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Research Article

Peripheral Rearterialization for Critical Limb Ischaemia and Antiseptic Resin or Honey Salve in Postoperative Ulcer Care Results in Healing Rate Of Leg Ulcers in Three Quarters of Cases. A Prospective Clinical Follow-up of 35 Patients with Preoperative Chronic Ulcer and 5 Patients with Post-Surgery Wound (Surgical Site Infection)

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Abstract

Objective and Approach: We evaluated the healing rate of leg ulcers in 35 patients with preoperative chronic ulcer and in 5 patients with postoperative complicated wound / "Surgical Site Infection" (SSI), after peripheral rearterialization for Critical Limb Ischemia (CLI). Consecutively, 20 patients received antiseptic resin salve and 20 honey salve for postoperative topical ulcer care. Ulcer healing was assessed clinically, or by evidence of a significant progressive reduction of ulcer size in linear regression analysis, over the available postoperative follow-up time.

Results: Ulcer healed clinically in 5 (25%) and 4 (20%) patients in resin and honey groups, respectively, i.e., in 9 (xx%; 95%CI: xx-cc) cases in total, the observed healing time being 61 ±41 days, median 56 days. Significant reduction of ulcer size occurred in 8 additional patients lost during the follow-up; the predicted healing time being 116 ±38 days, median 108 days. By principles of "intent-to-treat", 9 (45%, 95%CI: 23-67%) and 8 (40%; 95%CI: 19-61%) patients achieved treatment success in resin and honey groups, respectively. In "per protocol" analysis, 17 of 22 patients (77%; 95%CI: 60-95%) with sufficient data, ulcer showed objective evidence of treatment success; ulcer either healed or showed a significant trend to heal. When the SSI cases were excluded 14 of 19 patients (74%; 95%CI: 54-93%) with preoperative chronic foot ulcers in CLI healed after the revascularization, and with either resin or honey salve as postoperative ulcer care. In life-table analysis the probability of healing did not differ between the salve groups (log-rank test P=0.78). No adverse events occurred.

Conclusions: Statistical analyses by linear regression model on changes of ulcer size over the follow-up time improves the objectivity of the conclusions of leg ulcer healing in cases that otherwise would be classified as dropouts. By inclusion of a significant reduction of ulcer size also as a criterium, the healing of preoperative chronic leg ulcer occurs within 6 months in three quarters of CLI patients after the limb revascularization, and with antiseptic resin or honey salve as a postoperative topical ulcer care.

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Keywords: Chronic wound; Medical honey; Resin salve; Peripheral vascular surgery; Wound care

Abbreviations and Acronyms

β: Regression Coefficient In Linear Regression (statistical); BMI: Body Mass Index; CABG: Coronary Artery Bypass Grafting; CAD: Coronary Artery Disease; CE: Certification Mark In European Union; CI: Confidence Interval (Statistical); COPD: Chronic Obstructive Pulmonary Disease; CLI: Critical Limb Ischemia; ITT: Analysis By Intent-To-Treat Principle; P: P-Value; Probability Value (Statistical); post hoc: Statistical Analyses That Were Not Specified Before The Data Was Seen; PP: Analysis Per Protocol; PVI: Previous Vascular Intervention; PTA: Percutaneous Transluminal Angioplasty; r: Correlation Coefficient (statistical); R^2: Coefficient Of Determination (statistical); SSI: Surgical Site Infection

Introduction

Elective and emergency arterial surgery for lower limb revascularization (rearterialization) is a common, effective procedure for treating Critical Limb Ischemia (CLI). Despite surgery and successful revascularization, chronic ischaemic leg ulcers in CLI can take weeks or months to heal, and therefore, these ulcers are a challenge also for postoperative ulcer care [1,2]. In patients with CLI and poor peripheral circulation, the operation wounds are often complicated, which results in bacterial infections at the Surgical Site (SSI) [3-7]. Conditions that give rise to complicated SSI wounds and chronic ulcers in CLI are, however, multifactorial. A number of co-morbidities can contribute to complications and chronicity of the ulcers, including diabetes, chronic venous insufficiency, malnutrition, smoking, hyperglycaemia, older age, anemia, renal, cardiac or hepatic failure, malignancies, and the use of some drugs, like corticosteroids or immunosuppressives [8-10]. The incidence of SSIs varies after peripheral vascular surgery from 4% to 27% in patients with CLI [11], Like a slow healing of chronic ulcer in CLI, the healing of ulcers with SSI might be delayed in approximately 20% of patients after peripheral arterial revascularization, despite the restoration of perfusion and oxygen supply [1,2,10,12,13].

After revascularization surgery, conditions are not favourable for additional major surgical procedures; therefore, patients are typically referred postoperatively to topical wound care [14,15]. Although hundreds of wound care products and protocols are available, evidence remains tenuous of the effectiveness of different postoperative treatment strategies. Prospective trials on treatments success for CLI ulcers are also rare [16]. In recent years and due to the risk of appearance of resistant microbe strains, it has become important to find new antiseptic tools and safe natural alternatives for long-term topical wound care, instead of use of antibiotics. One is a resin prepared from natural coniferous Norway spruce (*Picea abies*) [17]. The other is refined medical grade honey, derived from the Manuka myrtle (*Leptospermum scoparium) [18]*. Both have been used for treating chronic skin wounds with or without clinical signs of infection [17-23]. The objective of this 6-month prospective, clinical study was to investigate the healing rate of chronic leg ulcers in patients that underwent peripheral arterial revascularization for CLI and who received post-surgical topical ulcer (wound) care with two optional antiseptic salves. The study aimed to determine the frequency of ulcer healing after revascularization surgery and discover whether the antiseptic resin and honey salves are effective and safe alternatives as topical postoperative ulcer care agents at home. We also aimed to provide preliminary data on whether resin and honey salves show any marked differences in efficacy as topical agents for ulcer healing in patients with CLI that had undergone the revascularization.

