing statement

The data for this article were sourced from the original evaluations. To access a copy of the data, please contact the authors.

Declaration of conflicting interests

The author declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: S.H. and F.E. provide consulting and speaker services to pharmaceutical and healthcare companies, including, but not limited to Sastomed GmbH.

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