

Clinical outcomes of punch-grafting for chronic leg and foot ulcers – A retrospective case series study

Master thesis in Medicine

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Abstract

Master Thesis/Degree project

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Introduction

Punch-grafting has been used at The Department of Dermatology since the mid 1990's for hard to heal ulcers of the leg and foot. These medical conditions are rather common, especially in the elderly population, and the prevalence is expected to increase due to longer life expectancy. Therefore evaluating this treatment method is necessary, something that has not been completely done before.

Aim

The aim of this study was to evaluate the clinical outcome of punch-grafting as a treatment for hard to heal leg and foot ulcers.

Methods

A single-center retrospective case-series study was performed to investigate the frequency of complete wound closures within 3 and 12 months after treatment. Data on case-subjects were collected manually from patient charts at Sahlgrenska University Hospital, primary care facilities or other forms of health care providers in charge of follow-up. Patients treated with punch-graft for one or several leg or foot ulcers at the Department of Dermatology between January 2004 and September 2013 were included in the case-group.

Results

A total of 213 patients with 284 ulcers were included and the mean age was 73.2 ± 13.6 years. At 3 months 18.7 % of the ulcers had healed and at 12 months 52.2 %. Mean time to healing was 136 days for all ulcers that healed and mean ulcer duration prior to punch-graft was 25 months. Analysis of possible correlation between ulcer duration before punch-graft and time to healing, patient age and time to healing and localization of the ulcer and time to healing had no significant result.

Conclusions/Implications

The healing rates in this study were somewhat lower than those in previous studies made on pinch-and punch-graft. Ulcers categorized as "others" had the shortest time to healing, indicating that these might be the most suitable ulcers for punch-graft. Even so, it is hard making any conclusions regarding these results due to the study design and further research is required before determining the future of this treatment method.

Key words

Chronic ulcer, Foot ulcer, Leg ulcer, Skin transplantation, Wound healing

Background

Introduction

The definition of chronic wounds is wounds that despite a timely and orderly reparative process for production of anatomical and functional integrity still fail to proceed during a 3-month period (1). European studies have shown point prevalence for open leg ulcers to be 1-4 per 1000 inhabitants (2-4). Urban populations in Sweden have been studied in two different Swedish studies resulting in a prevalence of 0.12 % regarding leg and foot ulcers (5, 6). Leg ulcers appear to be more common in the elderly population and about 60 % of the patients have turned 50 before they develop their first ulcer. Moreover, at an age over 85, 15-25 per 1000 individuals suffer from open ulcers (7). The aethiology is venous disease for many of these ulcers (8) and most venous ulcers are caused by a long duration of venous hypertension together with insufficiency. This does not only result in high costs for society but also in several negative effects on quality of life for each patient (9, 10).

In Sweden, an estimated calculation of the annual wound management costs was SEK 2.3 billion during the year of 1992 (11). In a study made by Phillips *et al.* 81 % out of 73 patients with chronic leg ulcers believed their ulcer had a great negative impact on their mobility. Moreover, they found a connection between the amount of time spent on the ulcer care and emotions like resentment and anger (12). Regardless of the aethiology of a leg ulcer, there is a risk of developing severe pain (13). For some patients, the pain makes a great impact on their daily life spending an average of 1.5 to 2 hours a day well-aware of their ulcer (14).

Classifications of chronic leg and foot ulcers

A ulcer located to the leg is not equivalent with a diagnosis, instead it should be considered as a sign of another underlying disease (15). In order to determine an adequate treatment, a

working diagnosis is of high clinical value (16).

The aethiology of chronic leg ulcers varies. In a study made by Callam *et al.* the cause of leg ulcers was determined in a general population. They studied 600 out of 1447 leg ulcer patients in a population of about a million Scottish inhabitants. When investigating aethiological factors of these leg and foot ulcers they found venous disease as a factor in 76% of the cases, arterial insufficiency in 22%, rheumatoid arthritis in 9% and diabetes in 5% of the patients (17). Moreover aethiologies in excess of these also existed but in a smaller quantity and also several ulcers had a multifactorial origin and did therefore not fit into any specific group.

Venous aethiology

It has been estimated that 1 % of the population will suffer from chronic leg ulceration at some point in life (18) and in a study made by Nelzén *et al.* 54 % out of 382 patients with active chronic leg ulcers suffered primarily from a venous disease (8). When diagnosing venous insufficiency it is almost always enough to study the skin searching for specific signs i.e. oedema, hyperkeratosis, hyperpigmentation, lipodermatosclerosis and atrophie blanche (19). In general, a long duration of venous hypertension together with insufficiency causes venous ulcers. These in turn are effects due to reflux because of incompetent valves which can be due to previous venous thrombosis (19).