Materials and Methods

Study population

The study population consisted of 40 consecutive patients with chronic ischaemic ulcers and CLI with or without preoperative chronic leg ulcer. All patients included had stage IV CLI, according to the Fontane classification of the European Society of Cardiovascular Surgery, or stage 5-6 CLI, according to the American Rutherford classification [22]. All patients underwent a clinically successful acute or elective rearterialization (see operation types and patient characteristics in Tables 1 and 2). All patients were considered eligible for postoperative topical ulcer care at home if they had a preoperative leg ulcer. By Fontane stage IV or Rutherford stage 5-6 CLI the leg ulcers are ischemic, necrotic, or gangrenous, and accompanied by diffuse pedal ischemia [22]. After study inclusion, the patients with preoperative leg ulcers (35 cases) were randomized to receive either a resin or honey salve for postoperative topical wound care at home. There also were patients (5 cases) who developed after surgery a complicated and infected wound at the Surgical Site (SSI). Also, these patients with SSI were similarly treated, randomized to the salve groups and followed-up postoperatively as the patients with a preoperative leg ulcer. Demographic data on patients in the resin and honey treatment groups are presented in Table 1. The operative procedures are given in Table 2. The majority of patients were older males, often with type 1 or 2 diabetes and a smoking history, as is typically the case in patients with CLI.

Characteristic	Resin group (n = 20)	Honey group (n = 20)	P value
Sex			
Male (%)	14 (70)	14 (70)	
Female (%)	6 (30)	6 (30)	1.000
Age years [range]	71±9.5 [46-91]	76±10.0 [57-93]	0.184
BMI kg/m^2 [range]	27.6±6.1 [18.5-39.8]	26.4±4.3 [15.9-32.9]	0.732
Smoker or ex-smoker (%)	13 (65)	15 (75)	0.49
Previous PVI (%)	6 (30)	11 (55)	0.11
Previous CABG (%)	5 (25)	2 (10)	0.212
Chronic diseases			
Diabetes type 1 or 2 (%)	13 (65)	9 (45)	0.20
CAD (%)	7 (35)	6 (30)	0.74
COPD (%)	4 (20)	3 (15)	0.68
kidney failure (%)	6 (30)	7 (35)	0.74
Alcoholism (%)	1 (5)	0	0.31
Rheumatoid arthritis (%)	0	1 (5)	0.31
Mobility			
normal (%)	18 (90)	15 (75)	
needs support (%)	2 (10)	4 (20)	
bedridden (%)	0	1 (5)	0.38
Permanent Chronic medication			
oral corticosteroid (%)	3 (15)	1 (5)	0.29
immunosuppressant (%)	2 (10)	2 (10)	1
Insulin (%)	9 (45)	5 (25)	0.19
oral diabetes medication (%)	5 (25)	6 (30)	0.72
diuretics (%)	12 (60)	13 (65)	0.74
warfarin (%)	3 (15)	8 (40)	0.08
antidepressant (%)	5 (25)	6 (30)	0.72
Plasma measures			
haemoglobin mg/l [range]	1.14±0.2 [0.73-1.47]	1.17±0.2 [0.0-1.64]	0.90
leucocytes 10^9/l [range]	8.6±2.9 [2.9-15.1]	8.9±3.1 [3.1-18.9]	0.88
C-reactive protein mg/l [range]	5 [3-50]	10 [4-23]	0.42
prealbumin mg/l [range]	220±7 [7-350]	180±7 [10-330]	0.07

Values denote the number of patients (percentage); the mean ± standard deviation [range]; or the median and [interquartile range]. BMI: Body Mass Index; CABG: Coronary Artery Bypass Grafting; CAD: Coronary Artery Disease; COPD: Chronic Obstructive Pulmonary Disease; PVI: Previous Vascular Intervention

Table 1: Baseline demographics, disease characteristics, permanent medications, and laboratory measures of patients that underwent CLI surgery, followed by self-care with a resin or honey salve.

Characteristic	Resin group (n = 19) [a]	Honey group (n = 20)	P value
Wound aetiology			
infected wound; SSI (%)	3 (16)	2 (10)	
ischaemic ulcer (%)	16 (84)	18 (90)	0.59
Wound size			
length mm [range]	48±32 [9-140]	39±25 [10-115]	0.44
width mm [range]	16±8 [3-33]	21±11 [2-45]	0.10
depth mm [range]	4±7 [20-34]	8±7 [1-15]	0.01
Urgency of operation			
Elective (%)	8 (41)	7 (35)	
Urgent (%)	11 (59)	13 (65)	0.65
Primary procedure			
femoro-popliteal bypass (%)	3 (16)	3 (15)	
distal bypass (%)	5 [(6)	2 (10)	
femoral endarterectomy (%)	1 (5)	1 (5)	
supragenicular PTA (%)	1 (5)	2 (10)	
infragenicular PTA (%)	3 (16)	8 (40)	
PTA + surgery (%)	3 [(6)	3 (15)	
other (%)	3 (16]	1 (5)	0.46
Combination procedure			
toe amputation (%)	2 (11)	0	
major revision (%)	1 (5)	0	
major amputation (%)	1 (5)	1 (5)	0.33
Initiation of treatment days[b] [range]	36 [5-64]	7 [1-44]	0.05
Follow-up time days[c] [range]	83±47 [30-183]	70±57 [8-183]	0.26
Final outcome			
fully healed (%)	5 (26)	4 (20)	

Values denote number of patients (percentage); the mean ± standard deviation [range]; or the median and [interquartile range]. PTA: percutaneous transluminal angioplasty. [a]Of the 20 patients that we intended to treat in the resin group, one man died after recruitment, but before the study start; this patient was dropped from the study. [b]Time from the surgical intervention to randomization. [c]Time from randomization to complete wound healing.

Table 2: Wound-related and vascular intervention-related baseline characteristics in patients with CLIs that underwent ulcer surgery, followed by self-care resin or honey salve treatment.

Estimation of study population size

The sizes (power analysis) of the resin and honey salve groups were estimated by a statistician. The endpoint was wound or ulcer healing within 6 months after surgery. We assumed that no patients would drop out of the study. The analysis was based on a binary outcome, head-to-head, superiority trial design. We used a two group c^2 -test with a 0.050 two-sided significance level and 80% power for observing the difference between a Group 1 proportion, p_1, of 0.900 and a Group 2 proportion, p_2, of 0.500 (odds ratio 0.111). The required sample size was estimated to be 20 patients in each group. The p_1 and p_2 values were arbitrarily chosen to exclude or include a marked, major difference in efficacy (90% versus 50%) between the test items in wound care. Simultaneously, we aimed to obtain moderately small-sized test groups. We assumed that this preliminary investigation would provide a more solid basis for calculating group sizes in future, larger investigations.

