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ORIGINAL RESEARCH

A proof of concept method to predict the feasibility of a new acellular synthetic matrix for improved venous leg ulcer healing time compared to standard care control

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A Single-Arm Trial Indirect Comparison Investigation: A Proof of Concept Method to Predict Venous Leg Ulcer Healing Time for a New Acellular Synthetic Matrix Matched to Standard Care Control

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Abstract:

PURPOSE: To compare data on time to healing from two separate cohorts: one treated with a new acellular synthetic matrix plus standard care and one matched from four large UK pragmatic, randomized controlled trials (venous leg ulcer evidence network). We introduce a new proof of concept strategy to a venous leg ulcer clinical evidence network, propensity score matching and sensitivity analysis to predict the feasibility of the new acellular synthetic matrix plus standard care for success in future randomized, controlled clinical trials.

SUBJECTS AND METHODS: Prospective data on chronic, venous leg ulcers from a safety and effectiveness study on an acellular synthetic matrix conducted in one wound center in the UK (17 patients) and three wound centers in Australia (36 patients) were compared retrospectively to propensity score matched data from patients with comparable leg ulcer disease etiology, age, baseline ulcer area, ulcer duration, multilayer compression bandaging and majority of care completed in specialist wound centers (avg. 1 visit per week) with the outcome measures at comparable follow-up periods, from patients enrolled in four prospective, multicenter, pragmatic, randomized studies of venous ulcers in the United Kingdom (the comparison group; venous leg ulcer evidence network).

RESULTS: Analysis using Kaplan Meier survival curves showed a mean healing time of 73.1 days for ASM plus SC (ASM) treated ulcers, in comparison to 83.5 days for comparison group ulcers treated with SC alone (Log rank test, χ^2 5.779, p = 0.016) within 12 weeks. Sensitivity analysis indicates that an unobserved covariate would have to change the odds of healing for SC by a factor of 1.1 to impact the baseline results.

CONCLUSIONS: Results from this study predict a significant effect on healing time when using a new ASM as an adjunct to standard care in the treatment of non-healing venous ulcers in the UK, but results are sensitive to unobserved covariates that may be important in healing time comparison.

Introduction

Chronic wounds represent a worldwide increasing medical and economic problem and the burden of hard-to-heal wounds, especially venous ulcers, is expected to increase in the ageing populations.[1, 2] Epidemiological data from recent quality cross-sectional general population studies in the United Kingdom indicate that at least 76% of all patients with vascular ulcers are of venous origin and 22% are of an arterial origin. Ten to 20% of patients are estimated to suffer from both arterial and venous insufficiency.[3, 4]

The estimated total treatment cost of venous leg ulcers (VLUs) is 1% of the total annual healthcare budget in western European countries.[5] The cost to the UK NHS for treating venous ulceration, mostly in primary care and through community nursing services, is at least £168-198 million per year.[6]

Internationally, VLUs are cared for by a variety of health care professionals, who use a wide range of treatments depending on their professional background and the care setting they work in. Standard care (SC) includes local wound care with dressings chosen to address wound conditions of slough, exudate or potential infection, and multilayer compression. Unfortunately, this standard of care has success rates between 30% and 60% after 24 weeks of treatment in clinical trials and the best success rates after a year of therapy range between 70% and 85%.[7, 8]

Whilst the initial cause of VLU, venous insufficiency, is treated with compression (sometimes followed by venous surgery), the local wound environment may also be important in potentially delaying wound healing, due to infection or other micro-environment imbalances. VLUs are often recurrent and can persist for several months or years.[9] Delayed healing is associated with prolonged inflammation.[10,11] Prolonged inflammation creates an environment where the extracellular matrix (ECM) does not form adequately or is continually damaged.[12,13] The ECM provides sites for attachment of skin cells. These sites are critical for cellular functions that are important for healing, including proliferation, migration and survival of skin cells, all of which are dependent on cell attachment.[14] Non-healing wounds over-express

proteases and cell attachment may not be possible when the balance between ECM production and degradation by proteases is skewed towards degradation. It is hypothesized that healing can progress more effectively if there is alteration of the cellular microenvironment.[15]

Recently, advanced treatments such as topical growth factors, biological dressings, and tissue engineered products have been developed in order to promote healing and decrease the time to healing. Nevertheless, if SC fails, there is no widely accepted, easy to use second-line treatment primarily because most of these advanced treatments are expensive and need further evidence of effectiveness in everyday clinical practice.[8, 16, 17] One such novel treatment, a synthetic, acellular matrix (VF-001, Factor Therapeutics Ltd., Level 19, 179 Turbot St, Brisbane Qld 4000, Australia (ASM)), has recently been developed as an ECM replacement. It consists of portions of an ECM protein, vitronectin, and of IGF-1 (insulin-like growth factor-1). It is intended for use as an adjunct to SC for ulcers that fail to heal in a timely manner with SC alone. In non-healing wounds, the rationale for the use of a scaffold is to provide an ECM replacement that, when placed in the wound bed, offers a temporary support onto which cells can attach, migrate and proliferate in an organized manner, thus leading to tissue regeneration and ultimately wound closure.[18]

The ASM comes in a liquid format, which facilitates application and enables coverage of the irregular surface of a wound bed. It is adhesive by design and binds rapidly to the wound bed forming a scaffold within minutes of application.[19] Fibroblasts and keratinocytes attach to the temporary scaffold and subsequently migrate onto the wound bed and proliferate.[19] The ASM is intended to be used once per week as an adjunct to SC in the treatment of chronic wounds, primarily venous leg ulcers.

The healing time effectiveness of the ASM plus SC was compared retrospectively to data from a German Registry of Chronic Wounds (DRCW) database containing patients with equivalent ulcer disease, patient age and gender, baseline ulcer area, ulcer duration, same healing outcome measures, and comparable follow-up periods. Results from this study predicts a highly significant effect of the ASM plus SC as an adjunct to standard care in the repair of non-healing venous and mixed leg ulcers in Germany.[20] This result has prompted further investigation in the United Kingdom to corroborate the German results.