In a population study made by Fowkes *et al.* no obvious risk factors for venous reflux according to life style was found. However, they found that some factors might play a role. For women, these factors were previous pregnancy, lower intake of oral contraceptives, overweight and amount of movement at work. For men body height was a factor (20).

An important aspect of venous disease is the effect on quality of life for each patient. This condition is both time consuming due to frequent consultations and nursing but also regarding the number of days away from work and in some cases even unemployment (21).

Regarding venous ulcers and adverse effects pain is one of the most frequently reported (22) and in an integrative review including 22 studies, Gonzalez-Consuegra *et al.* found that the most common factor affecting health-related quality of life was pain (23). Furthermore, ulcers causing severe pain are known to not heal (24). Besides great impact on the individual, these ulcers also have economic aspects. In developed countries, chronic leg ulceration accounts for approximately 1 % of the total cost for health care (25).

There are more women than men suffering from venous stasis ulcers and the incidence, regardless sex, increases with age (26). The different types of leg ulcers have, in most cases, characteristic localizations. Above the malleoli, in the ankle area, venous leg ulcers usually occur (27). When studying a venous ulcer it has characteristic borders with irregular shape and is badly defined. Furthermore, the bed of the wound is usually shallow and they mostly have a larger area than other types of chronic wounds, sometimes localized to the extremity circumferentially (28).

Arterial aethiology

About 25 % of leg ulcer patients suffer from arterial disease. Callam *et al.* showed similar data in a study were 21 % out of 600 patients with leg ulceration had an arterial disease (29).

The cause of arterial leg ulcers is arterial occlusion leading to local hypoxia. The most common pathology behind this occlusion of leg arteries is peripheral arterial occlusive disease (PAOD) (30). Moreover, arterial ulceration can be caused by diabetes, vasculitis, thalassemia, pyoderma gangrenosum and sickle cell disease. Risk factors regarding peripheral vascular

disease that can be modified include hypertension, hyperlipidemia, smoking, diabetes, obesity and lack of exercise. Some patients also have cardiovascular disease in their medical history such as angina pectoris, myocardial infarction, stroke and/or intermittent claudication (31).

Arterial ulcerations are often localized to the toes, heels and other protruding bony parts of the foot. Usually they have a round shape together with a distinct border and a base looking pale without granulation and in most cases necrosis occur. Oftentimes the ulcer is extremely painful, even at rest (31). During clinical examination cold feet, weak pulses when palpating dorsalis pedis arteries and extended capillary refill time in the toes indicate arterial insufficiency. Furthermore, examination of distal pulses with ultrasound (Doppler), arterial brachial index (ABPI) and toe brachial index (TBI) can verify a reduced arterial blood supply in the extremity (28). ABPI can be used as a measurement of the severity of the disease. Normally, ABPI is around 1.0 or slightly above. ABPI between 0.7-1.0 indicates mild arterial disease, an index between 0.5-0.7 mild to moderate and 0.3-0.5 is severe. An index of 0.3 or less does not only implicate severe arterial disease but also there is a risk of losing the extremity (31).

Mixed aethiology

Approximately 15 % of all leg ulcers constitute of combined venous and arterial disease, also known as mixed leg ulcers (8). A majority of these ulcers have a primary venous aethiology meanwhile the arterial occlusions makes the healing complicated (30). Callam *et al.* found that 176 out of 827 ulcerated legs had an arterial component. Of these 176 legs, 52 % had characteristic signs of chronic venous insufficiency and/or varicose veins (29).

Diabetes aethiology

In a retrospective cohort study made by Ramsey *et al.* the yearly incidence of foot ulcers was 2 % in a group of almost 9000 patients with diabetes mellitus type 1 or 2. Furthermore, 15.6 % of the patients developing a foot ulcer needed amputation (32). Diabetic ulcers are most common over pressure points and the distal metatarsal joints are particularly exposed (33). Boyko *et al.* found several factors associated with diabetic foot ulceration such as some deformities of the foot, massive body weight, decreased perfusion of the foot and lowered skin oxygenation and also neuropathy affecting both autonomic and sensory neurons (34). Numerous factors are known to influence diabetes wound healing, making it delayed. These factors include inadequate patient compliance regarding treatment, insufficient local oxygenation and deficient blood sugar control (35). Diabetic foot ulcers also have an economic aspect, due to extended and labour-consuming health care. Hospitalization is frequently required for patients with diabetes and it has been noted that these visits consume 59% more time for those diabetic patients having an ulcer than those without (36).