Inclusion criteria

The study population included patients that had undergone a surgical or percutaneous arterial revascularization for CLI, under either peripheral elective or emergency conditions, at the Clinic of Surgery in Kuopio University Hospital, Kuopio, Finland, between April 2014 and January 2015. Patients had a lower-limb (leg/foot) chronic ulcer of ischemic origin that was (a) infected or non-infected, but present prior to revascularization (35 patients, pre-surgery ulcer), or (b) postoperative wounds that were infected but appeared after surgery as complicated surgical chronic wounds that failed to heal postoperatively (i.e., SSI; 5 patients, post-surgery ulcer). In all cases, senior vascular surgeons considered that, after the operation and hospital stay, all ulcers required effective, active, postoperative topical care and follow-up for surveillance and control in the outpatient department. Exclusion criteria were a life expectancy of less than six months, advanced malignant disease, or an expected requirement of additional surgical revisions or plastic surgery, including minor skin transplantations. Patients were also excluded when they had a known hypersensitivity to resin or honey. When necessary, appropriate epicutaneous tests were arranged to detect the actual cause of suspected allergic reactions. All pre-surgery and post-surgery ulcers were considered to represent typical CLI-related leg wounds or ulcers that arose due to severe circulation failure in the lower limb. They were considered to require long-term, postoperative topical wound care, including intense, active postoperative attention and monitoring. After recruitment and randomization, we found that, of the 5 patients with SSIs, 4 were in the resin group and 1 was in the honey group. The average ulcer sizes were measured at the time of recruitment, after the operation procedures had been carried out (Table 2).

Randomization

Randomization to resin or honey salve group was performed by a statistician. Patient names were placed in permuted blocks of four with an allocation ratio of 1:1. The responsible physician allocated the patients to treatment groups, according to the randomization list (enclosed in envelopes). The treatments could not be blinded, because the resin and honey had discernible properties that could not be masked (e.g., color, fragrance, and consistency).

Study design, purpose of the study, and follow-up

The study group allocations and follow-up times are shown in Figure 1. The primary objective was to analyse the rate (frequency) of leg ulcer healing in the resin and honey groups, within the follow-up period of 6 months. All patients were scheduled to receive 5-7 follow-up examinations in the hospital. No significant differences between study groups were found in the distribution of completed follow-up visits or in demographic characteristics (Tables 1 and 2). Demographics, disease history, and follow-up data were recorded on clinical report forms by the responsible physician (TA). Forms were completed at the time of recruitment and at every follow-up visit throughout the 6-month period. All patients were advised to visit the surgical outpatient department, when clinically indicated and whenever the patients considered a visit justifiable. When the follow-up examination concluded that ulcer had clinically healed, the date of the last clinical report form was taken to indicate the time required for clinical healing of the ulcer (i.e., the primary objective was achieved). When the ulcer was not fully healed within 6 months at the last available follow-up visit, the treatment was considered clinically unsuccessful (i.e., the primary objective was not achieved). In those cases, a post hoc analysis of ulcer size was carried out with statistical linear modelling, when possible, and when sufficient follow-up data of ulcer size and follow-up time were available. The analyses aimed to obtain objective and statistically significant evidence of whether the ulcer was at a proper trend to heal, and to statistically (mathematically) estimate and predict the time point for final healing. Photographs of ulcers were taken at follow-up visits, when possible, but at least at the study initiation and at the study end (Figure 2). Any notable improvement, deterioration, or any other factor that might contribute to healing during the follow-up period (e.g., mechanical wound debridement, cleansing, or antibiotic treatment) was recorded on the clinical report form.

The topical treatments were started in the hospital and continued at home (self-care), according to instructions from a specialized nurse and the study protocol. Patients were instructed and prescheduled to contact the clinic at intervals of approximately 4 weeks, but at least whenever observable changes appeared, either

in ulcer status or in the general health of the patient. The resin or honey salve was the only topical ulcer care remedy allowed at home. Systemic antibiotics were given only during the hospital stay, when necessary, based on clinical grounds and at the discretion of the surgeon, but not during the home care period.

