

Dehydrated human amnion/chorion membrane treatment of venous leg ulcers: correlation between 4-week and 24-week outcomes

- **Objective:** To evaluate correct correlation between an intermediate rate of wound reduction (40% wound area reduction after 4-weeks treatment) and complete healing at 24 weeks in patients with a venous leg ulcer (VLU).
- **Method:** A retrospective follow-up study of patients completing a multicenter randomised controlled trial (RCT) comparing the number of patients achieving at least 40% healing of their VLU within 4-weeks of treatment with either dehydrated human amnion/chorion membrane (dHACM) allograft or multilayer compression only was conducted. Outcomes assessed were rates of complete healing within 24 weeks of enrolment and days to healing. Data were divided into two groups based on status at RCT completion (healed at least 40% yes or no). Correct correlation with status at 4 weeks and complete healing within 24 weeks was determined. Clinical characteristics were also compared for patients with and without correct correlation between 4-week and 24-week status.
- **Results:** We identified 55 patients at 5 study sites. Some 47 without complete healing during the initial study were eligible. As three patients were lost to follow-up we evaluated 44 records. Of these, 20 (45.4%) had reduced wound size of $\geq 40\%$ and 24 (55%) had $< 40\%$ reduction during the initial study. Complete healing occurred in 16/20 (80%) of the $\geq 40\%$ group at a mean of 46 days, $p=0.0027$ and 8/24 (33.3%) of the $< 40\%$ group at a mean of 103.6 days, $p=0.0023$. Overall, correct correlation of status at 4 weeks and ultimate healing status of VLU occurred in 32/44 patients (73%).
- **Conclusion:** These results confirm that the intermediate outcome used in our initial study is a viable predictor of ultimate VLU healing.
- **Declaration of interest:** Dr Thomas Serena has provided consultative services to MiMedx and has been a speaker on their behalf. The initial multicenter randomised trial was sponsored and funded by MiMedx Group, Inc., Marietta, GA.

amniotic membrane; healing rate; venous leg ulcers; intermediate outcomes

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Although complete healing is the ultimate goal when treating any venous leg ulcer (VLU), the protracted healing interval creates challenges in both the clinical and research arenas. Intermediate outcomes predictive of complete healing within a specified period of time can provide important information for evaluating treatment effectiveness.

In the clinical setting, early identification of patients unlikely to heal with standard therapy allows appropriate modification of treatment to include more advanced wound care products that have the potential to reduce suffering and morbidity.

In a research setting, the use of surrogate or intermediate markers allows for more rapid evaluation of treatment safety and potential benefits. When intermediate endpoints are used, clinical trials can be executed in a more efficient manner with less follow-up time needed and with a lower sample size required.¹ Both time and money are saved allowing for additional resources to be directed towards prod-

ucts or techniques showing the greatest potential as an effective treatment.

A proprietary PURION process of advanced tissue stabilisation and preservation has allowed for the widespread clinical use of human amniotic membrane in the form of a dehydrated human amnion/chorion membrane (dHACM) allograft.² dHACM allografts are comprised of amnion and chorion layers of the amniotic membrane. The source of amniotic membrane is donor placenta obtained following a caesarean section delivery, with informed consent from women who have acceptable infectious disease test results, as regulated by the Food and Drug Administration's (FDA) Good Tissue Practice and American Association of Tissue Banks (AATB). The dHACM is a minimally manipulated, non-viable cellular allograft containing numerous extracellular matrix proteins, growth factors, cytokines and other regulatory and structural proteins.³ Studies of dHACM reveal the ability of the tissue to recruit multiple stem cells relevant

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to wound repair and regeneration.⁴⁻⁶ The clinical and cost-effectiveness of dHACM has been established in randomised studies as a treatment for lower extremity ulcers in patients with diabetes.⁷⁻⁹ Moreover, published case studies and case series have also suggested its use in a variety of other wound types.¹⁰⁻¹²

Relevant to both patient care and clinical research, it has been demonstrated that the % change in wound area of a VLU at the fourth week of care can serve as an important intermediate marker predictive of complete wound healing after 24 weeks of care.^{1,13-15} Reduction of a VLU by approximately 40% over the first 4 weeks of treatment has been shown to correlate over 68% of the time with complete healing at 24 weeks.¹ Recently, a multicentre randomised trial was conducted to evaluate the efficacy of dHACM allograft (EpiFix, MiMedx Group, Marietta, GA) as a treatment for chronic VLU. The primary study outcome was an intermediate endpoint of the proportion of patients achieving at least 40% wound closure at 4 weeks.¹⁶ VLUs treated with dHACM showed a significant improvement in healing at 4 weeks compared to multilayer compression therapy (Coban2, 3M St. Paul, MN) alone. After 4 weeks, 62% of wounds treated with dHACM and 32% of controls had >40% wound closure (p=0.005).