In the evaluation of acellular matrices for treatment of chronic wounds, estimates of the relative effects and cost-effectiveness of competing treatments are rarely available from head-to-head trials. These effects must therefore be derived from evidence networks, matching-adjusted indirect comparisons and simulated treatment comparison methods. With this in view, there are few high quality studies in the United Kingdom comparing treatments for non-healing venous leg ulcers that maintain independence from commercial entities. One such evidence network is maintained by the University of York Clinical Trials Unit in York, United Kingdom. The University of York was commissioned by the National Institute for Health Research (NIHR) in the UK to complete several multicenter, pragmatic, randomized controlled trials to compare the clinical effectiveness and cost-effectiveness of VLU treatments in the UK.[21-28]

VenUS I evaluated the relative clinical and cost-effectiveness of four-layer and short-stretch compression bandaging for venous ulceration.[21, 22] VenUS II compared the clinical and cost-effectiveness of larval therapy with a standard debridement technique (hydrogel) for sloughy or necrotic leg ulcers.[23, 24] VenUS III assessed the clinical and cost-effectiveness of weekly delivery of low dose, high frequency therapeutic ultrasound in conjunction with standard care for hard-to-heal venous leg ulcers to standard care alone.[25,26] VenUS IV is the most recent study that investigated the clinical and cost-effectiveness of compression hosiery versus compression bandages in treatment of venous leg ulcers.[27,28]

The effectiveness of the ASM as an adjunct to standard care (ASM plus SC) has been evaluated in a single arm, prospective, non-controlled, multicenter observational study using healing (100% re-epithelialization) and wound area reduction as endpoints.[29] The purpose of our study was to compare healing times for VLUs based on their propensity to heal within 12-weeks and suitable for multilayer, high compression therapy treated with SC in everyday practice in the UK to healing times of matched patient and ulcers treated with the ASM plus SC. Data from the VenUS I [21, 22], VenUS II [23, 24], VenUS III [25, 26] and VenUS IV [27, 28] studies (VenUS RCTs) were found to be suitable for such comparative evaluation and predictive outcomes measuring due to their pragmatic design and comprehensive data on chronic VLU etiology, baseline VLU area, VLU duration, VLU area measurements, patient age, multilayer compression and time points of follow-up.

Material and Methods

Study Design

This is a 2-arm indirect assessment to compare healing times from a single arm, prospective, multicenter effectiveness and safety study (ASM plus SC) to healing times in a 1:1 propensity score matched VLU group treated with SC only pooled from an evidence network consisting of the VenUS I [21, 22], VenUS II [23, 24], VenUS III [25, 26] and VenUS IV [27, 28] patient databases.

The design included several data modeling steps and propensity score matching (Figure I) to facilitate a matched-pair base healing time comparison between patients and their respective reference ulcers in the control group and patients and their respective reference ulcers in the ASM plus SC treatment group. The basic equivalence criteria used were venous/non-venous ulcer etiology, baseline ulcer area and baseline ulcer duration, patient age, presence/absence of multilayer high compression therapy and majority of care completed in specialist wound centers (avg. 1 visit per week). Using Kaplan-Meier survival analysis, the baseline time to healing data was compared between the matched SC control group (VenUS-SC-53) and the treatment group ulcers treated with ASM plus SC. A sensitivity analysis for matched, censored survival outcomes was performed to evaluate hidden bias associated with missing covariates in the propensity score matching process that may have impact on healing time comparison.

Evidence Network and Interventions

The data sources included the intent-to-treat (ITT) population from the ASM plus SC study and the patients from the ITT populations treated with standard care alone in the VenUS-SC-870 pooled SC dataset (Figure I) who met the key selection criteria: 1) ankle brachial pressure index ≥ 0.7; 2) wound area at baseline ≤ 33.00 cm^2; 3) wound duration at baseline ≥ 4 weeks; 4) compression bandaging (multilayer high compression or short stretch high compression) and 5) patient has contact with a wound center specialist on average once per week.[29]

The phase II ASM plus SC study was a 12-week prospective, multicenter effectiveness and safety study in 53 patients with chronic venous leg ulcers.[29] It was conducted in one wound center in the UK (17

patients) and three wound centers in Australia (36 patients). All patients had to be suitable for multilayer high compression therapy (i.e. SC) [30-32], and all patients had been treated with SC for a minimum of four weeks prior to study entry. The purpose of the 4-week run-in period was to evaluate an enrollee's presence or absence of side effects, potential for completion of the study and non-healing status (no change in wound area over 4 weeks of SC treatment). All the ASM plus SC study patients were maintained on multilayer high compression bandages and dressings in accordance with local practice of the center in question; hence the only change in a patient's treatment upon entry into the ASM plus SC study was the addition of the ASM. Surgical debridement was performed, if necessary, to remove necrotic tissue prior to application of the ASM. Wound area tracings were performed at baseline and at each study visit until study end (12 weeks) or until the ulcer was considered completely healed (100% re-epithelialized).

The ITT population from the 53-patient ASM plus SC study was included in our comparative analysis. Mean age was 74.7 years (median = 76.0, Table 1), mean wound duration 33.4 months (median = 10 months, Table I) and mean baseline wound area 7.4 cm^2 (median = 4.5 cm^2, Table I). A total of 29 patients were male and 24 were female (Table I).[29] There were 16 ulcers healed by final trial visit in the phase II study. Another 3 ulcers achieved wound area reduction greater than 90% at their last study visit and healed between 72 days (12 weeks) and 90 days. Therefore, 19 out of 53 patients (35.9%) reference ulcers healed completely by 90 days.