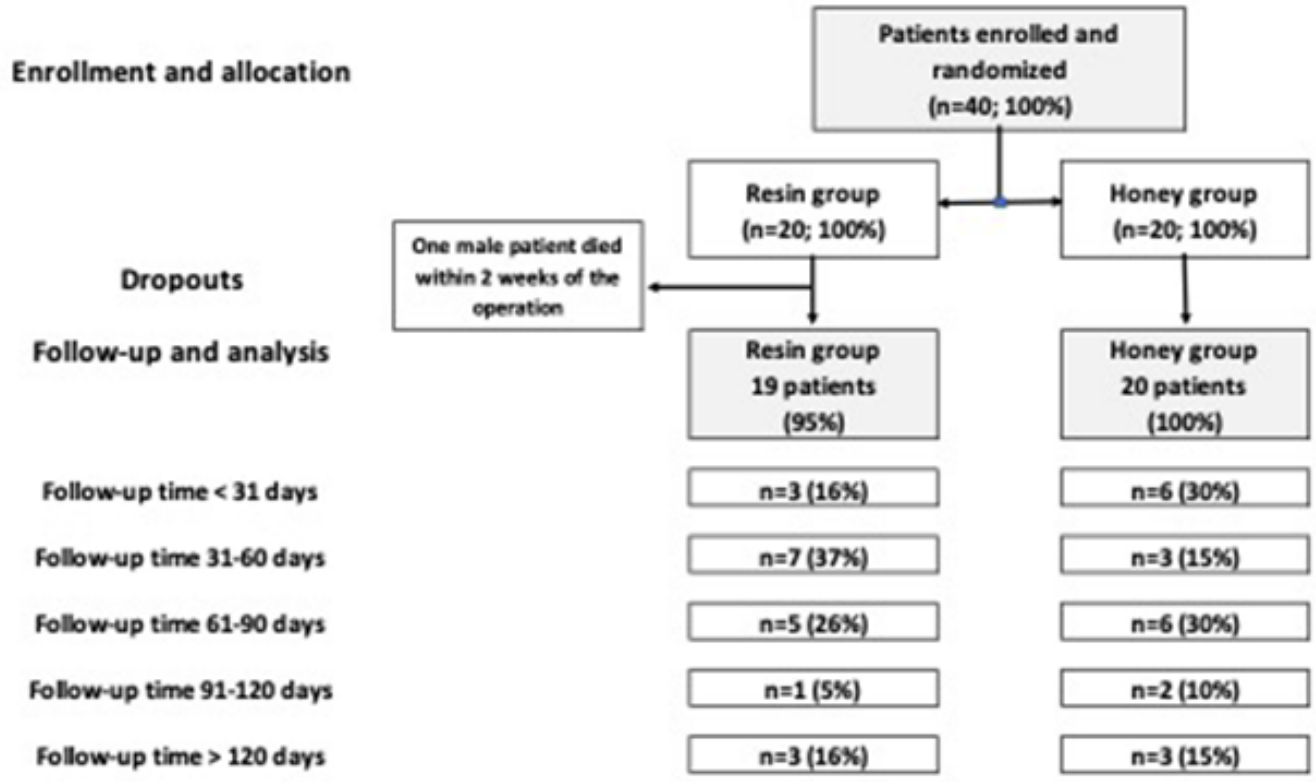

Figure 1: Flow diagram of the study. The follow-up times apply to corresponding rows in both study (salve) groups.

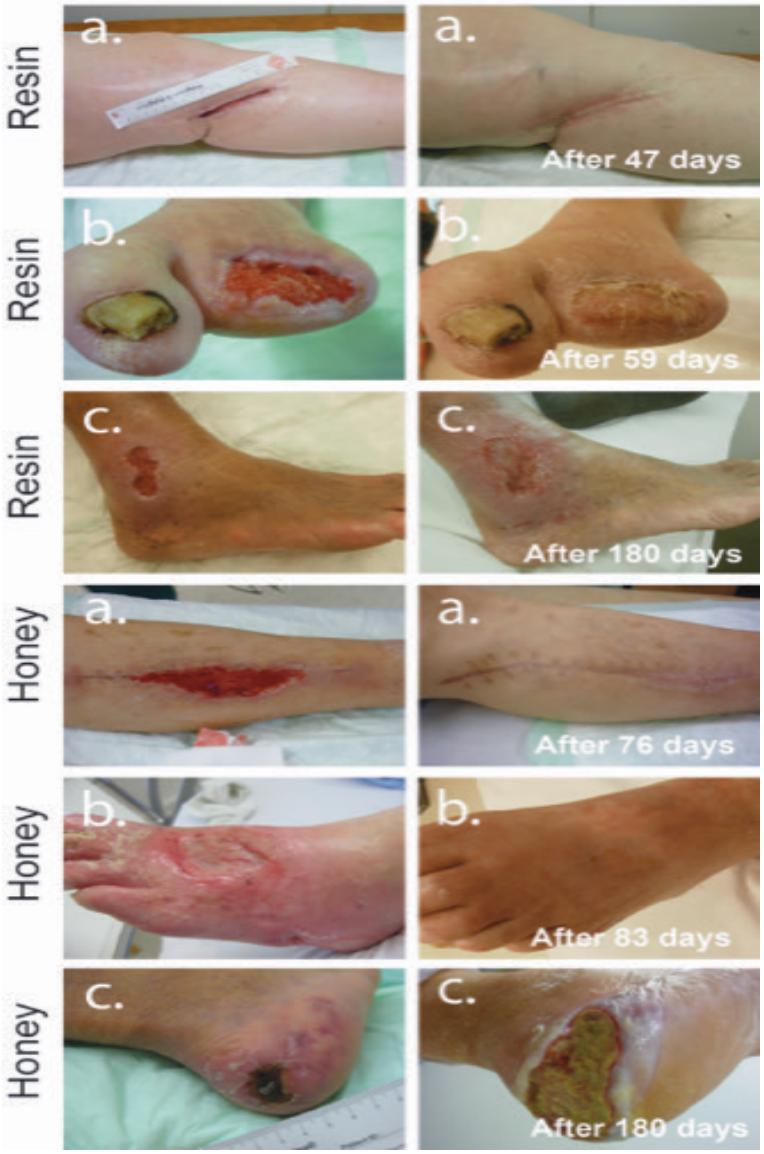

Figure 2: Photographs of acute and chronic wounds at the beginning (left) and end (right) of follow-up. Wounds were treated with either (top 3 rows) resin salve or (bottom 3 rows) medical honey. (a) Acute infected surgical wounds (SSIs) with successful outcome; (b) chronic ischaemic ulcers with successful outcome (ulcer healed); (c) chronic ischaemic ulcers with unsuccessful outcome (ulcer not healed within the 6-month treatment period). Treatment times are indicated in each case.

Data collection and statistics

Data collection, analyses, and results reporting were conducted according to the CONSORT-statement. Differences between parallel treatment groups were compared with the c^2-test or Fisher's exact test, as appropriate. Means and standard deviations were computed for continuous variables. In calculations of percentages, the means with 95% confidence intervals (95% CIs) were estimated. After determining the data distribution, proportions were compared with either the non-parametric Mann-Whitney U-test and log-rank test, with the parametric Student's t-test, or with the Z-test, as appropriate. The rates of ulcer healing were based on cumulative data. Assessments of mean and median healing times were performed with cohort tables of patients with healed ulcers in the two treatment groups over the treatment course. P-values <0.05 were considered statistically significant. Statistical calculations were performed with SPSS 20.0 (IBM Corp, Armonk, NY) and XLSTAT 2018.6 Excel software, iMac version (Addinsoft, Boston, USA).

Life table analyses

Life table analyses of the probability of ulcer healing were performed with XLSTAT 2018.6 Excel software (Addinsoft, Boston, USA), iMac version. The probability distribution function for ulcer healing over time was computed for patients in both the resin and honey groups. The observed time of clinical ulcer healing (cases in Figure 3), or the estimated healing time based on the linear model (cases in Figure 4), were used as end points of a favourable, successful event, in life-table computations. The log-rank test value was calculated to study the difference between study groups.