The purpose of the present study is to evaluate if the 4-week study outcome correctly correlated with rates of complete healing within 24 weeks.

Methods

We conducted a retrospective evaluation of data from patients enrolled in an institutional review board (IRB) approved multicentre randomised clinical trial (RCT), which evaluated the use of dHACM for the treatment of VLU.¹⁶ All patients provided signed consent and agreed to the publication of case histories, results of treatment, laboratory and pathological data, and photographs of wounds for scientific purposes. Included in the study were patients

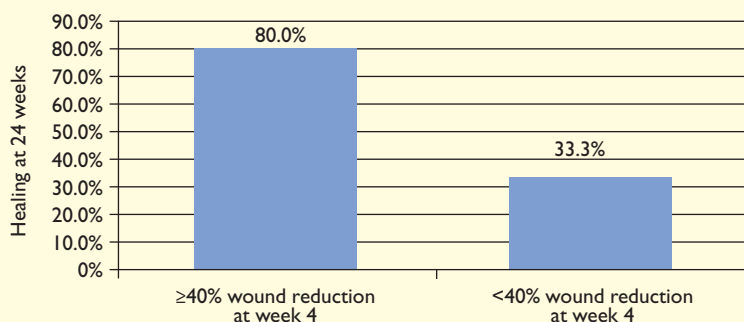
presenting for treatment of a VLU extending through the full-thickness of the skin but not down to muscle, tendon or bone, age 18 or older, with an ankle-brachial pressure index (ABPI) of >0.75. The VLU had to be 2–20cm² in size and present for at least one month. During the screening and run-in period all VLUs were treated with multilayer compression for at least 14 days and only those with <20% healing during that period advanced to randomisation. Within the 4-week study period, patients were randomised to receive one or two applications (day 0 and/or day 14) of dHACM allograft in addition to continuation of multilayer compression, or multilayer compression alone. In the RCT, the primary study outcome was the proportion of patients achieving at least 40% wound closure at 4 weeks. Full inclusion and exclusion criteria, study procedures and study outcomes have been previously reported.¹⁶ At conclusion, patients either continued to receive care at their respective study site or sought treatment elsewhere.

Of the study sites, five agreed to participate in the follow-up phase. Data on those patients who did not heal completely during the initial 4-week study period and continued to receive treatment were eligible. Chart reviews were conducted by the study coordinators at each site to determine if complete healing occurred within 24 weeks of initial study enrolment and date of healing. We sought to determine the relationship of the patients healing status at week 4, compared to their healing status at week 24. In the original study, outcomes were dichotomised into two groups at week 4: those with wound area reduction of ≥40%, and those whose wounds had not reduced by at least 40%. If patients with ≥40% wound reduction at 4 weeks were completely healed by week 24, we determined that their healing status at week 4 correctly correlated with complete healing within 24 weeks. If patients with <40% wound reduction at week 4 were not completely healed by week 24, we determined that their failure to heal by at least 40% at week 4 correctly correlated with failure to heal within 24 weeks. Time to healing for those healed was also examined, and a comparison of clinical characteristics for those with and without correct correlation of 4-week and 24-week outcomes was performed.

Statistical analysis

GraphPad InStat 3 was used to perform statistical testing. Rates of complete healing were compared using Fisher's exact test and time to healing compared using an unpaired t-test. Clinical characteristics were compared between those with and without correct correlation using parametric (Fisher's exact test, Student's t-test) and non-parametric (Mann-Whitney U) statistics as appropriate, with p<0.05 considered statistically significant.

Fig 1. Complete healing within 24 weeks. Patients with complete wound healing at 24 weeks according to level of wound reduction at 4 weeks (≥40% reduction or <40% wound reduction, p=0.0027)



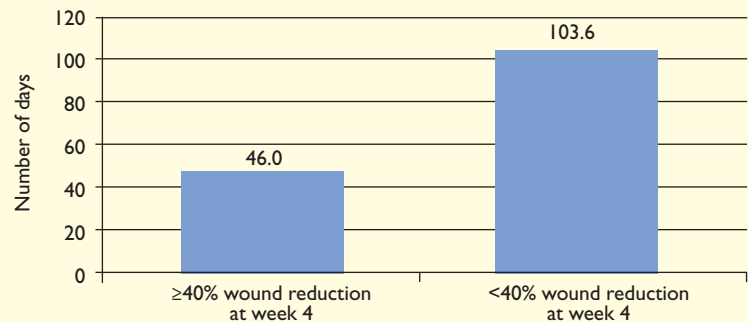
Results

There were 55 patients who participated and completed the initial 4-week study period. Of these, eight were excluded (six from the dHACM group and two from the multilayer compression only group) due to achieving complete healing during the initial 4 week trial, leaving 47 eligible for follow-up. After the initial study three patients discontinued care at the study site, leaving 44 evaluable records. Wound size reduction of $\geq 40\%$ was seen in 20 patients (45.5%) and 24 (54.5%) had $<40\%$ reduction during the initial study. The dichotomous outcome of wound reduction of at least 40% by treatment week 4 was predictive of healed or unhealed status at week 24 in 32 of 44 patients studied (72.7%).