A total of 387 adults with a non-healing VLU, receiving treatment either in primary care or as a hospital outpatient, were recruited to VenUS I prospective, randomized pragmatic study to either four-layer or short-stretch multilayer high compression bandages. Between April 1999 and December 2000, patients with VLUs treated in the community (leg ulcer services, district nursing or general practice) or as an outpatient (vascular surgery) were recruited from nine UK centers. Follow-up continued until the patient's reference leg was ulcer free or for a minimum of 12 months. The primary endpoint was time to healing of all ulcers on the reference leg. The dataset includes demographic and baseline data, monthly research nurse visits, non-trial healthcare visits necessary to maintain standard of care, patient quality of life, and

compression therapy and treatment outcomes data. Both treatment arms represent standard care therapy generally used in the United Kingdom including local wound care with dressings chosen to address wound conditions of slough, exudate or potential infection, and multilayer compression.[21, 22]

The VenUS II study was a pragmatic, three-arm, randomized controlled trial with an economic evaluation carried out in 22 centers in the United Kingdom from July 2004 to May 2007. The objectives of the trial were to compare the clinical effectiveness and cost-effectiveness of larval therapy with those of a standard debridement technique (hydrogel). The primary end point was complete healing of the largest eligible (the reference) ulcer and the primary outcome was time to complete healing of the reference ulcer. The setting was in community nursing services, community leg ulcer clinics and hospital outpatient leg ulcer clinics in a range of urban and rural settings. [23, 24]

The VenUS III study was a prospective, multicenter, pragmatic, two arm randomized study designed to compare the clinical effectiveness and cost-effectiveness of low-dose ultrasound as an adjunct to SC compared to SC alone in the treatment of hard-to-heal venous leg ulcers.[25, 26] The VenUS III database contains 337 patients with venous ulcers. Participants were recruited between March 2006 and December 2008 and were treated in community and district nurse led services, community leg ulcer clinics, and hospital outpatient leg ulcer clinics in 12 urban and rural settings (11 in the United Kingdom and one in the Republic of Ireland). The dataset includes demographic and baseline data and monthly wound area tracings and characteristics, patient quality of life, compression therapy, monthly research nurse visits, non-trial healthcare visits necessary to maintain standard of care with patients and treatment outcomes data. [25, 26]

The VenUS IV study was a pragmatic, randomized controlled trial in 34 centers in England and Northern Ireland. The centers were community nurse teams or services, family doctor practices, leg ulcer clinics, tissue viability clinics or services, and wound clinics.[27, 28] The VenUS IV study aimed to compare the clinical effectiveness and cost-effectiveness of two-layer hosiery with the four-layer bandage. The primary endpoint was time to healing of the reference ulcer. The VenUS IV database contains 457 patients with

non-healing VLUs. There were 230 patients allocated to two-layer hosiery and 227 to the four-layer bandage. The dataset includes demographic and baseline data, monthly research nurse visits, non-trial healthcare visits necessary to maintain standard of care, patient quality of life, and compression therapy and treatment outcomes data.

Standard Care Data Modeling

First, 870 patients from the VenUS RCT studies that were treated with SC alone including local wound care with dressings chosen to address wound conditions of slough, exudate or potential infection, and multilayer compression were selected from 1,448 patients (Figure I, Step 1). Patients that were treated with an experimental intervention plus SC were excluded from the data synthesis (larval therapy (VenUS II), therapeutic ultrasound (VenUS III) and compression hosiery (VenUS IV)). The results of larval therapy (VenUS II) were made public prior to 2010 and were not recommended for inclusion in evidence-based national venous leg ulcer clinical guidelines.[30] In VenUS II, larval therapy along with compression has been shown to be an effective method of debridement. However, the use of larval therapy did not increase the rate of healing for necrotic tissue or slough in leg ulcers compared with ulcers treated with hydrogel and compression.[23] Treatment with larval therapy cost, on average, £96.70 more per participant per year (95% confidence interval −£491.9 to £685.8) than treatment with hydrogel.[31] Therapeutic ultrasound has been found to stimulate a number of cellular effects associated with the acceleration of wound healing. These cellular effects have been well studied in vitro and include improvement of microcirculation, reduction of edema, and increases in cytokine and other protein levels that are active in the healing cascade.[32] Clinical trials studying the effects of ultrasound have reported positive results in the healing of a variety of chronic non-healing wounds.[33] Most therapeutic ultrasound protocols have recommended treatment sessions at higher frequency, such as three times weekly or once daily. However, this frequency of treatment would not be easily integrated into venous leg ulcer care, given the need for removal and reapplication of both dressings and compression therapy, and hence in the UK context weekly application was tested. VenUS III detected no evidence of any impact of ultrasound therapy on healing, which may have been expected if the key limitation of the trial was the dose of

ultrasound used. The VenUS IV trial reported that two-layer compression hosiery is a viable alternative to the four-layer bandage—it is equally as effective at healing venous leg ulcers. However, a higher rate of treatment changes in participants in the hosiery group than in the bandage group suggested that hosiery might not be suitable for all patients.[27] In this study, patients must be suitable to multilayer high compression for matched comparison to ASM plus SC patients (Figure I). We eliminated the treatment groups from further analysis due to exclusion from current clinical guidelines [30, 34] and potential suitability bias of compression hosiery[27].

In Figure I, Step 2, SC patients from the 870 patient group (VenUS-SC-870) were reduced to 177 patients (VenUS-SC-177) that met key inclusion criteria of the ASM plus SC study: 1) ankle brachial pressure index ≥ 0.7; 2) wound area at baseline ≤ 33.00 cm^2; 3) wound duration at baseline ≥ 4 weeks; 4) compression bandaging (suitable for multilayer high compression or short stretch high compression) and 5) majority of patient care completed in specialist wound centers (avg. 1 visit per week).

The VenUS-SC-177 control group (n=177) who met the key selection criteria were compared for statistical difference (p-value < 0.05) within the covariates of baseline VLU area, VLU duration and patient age to the same covariates in the ASM plus SC group . A balanced distribution (p-value < 0.05) of independent variables, such as VLU area, VLU duration and patient age, which can influence outcome (healing time) was considered important for the purpose of comparing the two populations for healing time effectiveness.[35-41]

The statistical comparative results in Step 3, Figure I, Table I showed statistical balance (p-value ≥ 0.05) between VenUS-SC-177 (n=177) to ASM plus SC (n=53) for baseline ulcer area (p-value = 0.650), however, not for ulcer duration (p-value < 0.006) and patient age (p-value = 0.026). Consequently, further matching was required to ensure that the two groups to be used for final comparative analysis were statistically balanced i.e. matched with respect to the three aforementioned key predictors for healing.