Statistical analyses of ulcer size

In 9 patients, the ulcers showed clinical signs of complete healing during the 6-month follow-up (Figure 3). In 13 patients, the ulcers had not healed, but follow-up data on ulcer size were available for at least three postoperative time points (Figure 4). A high number (17 cases) of patients had inadequate follow-up data (Figure 5); i.e., the ulcer had not healed and follow-up data were not complete or satisfactory for post hoc regression analysis. Furthermore, one male patient died after surgery but before entering the study. Therefore, we decided to extend the analysis of ulcer healing with a post hoc statistical approach, particularly for the 13 patients with ulcers that had not shown clinical evidence of complete healing at the last follow-up visit. In a linear analysis, statistically significant changes in ulcer size over time were considered objective predictors of the biological fate and course of the ulcer. That is, size changes indicated whether an ulcer was properly and progressively healing, even in cases where the ulcer had not completely closed (epithelized) at the last observation point.

Linear regression analysis

Ulcer size was calculated as the greatest length ´ the greatest width of the ulcer (i.e., surface area, mm^2). In linear regression analyses, the ulcer size and the follow-up time (days) were used as test parameters. The regression analysis was computed with commercial XLSTAT 2018.6 Excel software (Addinsoft, Boston, USA) in an iMac computer. We analysed data from all patients with healed ulcers (Figure 3) and from patients with follow-up data on unhealed ulcers, measured in at least three consecutive follow-up visits (Figure 4). In linear regression analyses, the variance tables disclosed R^2, the coefficient of determination, which indicated how well the variation of the dependent variable (ulcer size) could be explained by the explanatory variable (time). The closer the R^2 was to 1, the better the follow-up time could explain the changes in ulcer size; i.e., ulcer was at objective trend to heal. In linear regression, three parameters are computed: the F statistic, the value of Pr>F (i.e., indicating the significance [P] of the fit between the model and the observed data), and the regression coefficient. A negative regression coefficient indicates that an ulcer has progressively decreased in size, i.e., at trend to heal. In addition, the regression model allowed to estimate and predict the time to complete healing; i.e., the time to an ulcer size of 0 mm^2.

Study endpoints and criteria for successful ulcer healing in mathematical modelling

The primary study endpoint was total (complete) healing and "epithelization" of the ulcer or wound. This diagnosis was based on clinical examinations and evaluations of ulcer appearance by expert physicians at follow-up visits in the hospital. Examples of healed and unhealed chronic ulcers are shown in Figure 2. The regression analysis results for healed and unhealed cases are shown in Figures 3 and 4, respectively. The post hoc regression analysis was particularly focused on the 13 cases (Figure 4) of ulcers that were not fully healed clinically, but with reliable data on ulcer size from at least three follow-up visits. In these patients, ulcers were considered to be on a successful and appropriate healing course, when the linear regression analyses between ulcer size and follow-up time gave a negative regression coefficient, the correlation between ulcer size and time was high (³0.80), and the coefficient of determination (R^2) was ³0.80 and significant (Pr>F value £0.30). In these cases, the modelling also enabled the prediction of the healing time even though a proper healing of ulcer was not yet achieved at the last available follow-up time point.

Salves for wound care at home

Resin salve: In previous studies, the natural spruce resin salve is shown to exhibit substantial antimicrobial (antiseptic) properties in vitro, and additionally, it seems to enhance skin regeneration

and wound healing in vivo, according to observations from clinical trials in patients with pressure ulcers or complicated surgical wounds [17,23]. Resin salve is a 10% (w/w) mixture of coniferous purified spruce (*Picea abies*) resin suspended in a standard salve base ingredient. The coniferous resin (active ingredient) was collected manually from Finnish Lapland, mechanically purified, chemically unmodified, and mixed into salve form, in accordance with GMP standards. The product carried the CE mark for approved medical devices, and it is sold in pharmacies in Europe (Abilar® 10% Resin Salve, Repolar Pharmaceuticals Oy, Espoo, Finland).

Honey salve: For medical purposes, honey is generated by bees that have fed on flowers of the Manuka myrtle (*Leptospermum scoparium)* in New Zealand and southeast Australia. Honey has been shown to possess a wide spectrum of antimicrobial qualities [19]. Several honey products, prepared for wound care, are widely available all over the world. We used refined Manuka honey, prepared for medical use, which carried the CE mark (Activon Tube® 25 g, Advancis Medical Ltd., Nottingham, England). Patients were instructed to use the resin or honey salves according to manufacturer guidelines. Briefly, the salves were spread directly onto the clean wound (e.g., cleansed with saline), then the wound was covered with a bandage or gauze. When skin damage was widespread or included cavities or fistulae, the salve was spread at a thickness of at least 1 mm onto a gauze or gauze ribbon, which was then used to fill the cavity or fistula channel. Bandages were changed every 1–3 days, depending on the degree of infection and the amount of wound secretion. In cases of profuse wound secretion, which increased the risk of skin breakdown, a liquid barrier film (Cavilon®, 3M Skin and Wound Care, St. Paul, MN, U.S.A) was applied temporarily to protect wound edges from maceration.

Ethics, registration, and approval: The study protocol was approved by the Ethics Committee of the Kuopio University Hospital (KUH15101075), and it conformed to the guidelines of the Helsinki declaration. This study was registered at ClinicalTrials.gov (identifier: NCT01868412). All patients were verbally informed of the study course, and all provided written informed consent. For ethical reasons, we could not include a control group that did not receive any active home-care treatment. All included patients had marked, severe skin ulcers or wounds that required attention and active treatment.

Results

All available data of ulcer surface size (area; mm^2) and follow-up times (days) for all patients in both salve treatment groups are presented in table format in Figures 3-5. Figure 3 shows data for 9 patients with ulcers that fully healed clinically during the 6-month follow-up (patients symbolized with green color). Figure 4 presents data from 13 patients with ulcers that was not recorded to been healed within the 6-month follow-up. In this group, ulcer size data and follow-up times were, however, available from at least 3 follow-up visits enabling the statistical regression analysis; therefore, in this post hoc regression analysis, we could compute the relationship between ulcer size and follow-up time. For 8 patients, the regression analysis showed statistical evidence of a progressive reduction in ulcer size over time (highlighted in yellow, Figure 4). We concluded that these cases were on a successful, appropriate healing course and showed objective evidence of treatment success, even though the complete ulcer healing had not been clinically achieved and recorded at the last follow-up visit. Figure 5 presents data on 17 patients lost to follow-up. These patients had insufficient follow-up data (£2 follow-up visits) for determining objectively and statistically whether the ulcer was on a proper trend of healing. Among these 17 cases lost to sufficient follow-up, reasons for discontinuing the follow-up were not recorded. However, the demographic and background factors were not significantly different between the dropouts and the patients included in the treatment success analyses.