At 24 weeks, complete healing had occurred in 24 of the 44 patients (54.5%). Wound with reduction of $\geq 40\%$ within the first 4 weeks of treatment were more likely to be completely healed within 24 weeks 16/20 (80%), compared with those that had $<40\%$ healing the first 4 weeks 8/24 (33%; $p=0.0027$; Fig 1). For patients that experienced complete healing within 24 weeks, time to healing was significantly faster for patients that experienced $\geq 40\%$ healing within the first 4 weeks of treatment at 46 ± 35.1 days versus 103.6 ± 44.9 days for patients with $<40\%$ healing after 4 weeks ($p=0.0023$; Fig 2).

A comparison of clinical characteristics for those with and without correct correlation between the 4-week intermediate outcome and wound healing status at 24 weeks is presented in Table 1. No difference was observed for those with or without correct correlation for age, gender, race, body mass index

Fig 2. Mean number of days to complete healing according to level of wound reduction at 4 weeks, $p=0.0023$



(BMI), duration of wound, or treatment received in the initial study. Interestingly, patients with correct correlation between wound status at 4 and 24 weeks had significantly larger wounds at initiation of the study than those without correct correlation.

Discussion

Surrogate endpoints that can predict the ultimate outcome of treatment are beneficial for research in new wound healing products, allowing for more rapid evaluation of potentially promising innovations.^{6,9} In order to quickly evaluate the potential efficacy of using dHACM as a treatment for VLU an intermediate endpoint of $\geq 40\%$ healing at 4 weeks was used in our previous study.¹⁶ The results of the current follow-up study validate the intermediate endpoint used, showing that healing status at 4 weeks correlated correctly with rates of ultimate healing within 24 weeks 72.7% of the time.

Our results are similar to those reported by Gel-

Table 1. Comparison of clinical characteristics for patients with and without correct correlation of wound status at 4 weeks and complete healing within 24 weeks

	Correct correlation with status at 4 weeks (n=32)	Incorrect correlation with status at 4 weeks (n=12)	p-value
Age (years)	62.9 \pm 16.3	63.1 \pm 11.7	0.97
Male	17 (39%)	3 (7%)	0.17
Non-caucasian	4 (9%)	1 (2%)	1.00
Body mass index	38.0 \pm 13.4 35.7 (17.3, 80.7)	37.6 \pm 8.9 37.5 (26.3, 51.2)	0.78
Duration of wound at RCT enrolment (weeks)	18.1 \pm 24.3 5.5 (1, 96)	9.0 \pm 7.5 8.0 (0.9, 18)	0.35
Wound size at RCT enrolment (cm ²)	7.0 \pm 4.8 5.2 (2.0, 17.2)	3.8 \pm 1.7 3.4 (1.9, 7.6)	0.03
Wound size at end of RCT (cm ²)	5.6 \pm 6.3 4.0 (0.1, 24)	3.1 \pm 1.8 2.8 (0.5, 5.4)	0.38
Received dHACM in initial RCT	22 (69%)	9 (75%)	1.00

Data presented as mean \pm standard deviation, median (minimum, maximum), or number (%) as indicated.
RCT – randomised controlled trial; dHACM – dehydrated human amnion/chorion membrane

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and, et al.¹ who reported correct correlation of 68% between the % reduction at 4 weeks and the complete healing rates 24 weeks. Their work provides further validity and reassurance when using intermediate outcomes in investigations of new or novel treatments for VLU.

Limitations

There are limitations of the present study. During the follow-up period after completion of the initial 4-week RCT, patients received various treatments that may or may not have included initiation of, or additional application of dHACM, or other advanced treatments. Also, in the initial RCT, dHACM was only applied once or twice during the study period, which may not be reflective of how the treatment is used in a real world setting. As the goal of the present study was to evaluate the intermediate outcome and not the treatment

received, we did not control for treatment and chose to simply evaluate correct correlation between status at 4 weeks and 24 weeks without regard to treatment received in the initial study, or after the study was completed. We believe this approach makes these data more able to be generalised to a larger population of patients with VLU receiving a variety of treatment modalities.

Conclusions

While larger studies are underway to determine efficacy of repeated dHACM applications, as well as rates and time to complete healing of VLU, this follow-up analysis validates the intermediate outcome used in the initial RCT. These data provide further validation that clinical evaluation of VLU healing after 4 weeks of treatment is a simple, useful tool for identification of patients unlikely to heal within 24 weeks. ■

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