Uncommon aethiologies

There are also more uncommon aethiologies of hard to heal ulcers such as vasculitis and pyoderma gangrenosum. Although vasculitis can be described as a heterogenous group of conditions they have one thing in common; vessel damage due to inflammation. Based on clinical findings, type of infiltrate or size of the vessels a number of different subdivisions are possible (37). Pyoderma gangrenosum is an ulcerating dermatosis causing ulcers that are deep and necrotic together with an elevated, violet border. Since the cause of pyoderma gangrenosum is not known, clinical findings are helpful to diagnose this condition. Different kinds of immunomodulatory drugs are the only alternative when treating this process and without treatment the ulceration proceeds (27).

Treatment of hard to heal ulcers of the foot and leg

Pinch-grafting is an alternative when treating chronic foot and leg ulcers, a method that was first described by a surgeon named Reverdin in 1869. When harvesting the pinch-grafts, during local anesthesia, a scalpel and nipper are used for excision meanwhile the skin is elevated (38). In practice it is rather easy to perform and can easily be done in the primary care (39). Furthermore, it has been shown that the cost for this procedure is 3.3-5.9 times lower in the primary care compared with in hospital care (40). It is not clear how skin grafts are able to enhance healing but it is suggested that the grafts both replace old tissue and also provides with essential components stimulating healing (41). In addition to pinch-grafting there is also punch-grafting where a biopsy punch instead of a nipper is used. However the excision still requires a scalpel. Local anesthesia is enough, allowing this procedure in both primary and hospital care (42).

The preparation of the bed of the wound plays an important role when it comes to the survival of the graft. The bed needs to have sufficient vascularization for the survival of the skin graft. Achieving this requires total loss of foreign material, devitalized tissue and biofilms. Therefore it is beneficial with a radical debridement before skin grafting (43). There are different methods regarding debridement such as enzymatic, autolytic, surgical or mechanical. When it comes to autolytic debridement interactive dressings are used, for example hydrocolloids, hydrogels and alginates (44).

Several reports have described enhanced healing when pinch-grafting has been done (39, 45-47). A report from Öien *et al.* showed healed ulcers in 33 % of the cases after 12 weeks (45). Furthermore, Christiansen *et al.* had similar results with 22 % healed at 8 weeks follow-up (47). Nevertheless, there are just a few randomized controlled trials investigating autologous

skin graft transplantation compared to other forms of treatment regarding hard to heal ulcers. One of these made by Jakunas *et al.* (48) comparing skin grafting with standard of care when treating chronic venous ulcers. The results showed that within 6 months 67.5 % of the ulcers being grafted were healed. At the same time none of the ulcers in the standard of care group had healed. In another study made by Warburg *et al.* skin grafting, using meshed split skin, was compared with standard of care in venous ulcer patients that had undergone vascular surgery. However, they did not find any correlation with enhanced healing (49). These two studies were included in a Cochrane review made by Jones *et al.* investigating skin grafting as a treatment for venous leg ulcers. In summary, neither of these studies had enough evidence to prioritize autologous skin graft before standard of care. Due to this result, more research is required for evaluation of the effects of skin grafting on ulcer healing (50).

Punch-grafting has been used since the mid 1990's at the Department of Dermatology at Sahlgrenska University Hospital. This method has primarily been used for ulcers where conservative treatment using wound dressing has been unsuccessful. During these years it has been practiced in both inpatient and outpatient care with the aim to promote wound healing and also decrease pain associated with the wound. Based on experience superficial wounds without extensive oozing together with a bed of granulation tissue are considered most suitable for punch-grafting. A clean wound without infection is necessary before punch-grafting to ensure efficiency regarding healing. In practice, a number of viable biopsies consisting of autologous skin tissue are extracted with the assistance of a 4-millimeter biopsy punch. The donor site, usually the thigh, is locally anesthetized using lidocaine with epinephrine. After harvesting the grafts they are placed with at least an interspace of 4 millimeters in the ulcer. Thereafter, paraffin gauze compresses (Jelonet, Smith&Nephew, Sweden) dresses the ulcer making sure the grafts are in direct contact with the bed of the