Observations	Patient 1		Patient 3		Patient 4		Patient 5		Patient 6		Patient 20		Patient 21		Patient 27		Patient 31	
Treatment group	Resin		Resin		Resin		Resin		Resin		Honey		Honey		Honey		Honey	
Ulcer size (mm^2) and	Size	Time	Size	Time	Size	Time	Size	Time	Size	Time	Size	Time	Size	Time	Size	Time	Size	Time
follow-up time (days)	48	14	189	28	0	119	495	30	1005	20	595	29	600	28	2530	28	20	29
	18	28	825	56			100	49	0	30	147	57	220	56	400	63	0	39
	5	42	360	86			0	59			0	67	0	56	25	91		
	4	42	143	116											0	101		
	0	54	55	143														
			0	143														
Ulcer subtype									SSI									
Statistics:																		
Linear regression *																		
R^2	0.90		0.36				0.98				1.00		0.87		0.87			
Significance P (Pr>F)	0.01		0.21		Statistics		0.10		Statistics		0.01		0.24		0.07		Statistics	
Regression coefficient β	-1.21		-3.86		not valid		-17.56		not valid		-15.73		-17.50		-34.47		not valid	
Correlation coefficient; r	-0.95		-0.60				-0.99				-1.00		-0.93		-0.93			
Predicted time (days) for healing by mod	50		155				55						60		90			

* Linear regression model: Size=α +β*Time; where α is computer estimated ulcer size at time point 0 and β is estimated regression coefficient.
R^2= coefficient of determination

Figure 3: Data of ulcer size at each available follow-up visit and the results obtained from statistical modelling of the ulcer data for patients with clinically healed foot/leg ulcers (patients symbolized with green color). Upper panel: observed ulcer sizes at indicated time points. Lower panel: statistical significance indicators of ulcer size from linear regression modelling (for details, see the Material and Methods). The observed time of ulcer healing is indicated by ulcer size = 0 in the upper panel. The healing time predicted by the linear regression model is shown in the lower panel.

Observations	Patient 2		Patient 7		Patient 9		Patient 10		Patient 15		Patient 22		Patient 23	
Treatment group	Resin		Resin		Resin		Resin		Resin		Honey		Honey	
Ulcer size (mm^2) and	Size	Time	Size	Time	Size	Time	Size	Time	Size	Time	Size	Time	Size	Time
follow-up time (days)	1200	7	625	27	465	30	490	23	867	14	400	25	1680	24
	1040	28	272	54	616	59	342	52	525	37	625	41	1575	59
	333	76	256	84	731	89	68	112	100	71	1050	56	455	86
			130	114	697	119					1440	81	270	114
			15	129	1250	141					1575	93	195	144
					2200	189					1376	107	55	172
											1376	140		
Ulcer subtype	SSI													
Statistics														
Linear regression *														
R^2	0.98		0.88		0.81		1.00		1.00		0.69		0.83	
Significance P (Pr>F)	0.08		0.02		0.02		0.02		0.03		0.02		0.01	
Regression coefficient β	-12.94		-5.12		+10.13		-4.72		-13.38		+9.30		-12.13	
Correlation coefficient r	-0.99		-0.94		0,90		-1.00		-1.00		+0.83		-0.91	
Predicted time (days) for healing by model	105		135				130		80				195	

cont.

Observations	Patient 28		Patient 29		Patient 32		Patient 36		Patient 37		Patient 39	
Treatment group	Honey		Honey		Honey		Honey		Honey		Honey	
Ulcer size (mm^2) and	Size	Time	Size	Time	Size	Time	Size	Time	Size	Time	Size	Time
follow-up time (days)	1925	27	420	30	2000	33	1300	14	153	21	2475	27
	3630	63	120	61	2000	40	300	37	90	37	3819	58
	720	88	20	91	2000	54	80	71	75	50	3944	88
			28	101	2400	64			50	83	2000	118
											3575	125
											3575	175
											1408	154
									SSI			
Statistics												
Linear regression *												
R^2	0.10		0.88		0.61		0.80		0.82		0.028	
Significance P (Pr>F)	0.80		0.02		0.22		0.30		0.09		0.72	
Regression coefficient β	-15.04		-5.12		+11.19		-20.27		-1.50		-3.20	
Correlation coefficient r	-0.32		-0.94		+0.78		-0.89		-0.90		-0.17	
Predicted time (days) for healing by model	200		100				75		110			

* Linear regression model: Size=α+β*Time; where α is computer estimated ulcer size at time point 0 and β is estimated regression coefficient.
R^2= coefficient of determination

Figure 4: Data of ulcer size and the results obtained from statistical modelling of ulcer data for patients with unhealed foot/leg ulcers but with data available on ulcer size for at least three follow-up visits. Upper panel: observed ulcer size data at indicated time points. Lower panel: statistical significance indicators of ulcer size from linear regression modelling. Eight patients showed objective, significant evidence of a trend in ulcer healing; these are marked in yellow.

Observations	Patient 8		Patient 11		Patient 12		Patient 13		Patient 14		Patient 16		Patient 17		Patient 18		Patient 19	
Treatment group	Resin		Resin		Resin		Resin		Resin		Resin		Resin		Resin		Resin	
Ulcer size (mm^2) and follow-up time (days)	Size	Time	Size	Time	Size	Time	Size	Time	Size	Time	Size	Time	Size	Time	Size	Time	Size	Time
	800	29	435	30	2550	30	45	24	360	38	140	31	2520	28	632	22	990	17
	114	59	50	52	2550	62	6	53	20	82	50	57	450	63	217	47	1248	28
Ulcer subtype													SSI		SSI			

cont.

Observations	Patient 24		Patient 25		Patient 26		Patient 30		Patient 33		Patient 34		Patient 35		Patient 38	
Treatment group	Honey		Honey		Honey		Honey		Honey		Honey		Honey		Honey	
Ulcer size (mm^2) and follow-up time (days)	Size	Time	Size	Time	Size	Time	Size	Time	Size	Time	Size	Time	Size	Time	Size	Time
	735	25	600	25	1638	25	144	30	192	29	450	35	400	28	1120	30
											6	45			385	66
Ulcer subtype																

Figure 5: Data of ulcer size in patients lost to follow-up (i.e., dropouts with insufficient data; patients symbolized with grey colour). None of the leg ulcers were healed. Data on ulcer size and follow-up times were only available from two follow-up visits at most. Objective conclusions of ulcer fate and statistical analyses were not possible.