Propensity Score Group Matching

In the next step, Figure I, Step 4, (i) patient age [41] and (ii) extent of ulcer duration (1 = ≤ 6 months, 0 = > 6 months) [35], as well as (iii) extent of ulcer area at baseline (1 = ≤ 5 cm^2, 0 = > 5 cm^2) [35] were used for propensity score matching for causal inference. A nearest neighbor propensity score matching algorithm (MATCHIT (version 2.4-21, http://gking.harvard.edu/matchit) [42, 43] in r (version 2.150) [44] was integrated into IBM-SPSS Statistical software, version 22.0 to complete the matching.[45]

A 1:1 nearest neighbor matching algorithm was selected to ascertain the best control matches for each individual and ulcer in the ASM plus SC treatment group. Matching was completed using a distance measure specified by the distance option (we used the default option=logit). Matches were chosen for each treated unit one at a time without replacement, with the order specified by the m.order command (we used the default=largest to smallest). At each matching step the control unit that is not yet matched but is closest to the treated unit on the distance measure was chosen. Using this validated balancing method [46, 47,] a VenUS-SC-177 patient from the control group and corresponding study ulcer was identified for each ASM plus SC patient based on patient age, baseline ulcer area category (1 = ≤ 5 cm^2, 0 = > 5 cm^2) and ulcer duration category (1 = ≤ 6 months, 0 = > 6 months). Diagnostic information from the matching included balance statistics and graphics to assess achieved balance on the covariates (Figure I, Step 4).

Ethical Considerations

Approval for the ASM study was obtained from the Medicines and Healthcare products (MHRA) in the UK and the Therapeutics Goods Administration (TGA) in Australia, as well as the local ethics committees for all the study sites. Written informed consent was obtained from all patients and study records were de-identified for confidentiality. The VenUS RCT studies were all approved by York Multicenter Research Ethics Committee (MREC), local research ethics committees, and research and development departments. Participant study records were de-identified for confidentiality.

Statistical Analysis

Statistical and predictive healing time analysis was completed using IBM-SPSS Statistics for Windows, Version 22.0. Armonk, NY and SigmaPlot™ 13 Graphical and Data Analysis software, Systat Software, a subsidiary of Cranes Software International Ltd. In all inferential statistics p-values ≤ 0.05 were considered significant. Group matching was established on the basis of ulcer duration category (1 = ≤ 6 months, 0 = > 6 months),[35] baseline ulcer area category (1 = ≤ 5 cm^2, 0 = > 5 cm^2) [35] and patient age [41] data that have been shown to influence healing time outcomes of patients with leg ulcers.[35-41] A Mann-Whitney Rank Sum Test was used to assess statistical differences between the matched control group and the ASM plus SC group for the main factors that influence healing outcomes (baseline ulcer area, ulcer duration and patient age).[48] An independent chi-square test was performed to determine statistical differences in gender between groups and difference of categorical variables level of baseline ulcer area (1 = ≤ 5 cm^2, 0 = > 5 cm^2) and ulcer duration (1 = ≤ 6 months, 0 = > 6 months).[48]

Kaplan-Meier survival curves (healing trajectories) and median and mean time to healing with 95% confidence intervals (CI) were calculated for each group and a Log rank test was used to compare equality of survivor functions.[48-53]

Sensitivity Analysis

A sensitivity analysis for matched, censored survival outcomes[54] was performed to evaluate hidden bias associated with missing covariates in the propensity score matching process that may have impact on the magnitude of healing time comparison. A hidden bias may exist in this study as we were not able to collect information (e.g., patient comorbidities) from the evidence network that may have an impact in propensity score matching result. A sensitivity analysis for matched samples and censored survival outcomes is used to answer: How would inferences about treatment healing time effects be altered by hidden biases or unobserved covariates of various magnitudes? The “Winners” and “Losers” for each paired, matched censored samples are ascertained in the analysis:

1. The number of pairs in which the “Winner” (longer healing time) can be conclusively determined.

2. Number of pairs with a clear “winner” in which the patient with ASM + SC healed the ulcer faster than the patient with standard care alone.

A sensitivity parameter (gamma), Γ is the measure of the degree of departure from a study that is free of hidden bias. A Microsoft EXCEL spreadsheet version 13.0 (Microsoft Corporation, Redmond, WA USA) is used to calculate the gamma value (Figure II). The spreadsheet automatically completes the calculations for gamma values between 1.0 (i.e., no hidden bias) and 6.0, stepping by 0.5.

Results

A total of 53 patients from the VenUS-SC-177 control group were matched to the 53 patients in the ASM plus SC study group using a 1:1 nearest neighbor matching algorithm without replacement [42-45] and used for comparative analysis of healing time effectiveness (Figure I, Step 5).

No covariate exhibited a large imbalance in either control group (standardized bias: $|d| > 0.25$) to the ASM plus SC treatment group.[42] Figures III and IV show that covariate balance was much improved in the matched group after propensity score 1:1 matching process.

The baseline characteristics of all patients (Table II) from the ASM plus SC and the control matched study group showed no evidence of a statistical difference between wound factors known to influence healing time: (baseline ulcer area: p-value = 0.997), ulcer duration: (p-value = 0.283), patient age: (p-value = 0.359) and the categorical variables for level of ulcer duration (1 = ≤ 6 months, 0 = > 6 months; (χ^2 0.164 p-value = 0.685)) and level of ulcer area (1 = ≤ 5 cm^2, 0 = > 5 cm^2; (χ^2 0.340 p-value = 0.560)) indicating no statistical differences in both baseline ulcer area and ulcer duration. Gender was also equivalent (gender: χ^2 0.151 (p-value = 0.697).