ulcer. On top of this dressing, another dressing (Aquacel, ConvaTec Wound Therapeutics, Sweden) is applied. Finally, if the arterial circulation allows it and the patient is fine with it, a compression bandage is used. Today it is more common using negative pressure wound therapy after punch-grafting due to the initial restrictions following the procedure together with the difficulty to optimize graft conditions for patients with self-care at home. At first it is important for the patient to stay as immobilized as possible and also keeping the leg elevated. After 3 days the inner dressing is changed for the first time. However, the outer dressing can be changed on a daily basis or even more often if needed. Furthermore, the wound is ventilated without the dressing that is on top for 20-30 minutes every day. This is how the nurses at the Department of Dermatology at Sahlgrenska University hospital have been performing the post punch treatment for over ten years (51).

Dressings used for patients treated with standard of care instead of punch-grafting are in principle the same as the ones that are used after punch-graft. Also the basic strategy regarding the dressings is basically the same. However, different nurses and physicians might have individual prioritization. One thing that might differ between the treatment options is the method of debridement. Ulcers not being punched can be debrided with a pharmaceutical substance reminding of gel, also known as Cadomerejod (Iodosorb, Smith&Nephew Sweden). Ulcers containing punch-grafts can only be debrided by sharp debridement since addition of more fluid to the ulcer bed might result in the grafts not taking. When performing this sharp debridement with curette it is very important to be careful using a small instrument minimizing the risk of graft disruption. For the same reason local anesthetics, e.g. Xylocain, are carefully used. Therefore the debridement might be more painful for the punch-grafted patients sometimes resulting in a less thorough debridement (51).

Although punch-grafting has been used for nearly 20 years at the Department of Dermatology at Sahlgrenska University Hospital no guidelines have been defined for the method. Instead each physician has decided when to practice this method. Furthermore, no analysis has been made evaluating the outcome of this procedure except for one study made by Nordström and Hansson in 2008 (52) where 22 patients with chronic leg and foot ulcers were included and treated with punch-grafting resulting in 50 % ulcers healing rate at a mean of 2.5 months. These results were consistent with results from studies on pinch-grafting (47, 53, 54).

Aim

The aim of the current study was to evaluate the clinical outcome of punch-grafting as a treatment for hard to heal leg and foot ulcers.

Material and Methods

Study design

The design of this study was a single center retrospective case series study for investigation of the frequency of complete wound closures within 3 and 12 months after punch-grafting. The data on case subjects were acquired by a manual retrospective data collection from medical records at Sahlgrenska University Hospital, primary care facilities and also home care services or other forms of health care providers in charge of follow-up. Patients included in the case-group were all of them who had been treated with punch-graft for one or several ulcers located to the leg or foot at the Department of Dermatology at Sahlgrenska University Hospital during the period between January 2004 and September 2013. For assessment of a 12-month follow-up period copies of the included patients charts were requested from the respective primary care unit, homecare service or other health care providers. Thereafter collection of study data was made during a manually process.

Study population

Through an automated search of electronic patient charts at the Department of Dermatology at Sahlgrenska University Hospital on potential treatment-codes regarding punch-graft treatment and diagnosis codes for potential ulcers (Table 1), possible case-subjects were identified.

Thereafter these electronic charts of potential case-subjects were analyzed during a manual process selecting the case-group with the exclusion and inclusion criteria in mind. Subjects included in this case group had clinically been diagnosed with chronic ulcers located to the leg or foot and had also been treated with punch-graft for at least one leg or foot ulcer at the Department of Dermatology between January 2004 and September 2013. Patients receiving this treatment more than once were only included regarding their first treatment. For

exclusion criteria see Table 2. Altogether 213 case-subjects were included with a total of 284 punch-grafted ulcers.

Table 1. Main diagnosis codes describing ulcers potentially includable in the study

Diagnosis code	
L979	Ulcer of lower limb, not elsewhere classified
I702C	Atherosclerosis of arteries of extremities with ulceration
I830	Varicose veins of lower extremities with ulceration
I832	Varicose veins of lower extremities with both ulcer and inflammation
E10.6D	Type 1 diabetes mellitus with (diabetic) foot ulcer
E11.6D	Type 2 diabetes mellitus with (diabetic) foot ulcer