Healing rate

Ulcers were fully healed clinically in 9 patients during the 6-month follow-up in total (Figure 3). An additional 8 patients displayed a statistically objective trend of ulcer healing, according to the linear regression analyses (Figure 4). Thereby, based on the intent-to-treat (ITT) principle, a significant and objective ulcer healing occurred altogether in 9 of 20 patients (45%; 95%CI: 23-67%) in the resin group and in 8 of 20 patients (40%; 95%CI: 19-61%) in the honey group (Figures 3 and 4). Alternatively, based on a per-protocol (PP) analysis, after excluding the 17 cases without sufficient follow-up data or postoperative death (one case), we found successful, proper ulcer healing in 9 of 10 (90%; 95%CI: 71-100%) patients and in 8 of 12 (67%; 95%CI: 40-93%) patients in the resin and honey salve groups, respectively (difference not significant). In the entire study population with sufficient follow-up data (analysis per protocol; PP), healing occurred in 17 of 22 (77%; 95%CI: 60-95%) patients. In one patient in resin group and in two patients in honey group, the ulcer showed a significant trend toward worsening (Figure 4). Moreover, in two patients in honey group, the ulcer did not show any significant trend towards either healing or worsening (Figure 4).

Ulcers due to SSI versus the leg ulcers present already before surgery

In 5 patients, ulcers were initially classified as complicated operation wounds; i.e., SSIs. However, sufficient follow-up data was available for only 3 of these patients. In all three, the ulcer (wound) either healed or showed a trend toward healing (Figures 3 and 4). When the SSI cases were excluded from treatment success calculations, 14 of 19 patients (74%; 95%CI: 54-93%) with ordinary foot ulcers in CLI healed after revascularization, with either resin or honey salve as a postoperative ulcer care.

Life table analyses

The life table analyses indicated the probability of ulcers healing in the resin and honey groups (Figure 6). We found no difference in probability distributions between the two salve groups, when clinical ulcer healing was chosen to indicate the positive favourable event (Log-rank P=0.783). Similarly, no difference was found between the groups when statistical evidence of successful ulcer healing (patients with evidence of ulcer healing by the regression analysis; Figure 4) were also included as favourable events (Log-rank P=0.567).

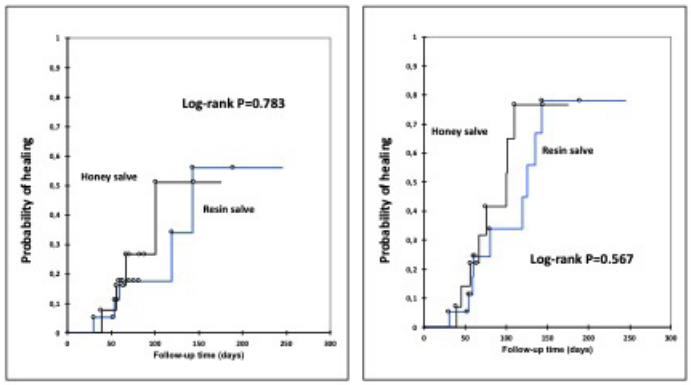

Figure 6: Life-table analyses on the probability of healing foot/leg ulcers with resin (black lines) and honey (blue lines) salve treatments. Left: only clinically healed ulcers (see Figure 3) were considered positive events. Right: both healed and healing ulcers (highlighted in green and yellow in Figures 3 and 4) were considered positive events.

Healing time

The observed and predicted healing times are shown for all patients with healed or successfully healing ulcers in Figures 3 and 4. In total, the mean healing time was 87 ±48 days (median 80 days), as observed clinically or estimated with the linear regression model. The mean observed healing time (Figure 3) was 61 ±41 days (median 56 days) in patients with the clinically healed ulcer. The mean estimated healing time (liner regression model) was 116 ±38 days (median 108 days) in patients with the statistically significant trend of ulcer to heal (Figure 4).

The healing time in patients with clinically healed ulcers (Figure 3) was not significantly correlated with the initial ulcer size (correlation coefficient r = 0.29; P=0.447). This was also the case (r = 0.33; P=0.436) in patients with the statistical trend of ulcer to heal (Figure 4), when the healing time was predicted by the linear regression model.

Side effects

One patient in the resin group reported signs of local skin irritation at the ulcer site at the end of follow-up. However, an epicutaneous test did not reveal hypersensitivity to coniferous resin (colophony allergy); the final diagnosis was infectious eczema, based on skin patch test and examination by a consulting dermatologist.

Discussion

The present observations show that the treatment of leg ulcers with arterial revascularization for CLI and with a postoperative antiseptic resin or honey salve as a postoperative topical ulcer care agent resulted in healing of leg ulcers in a markedly high number of cases, even in three quarters of patients. Based on PP principles, 17 of 22 (77%; 95%CI: 60-95%) patients with chronic leg ulcer or SSI showed objective evidence of ulcer healing. When the SSI cases were excluded, 14 of 19 patients (74%; 95%CI: 54-93%) with preoperative chronic foot ulcers in CLI healed after the revascularization, and with either resin or honey salve as the postoperative ulcer care. In these cases, ulcer was clinically healed or showed statistical evidence that it was progressively and significantly decreasing in size. However, a high number of cases were lost from reliable analyses (18 of 40 patients, 45%) due to insufficient follow-up; thus, the healing rate in an ITT analysis remained relatively low; 17 of the initial 40 cases displayed evidence of objective healing (43%; 95%CI: 27-58%).