Analysis of baseline healing time between the ASM plus SC group (n=53) with the matched SC control (n =53) indicates a significant statistical difference in time to healing for patients treated with the ASM plus SC, in comparison to matched patients treated with SC alone in the VenUS-SC-53 group (Log rank test,

χ^2 5.779, $p < 0.016$) within 12 weeks. Mean time to healing in ASM plus SC was 73.10 days compared with 83.5 days in the comparison cohort. Table 3 contains mean, median and 95% confidence intervals for each group. Figure V shows the Kaplan Meier survival curves of time to healing for the matched SC control group and ASM plus SC group over 12 weeks.

Sensitivity analysis for matched, censored survival outcomes between the ASM plus SC group (n=53) and matched SC control (n =53) shows that the sensitivity parameter Γ tips over significance at the two-tailed $\alpha = 0.05$ level somewhere between $\Gamma = 1.0$ and $\Gamma = 1.2$ (2-tailed p-value [0.0147 lower bound, 0.0495 upper bound]) which indicates that a hidden bias or unobserved covariate in the SC group would need to increase the odds of healing by more than a factor of 1.1 to change the baseline result, Table 4. There are 25 matched pairs where there is a clear winner in healing time with 18 of the matched pairs associated with the use of ASM + SC.

Discussion

Evidence network[55] and propensity score matching-adjusted indirect comparison methods [43-47] have been developed to address the challenges of investigating the relative effects and cost-effectiveness [56-59] of competing treatments when results are not available from head-to-head trials. These focus on comparisons of outcomes for two specific treatments of interest by using patient-level data for one treatment and patient-level trial data for the other treatment from compatible studies, taking into account possible confounding due to population differences for a small number of variables.

The evidence network consisting of patient level data from VenUS I [21, 22], VenUS II [23, 24], VenUS III [25, 26] and VenUS IV [27, 28] patient databases (VenUS-SC-870 pooled dataset) contained a sufficient number of patients treated with SC that met key inclusion criteria to the ASM plus SC treatment group to simulate a matching-adjusted indirect comparison of healing time effectiveness (Figure I, Step 2).

For efficient causal inference and good estimation of the unobserved potential outcomes, we needed to compare treated and control groups that are as similar as possible. Propensity score matching entails forming matched sets of treated and untreated subjects who share a similar value of the propensity score.[60, 61] Moreover, propensity score methods often yield more reliable estimates of treatment effects than traditional methods, such as regression adjustment.[62] Propensity score matching allows one to estimate the "average effect of the treatment on the treated" (ATT).[63] The most common implementation of propensity score matching is one-to-one or pair matching, in which pairs of treated and untreated subjects are formed, such that matched subjects have similar values of the propensity score. Although one-to-one matching appears to be the most common approach to propensity score matching, other approaches can be used.[64] The primary advice to this point has been to select the method that yields the best balance.[43, 65, 66]

We utilized a 1:1 nearest neighbor propensity score matching algorithm to balance the groups on baseline ulcer area, ulcer duration and patient age to minimize selection bias and confounding in our patient and ulcer selection process (Figure I, Step 4).[42, 43] This method was selected as it 1) yields the smallest standardized difference of means across the largest number of covariates, 2) minimizes the standardized difference of means of a few particularly prognostic covariates, and 3) results in the fewest number of "large" standardized differences of means (greater than 0.25). This is generally the most effective method for settings where the goal is to select individuals for follow-up. Nearest neighbor matching nearly always estimates the ATT, which is the effect for those in the treatment group, and the "average treatment effect" (ATE), which is the effect on all individuals (treatment and control), as it matches control individuals to the treated group and discards controls who are not selected as matches.[63, 67, 68] Nearest neighbor matching is the easiest to implement and understand.

We also chose to use matching without replacement in our 1:1 nearest neighbor matching algorithm. Matching with replacement can often decrease bias because controls that look similar to many treated individuals can be used multiple times. This is particularly helpful in settings where there are few control

individuals comparable to the treated individuals.[69] However, our control database included a higher number of patients for selection than treatment patients therefore replacement was disregarded.

Results of the matching indicated the overall balance statistics between the standardized means of the prognostic covariates is good and no covariate exhibited a large imbalance in either control group to the ASM plus SC treatment group. Figures III and IV show that covariate balance was much improved in the matched groups. The baseline characteristics of all patients (Table II) from the ASM plus SC and the control matched study group showed no evidence of difference between patient and wound factors known to influence healing time after matching. [35-41]

A common complaint regarding nearest neighbor matching is that it can discard a large number of observations and thus would apparently lead to reduced power. However, the reduction in power is often minimal, for two main reasons. First, in a two-sample comparison of means, the precision is largely driven by the smaller group size.[70] So if the treatment group stays the same size, and only the control group decreases in size, the overall power may not actually be reduced very much.[43] Second, the power increases when the groups are more similar because of the reduced extrapolation and higher precision that is obtained when comparing groups that are similar versus groups that are quite different.[71]

Sensitivity analysis indicates that an unobserved covariate would have to change the odds of healing for SC by a factor of 1.1 to impact the baseline results. It is plausible that comorbidities, location of care, person giving the care, compliance to care and others could influence the outcome in this investigation. A future randomized, blinded clinical study with a control is important next step in the proof of healing time effectiveness.

Limitations

The present analysis shares the limitations of retrospective comparisons of treatment approaches from patient databases including the inability to retrieve important variables that may not have been collected or recorded and chart entries that are confusing, inaccurate, or incomplete. Retrospective studies have

disadvantages vis-a-vis prospective studies. Underlying diseases, personal circumstances, and other factors can all contribute to a wound healing poorly. In retrospective studies it is more difficult to assess these issues than in prospective studies.

The major limitation of data modeling is that it requires investigator judgment for many decisions.[72] The senior investigator (RS) followed several data modeling steps to minimize bias in selection of venous leg ulcer patients and ulcers suitable for comparative analysis (Figure I, Steps 1 to 5).

It is also important to point out that, even though propensity score matching can balance observed baseline covariates between groups, they do nothing to balance unmeasured characteristics and confounders. Hence, as with all observational studies and unlike randomized controlled trials, propensity score matching analyses have the limitation that remaining unmeasured confounding may still be present. For example, patients from the control group were selected for matched healing comparison to ASM plus SC based on predictive healing factors of baseline ulcer area, ulcer duration and patient age. Although all prognostic factors were statistically equivalent between groups after matching we understand that other patient factors (e.g., comorbidities, medicines, nutrition, compliance etc.), which aren't recorded in clinical trials, would be important in minimizing bias in selection of patients in the control groups for comparison. The use of other patient factors especially comorbidities, would improve the performance of propensity score matching leading to greatly reduced bias and closer matches.

There also exists systematic differences in the patterns of care in the ASM plus SC versus pragmatic trial 'control' cohorts (e.g., attention bias, better care in a trial of a novel intervention, as well as country and regional differences which may include potentially better compression bandaging technique amongst nurses / clinicians in ASM plus SC cohort). Although, patients from the control group selected for matched healing comparison were identified as having majority of wound care completed in a wound specialist clinic similar to the ASM + SC study group.

The usual tradeoff between bias and efficiency arises in selecting a propensity score matching algorithm. By choosing only one nearest neighbor (1:1), we minimized bias by using the most similar observation.[64] However, this ignores a great deal of information, and thus may yield less efficient estimates in our analysis. Additional k:1 analysis was used to increase efficiency without restrictions. We created matches at 2:1 and 3:1 but diagnostics gave poor standardized means or less common space between the groups. We imposed restrictions for 2:1 and 3:1 using calipers (.01 to 0.1) in order to reduce poor matches. This also led to reduced standardized means balance diagnostics between groups compared to 1:1 matching. However, we did see lower p-values in eventual healing time comparisons which would be expected with larger control sample size.

In our dataset, outcomes data was available for both control and treated groups. One limitation of k:1 nearest neighbor matching is that it does not necessarily use all the data, in that some control individuals, even some of those with propensity scores in the range of the treatment groups' scores, are discarded and were not used in the analysis. Weighting, full matching, and sub classification methods instead use all individuals. These methods can be thought of as giving all individuals (either implicit or explicit) weights between 0 and 1, in contrast with nearest neighbor matching, in which individuals essentially receive a weight of either 0 or 1 (depending on whether or not they are selected as a match). The three methods represent a continuum in terms of the number of groupings formed, with weighting as the limit of sub classification as the number of observations and subclasses go to infinity and full matching in between.[73]

Our MATCHIT software in SPSS has limitations in analysis. We did not have access to optimal matching, full matching, coarsened exact matching, genetic matching, matching, or entropy matching.[74-79] Work is being conducted to address these limitations and include other matching and estimation techniques in our proof of concept model. We will report results when all options have been used.

Measurement of wound area (cm^2) was not the same throughout all studies. Typically, ulcer measurements were recorded via transparent film followed by different tools to measure ulcer area

(cm^2).[48, 80-82] However, it did not appear to affect the scoring process in this evaluation for predicting healing using the scoring method by Margolis et. al.[35] We found the difference of ulcer area measurements (1 = ≤ 5 cm^2, 0 = > 5 cm^2) between ulcers in the VenUS III study [25] with "Mouseyes" [82] and "VISITRAK™" [48] to be small (8.6%). We assume the same margin of error with ulcer measurements in the VenUS I, VenUS II and VenUS IV studies.

The primary endpoint of wound treatment trials should be complete ulcer healing, and, preferably, the primary outcome should be time to healing.[7] Assessment of outcomes should be undertaken either by assessors masked to trial treatment, or independently confirmed by assessors masked to treatment.[7] The VenUS RCT studies used blinded, verified outcome by photographs to avoid bias in this regard. The ASM plus SC study required digital photographs of all reference ulcers at every visit which included a date when the image was taken.[29] Digital photographs were taken after last study visit by trial sites especially for ulcers that were over 90% re-epithelialized at last visit. The photographs in ASM study served as a means to validate 100% healing and time by external, qualified wound care professionals who were not able to be blind to assessment.

A comparative healing time analysis between matched groups of 53 patients from the VenUS-SC-177 group to 53 patients in the ASM plus SC study group indicates a statistically significant difference in healing time (Figure V). The chance of this result may be minimized due to a small sample size controlled by the ASM plus SC study. However, the statistical power increases when the groups are more similar because of the reduced extrapolation and higher precision that is obtained when comparing groups that are similar "balanced" versus groups that are quite different.[43, 70-71]

Conclusion

The York Clinical Trial VLU evidence network contains high quality data that facilitates model building, propensity score matching and comparative statistical analysis to investigate the "potential" healing time effectiveness of acellular matrices and other wound healing therapies for the management of venous leg ulcers in medical practice. The results of our investigation suggest that providing SC and replacing the

ECM with the ASM may significantly reduce healing time compared to providing SC alone during the first 12-weeks of treatment, but the results are sensitive to unobserved covariates not included in the propensity score matching process that may impact outcome in this investigation. The next step is to estimate any actual difference in a head-to-head, randomized clinical trials.

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Disclosure

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Table I Baseline characteristics of patients and leg ulcers in control group (N=177) that met key inclusion criteria and ASM plus SC study group (N=53) before propensity score matching. Values are numbers (percentages) of participants unless stated otherwise

Characteristics	**VenUS-SC-177 (N=177)**	**ASM Plus SC (N=53)**	**P value***
Men	88 (49.7)	29 (54.7)	0.523
Patient Age			
Mean age in years (SD)	69.4 (13.6)	74.7 (11.5)	
Median age in years	72.0	76.0	0.026
Area of Ulcer (cm^2)			
≤ 5	96 (54.2)	24 (45.3)	0.253
> 5	81 (45.8)	29 (54.7)	
Mean area of ulcer in cm^2 (SD)	7.4 (5.9)	7.4 (7.0)	
Median area of ulcer in cm^2	5.5	4.5	0.650
Duration (months)			
≤ 6	78 (44.1)	35 (66.0)	0.005
> 6	99 (55.9)	18 (34.0)	
Mean duration of ulcer in months (SD)	17.6 (35.9)	33.4 (59.5)	
Median duration of ulcer in months	6	10	0.006