In the present study, we could not determine how much the vascular surgery and how much the postoperative topical ulcer care with resin or honey salve contributed to treatment success. In our study population, all chronic leg/foot ulcers in CLI were severe ischaemic lesions, which are classified to stage IV CLIs in the Fontane classification and to stage 5-6 CLIs in the Rutherford classification [22]. The ulcers in sever CLI are necrotic, gangrenous lesions. Therefore, they also require attention and active, serious postoperative ulcer care [24,25]. Unfortunately, due to severe nature of the ulcers, it was not ethically accepted to arrange control groups without any active postoperative ulcer care. Antiseptic resin and honey salves were considered acceptable active options; they are safe, even for long-term topical ulcer care at home, and do not induce resistant microbe strains, and are shown to be beneficial in previous trials on various skin ulcers and wounds [17,20,21]. It is, however, conceivable that the postoperative ulcer care with either the resin or honey salve contributed positively to good postoperative ulcer healing. The present 35 patients had a preoperative history of ulcers that had persisted for months or even years, but then, healed within about 3 months after the limb revascularization combined with the topical postoperative antiseptic salve treatment. On its own, peripheral limb rearterialization is, without doubt, an effective surgical therapy in CLI; indeed, it is certainly the basic requirement for good healing results. In most recent clinical trials on ischaemic leg ulcers after rearterialization for CLI, the healing rates (frequency of cases healed) are reported to vary from 36% to 96% in follow-ups of 6-12 months, and the mean rate being around 60% [25-30]. In present study, the healing rate up to 77% within 6 months (PP analysis) is consistent with the literature and corresponded to a good treatment result.

In present study population, the significant reduction in ulcer size over time in linear regression modelling occurred in 5 of the 6 patients with a clinically healed ulcer (Figure 3). Similarly,

the regression modelling of data of ulcer size and follow-up time revealed additional 8 ulcer patients considered to be on a similar proper and successful healing course than the patients with the clinically healed ulcer even though the ulcer in these cases was not fully healed (closured) at the last available follow-up visit (Figure 4). The application of statistics and mathematical analyses of changes in ulcer size (surface area) over the available follow-up time provides an opportunity to improve the objectivity for conclusions about whether an ulcer in question is really healing or not. Thus, the statistical "post hoc" regression analyses of changes in wound/ulcer size could be useful in cases that have not achieved final ulcer closure, for whatever reason, during the follow-up. Hence, the imputations of the "post hoc" analyses with mathematical regression models could increase the reliability and objectivity of conclusions about the ulcer fate in cases that otherwise would be excluded from ITT-type evaluations and "censored" from the life-table analyses. Both resin and honey salves have long histories in wound care; thus, they are plausible biologically active agents. Coniferous natural resin collected from Norway spruce (*Picea abies*) has been used as a salve mixed with fat or butter for centuries in Nordic countries for treating skin wounds, sores, pressure ulcers, punctured abscesses, suppurating burns, onychomycosis, and paronychia; it has even been used as a chewing gum [17]. The acids of coniferous natural resin are strongly antimicrobial (antiseptic) [17]. They prevent and destroy bacterial biofilms in in vivo studies, and they might possess general anti-inflammatory and cell-regenerative properties; e.g., the capacity to enhance skin epithelialization [17]. In in vitro tests, this strong antiseptic activity is directed against a wide range of bacteria, yeasts, and fungi, including methicillin-resistant Staphylococcus aureus, vancomycin-resistant enterococci, and even highly drug-resistant "super bacteria". In clinical trials, the resin salve is shown to enhance the ulcer healing in patients with pressure ulcers, complicated surgical ulcers, or heals paronychia [17]. This effect probably arises from antiseptic mechanisms and/or from influences on tissue cytokines and tissue growth factors [17].

Honey has also been used for decades in wound care. In general, honey consists of concentrated sugar, which induces high osmolality at the site of application. Together, the low water and high sugar contents restrict microbial growth and might promote wound healing [18,19]. Medical grade honey is generated from Leptospermum species (i.e., Manuka honey). Apart from osmolality, Manuka honey is reported to have wound-healing properties, including anti-infectious, anti-inflammatory, anti-exudative, antioxidant, and wound debriding properties, in addition to its nutritional activities [18,19]. We did not observe any significant major differences in healing rates between resin or honey salve as postoperative ulcer care agent in the present study, either in standard statistical analyses or in life table analyses. Much larger study populations are needed to detect minor differences in efficacy, if any, between the resin and honey salves as postoperative ulcer care in CLI. The rate of dropouts tends to be high, for manifold reasons, in long-lasting clinical follow-ups, particularly in studies on severe diseases among old people at home care. In present investigation this dropout rate was 18 of 40 cases (45%). Our initial power analysis and the estimation of study group sizes were overly optimistic. Therefore, we were limited in the ability to detect significant changes in efficacy, if any, between the two test salves (coniferous resin vs. medical honey).

Finally, a total of 18 cases (including one patient included into the study population but who died before the study start) were lost and excluded from the analyses. However, they might not have markedly altered the general conclusions about the overall treatment results. The patients excluded from were not significantly different regarding the initial ulcer size, demographic characteristics, or clinical observations, compared to the patients included in analyses. The inclusion of two wound types, i.e., ordinary chronic ischaemic ulcers and complicated operation wounds (SSIs), might be another source for interpretation biases, even though both of these ulcer subtypes are intimately linked to CLI. However, when all 5 patients with SSIs were excluded from the analyses, the main conclusions about the favourable treatment success did not change. Another limitation is that the study could not be blinded, due to the specific, readily recognizable features of resin and honey salves. The study neither had a control group without any active postoperative ulcer care. The severity of necrotic ulcers in CLI did not allow to do that.

Innovation

Surgical limb revascularization for CLI in patients with chronic leg ulcer and the postoperative ulcer care with natural resin or honey salve will result in healing of ulcers even in three quarters of cases within 6 months. This can be demonstrated if the calculations also include the "missed" cases in which data of ulcer size are available from three follow-up visits at least. In these cases, the statistical linear regression modelling of the ulcer size (mean area) over the follow-up time can be applied as an objective criterium of whether or not an ulcer in question is at a trend to heal even though the ulcer would not be clinically closured at the last available time point of the follow-up.

Conclusions

Peripheral arterial revascularization for CLI in patients with chronic leg ulcer or SSI, and with postoperative topical ulcer care with natural coniferous resin or medical honey salve, results in

ulcer healing in about three quarters of the cases within 6 months. In this treatment strategy, resin and honey salves have equivalent effect as postoperative topical ulcer care agents. There are no major differences in their efficacy as a postoperative ulcer care agent. A linear regression analysis of data of ulcer size over the postoperative follow-up time increases the objectivity of conclusions about whether an ulcer in question is healing properly or not.

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