* Statistical difference between groups at the < 0.05 p-value threshold

Table II Baseline characteristics of patients and leg ulcers in matched control group (N=53) and ASM plus SC study group (N=53) after 1:1 propensity score matching. Values are numbers (percentages) of participants unless stated otherwise

Characteristics	**VenUS-SC-53 (N=53)**	**ASM Plus SC (N=53)**	**P value***
Men	27 (50.9)	29 (54.7)	0.697
Patient Age			
Mean age in years (SD)	76.1 (10.3)	74.7 (11.5)	
Median age in years	77.0	76.0	0.359
Area of Ulcer (cm^2)			
≤ 5	27 (50.9)	24 (45.3)	0.560
> 5	26 (49.1)	29 (54.7)	
Mean area of ulcer in cm^2 (SD)	7.3 (6.0)	7.4 (7.0)	
Median area of ulcer in cm^2	5.1	4.5	0.997
Duration (months)			
≤ 6	33 (62.3)	35 (66.0)	0.685
> 6	20 (37.7)	18 (34.0)	
Mean duration of ulcer in months (SD)	23.4 (42.9)	33.4 (59.5)	
Median duration of ulcer in months	8	10	0.283

* Statistical difference between groups at the < 0.05 p-value threshold

Table III Mean, median and 95% confidence intervals for the matched SC control and ASM plus SC treatment groups at 12-weeks

Characteristics	VenUS-SC-53 (N=53)	ASM Plus SC (N=53)
Mean (95% CI), Days	83.5 (79.5 – 87.5)	73.1 (66.4 – 79.9)
Median (95% CI), Days**	----------	89.0 (89.0 – 89.0)
P-value*	----------	0.016

***** Statistical difference between groups at the < 0.05 p-value threshold

** The median cannot be estimated when less than 50% events occur. Likewise you can't estimate confidence intervals for the median if you don't have enough events to estimate a standard error for the median survival time.

Table IV Sensitivity analysis for matched, censored survival outcomes between the ASM plus SC group (n=53) and matched SC control (n =53)

Gamma Value (Γ)	2-tail P value (lower bound)	2-tail P value (upper bound)	P+	P-	E(T+)	E(T-)	SD(T+)
1.1	**0.0147**	**0.0495**	0.476	0.524	11.90	13.10	2.50
1.2	0.0077	0.0797	0.455	0.545	11.36	13.64	2.49
1.3	0.0040	0.1185	0.435	0.565	10.87	14.13	2.48

Figure I Evidence network and data modeling process of the VenUS - RCT database to identify 1:1 matched standard care control study group to the ASM plus SC treatment study group.

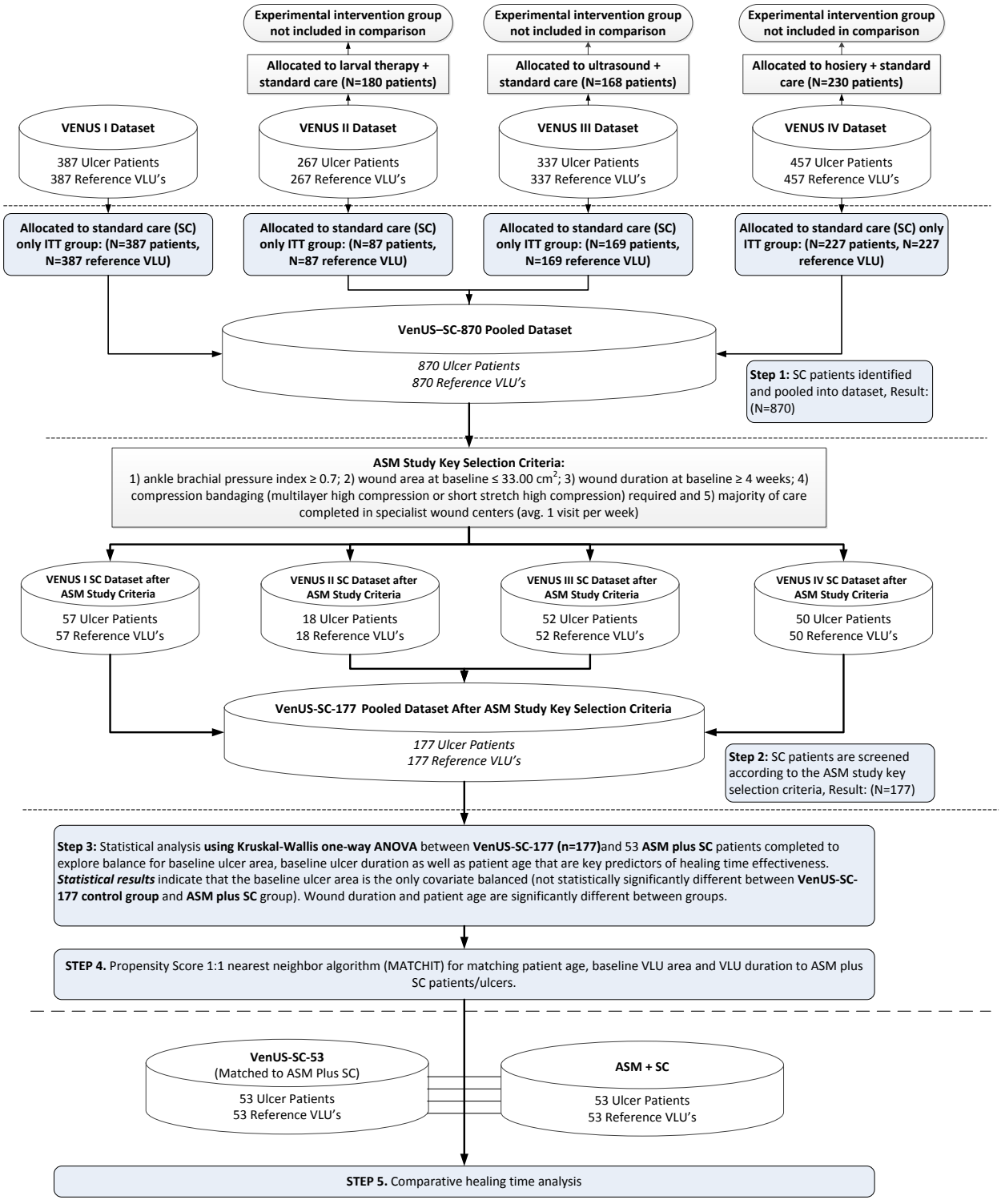

Figure II Spreadsheet calculation for unobserved covariate and hidden bias sensitivity analysis

Data

Total # of Pairs With A Clear Winner	**25**
T = # of Pairs Where Exposed Outlives Control	**18**

Sensitivity Analysis

Gamma Values	2-tail P value (lower bound)	2-tail P value (upper bound)	P+	P-	E(T+)	E(T-)	SD(T+)
1.0	**0.0278**	**0.0278**	0.500	0.500	12.50	12.50	2.50
1.5	**0.0011**	**0.2207**	0.400	0.600	10.00	15.00	2.45
2.0	**0.0000**	**0.5716**	0.333	0.667	8.33	16.67	2.36
2.5	**0.0000**	**0.9496**	0.286	0.714	7.14	17.86	2.26
3.0	**0.0000**	**1.0000**	0.250	0.750	6.25	18.75	2.17
3.5	**0.0000**	**1.0000**	0.222	0.778	5.56	19.44	2.08
4.0	**0.0000**	**1.0000**	0.200	0.800	5.00	20.00	2.00
4.5	**0.0000**	**1.0000**	0.182	0.818	4.55	20.45	1.93
5.0	**0.0000**	**1.0000**	0.167	0.833	4.17	20.83	1.86
5.5	**0.0000**	**1.0000**	0.154	0.846	3.85	21.15	1.80
6.0	**0.0000**	**1.0000**	0.143	0.857	3.57	21.43	1.75

Insert Gamma Value Below	2-tail P value (lower bound)	2-tail P value (upper bound)	P+	P-	E(T+)	E(T-)	SD(T+)
1.1	**0.0147**	**0.0495**	0.476	0.524	11.90	13.10	2.50

Figure III Standardized mean differences for VenUS-SC-53 and ASM plus SC matched groups before and after 1:1 propensity score matching

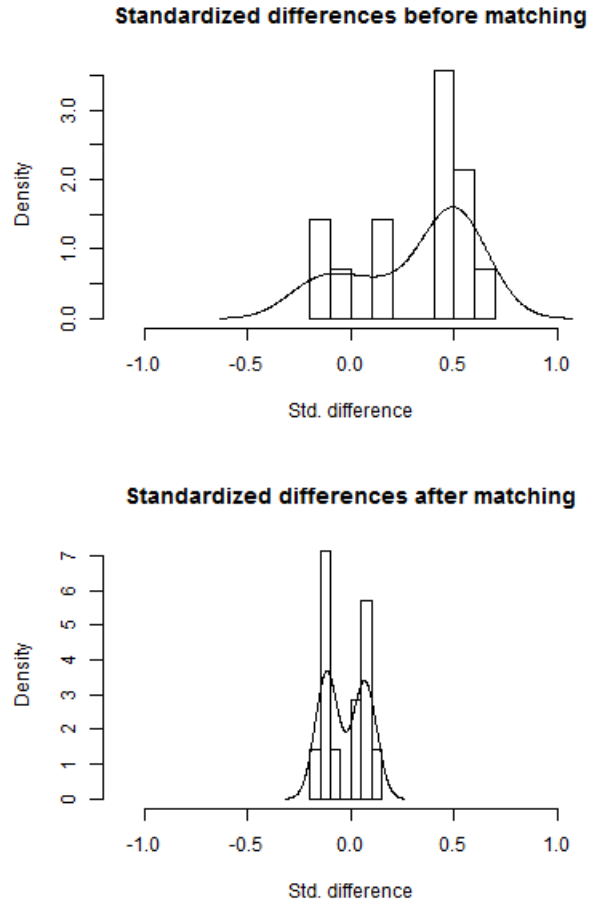

Figure IV Dot plots of standardized mean differences (Cohen's d) for VenUS-SC-53 and ASM plus SC matched groups before and after 1:1 propensity score matching

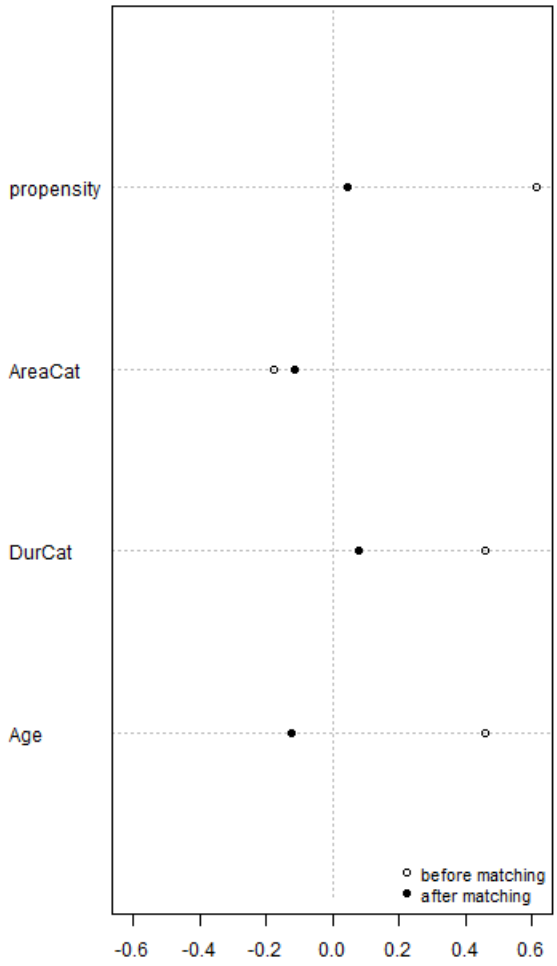

Figure V Kaplan–Meier survival curves for time to healing of venous leg ulcers with 12-week treatment of ASM plus SC (N=53) healers and matched SC control group (N=53)