Table 2. Exclusion criteria

		Subjects before exclusion n=244
		N (%)
<i>Excluded patients</i>		31 (12.7%)
<i>Exclusion criteria</i>		
	<i>Death within 3 months from baseline</i>	4 (12.9%)
	<i>Follow-up data at 3 months not available</i>	2 (6.5%)
	<i>Vascular surgery within 3 months from baseline</i>	3 (9.7%)
	<i>Ulcer duration < 2 months</i>	8 (25.8%)
	<i>Unknown aetiology</i>	10 (32.3%)
	<i>Ulcer location elsewhere</i>	2 (6.5%)
	<i>Missing baseline data</i>	1 (3.2%)
	<i>Other</i>	1 (3.2%)

Data collection

Based on the diagnosis code or notes made by the physician in the medical records an aetiological diagnosis was found for all the study subjects. A majority of the patients had been examined with a hand held Doppler resulting in an ankle blood pressure. Furthermore, some of the patients visited the Department of Clinical Physiology for extended examination regarding the arterial and venous circulation determining toe pressure and venous reflux respectively. In those cases where vasculitis might be the cause biopsies of the skin were sent for further examination if the physician believed the donor site would be able to heal adequate.

In this study the classifications venous, arterial, pressure, diabetic, mixed or “other” ulcers were used. Aetiologies of more uncommon kinds ended up in the group called “other” ulcers to simplify the data managing process and consisted of vasculitic ulcers, ulcers caused by pyoderma gangrenosum, traumatic ulcers etc. Further information such as sex, year of birth, location and duration of the current ulcer were gathered from the medical records. Baseline was set as the date of the first punch-graft transplantation.

Patients with more than one ulcer treated with punch-graft had each wound registered as a separate case. A number of ulcers were grafted more than once; however calculation of time to wound closure was based on the date for the first punch-graft treatment since this was set as baseline.

Evaluation of healing frequency was made after 3 and 12 months from baseline. Wounds not healed within this period did not have further follow-up.

All ulcers that healed within 12 months had time to wound closure calculated. Regarding wound closure a single measurement could not be used for all the patients. Instead the medical records were used as guideline looking for ulcer descriptions or notes from physicians or nurses regarding wound healing. Description of re-epithelialization of the ulcer, no need for further treatment with dressings or notes saying the ulcer had healed was defined as complete wound closure.

Data regarding history of diabetes, cardiovascular disease, vascular surgery, deep venous thrombosis, systolic blood pressure, ankle- and toe-pressure, body weight and height, levels of fasting blood glucose and HbA1c was obtained from the patient charts. Moreover the frequency of hospitalizations and also outpatient visits at the Department of Dermatology

regarding the punch-grafted ulcer within a period of 12 months from baseline was noted together with any arterial or venous surgery within 3-12 months after baseline. The quantity of antibiotic prescriptions within the first 3 months, initiation of or ongoing treatment with antibiotics at baseline and type/dosage of analgesic medication at baseline and 3 months ahead was also noted. Finally, the number of repeated punch-graft treatments for the same ulcer within 12 months was obtained.

Statistical methods

Fisher's exact test was used to compare proportions between groups. Wilcoxon's rank sum test was used for two-sample comparisons between groups. A Cox' proportional hazards model was used with the wounds "time to healed" as the dependent variable. If the time to healing was more than 365 days, the time was treated as censored at 365 days. Ulcer duration before treatment, type of wound, patient age and localization was used as predictors. Also a Cox' proportional hazard model was used for each subgroup defined by wound type with the same dependent variable and predictors as above (excluding wound type) and with year of treatment (categorized as 2004-2008 or 2009-2013) added as a predictor. All tests were two-sided. Furthermore a p-value <0.05 was considered as statistically significant.

Ethics

Prior to initiating this study a research protocol including description of the design and execution of the project was committed and send to the regional ethical review board for consideration. Their evaluation was that no approval of the board was necessary based on the fact that the study is a retrospective quality evaluation of a treatment method given by the Department of Dermatology at Sahlgrenska University Hospital. Furthermore Helena Gustafson, The Head of the Department of Dermatology, gave permission to collect data from the patient records.

Since only the investigators together with authorized personnel being a part of the study had the possibility to access original data files, the confidentiality regarding study materials were protected. All patients were assigned with a number impossible to connect to the individual and the obtained data was anonymously processed with no connections between patients and analyzed data.

Incoming data from other units than the Department of Dermatology sometimes contained information regarding other conditions than the wound. Due to ethical and patient related considerations only information essential for the study were obtained. It was taken into consideration the possibility of insulting patient privacy by overcoming irrelevant fact when carrying out the study. However, the positive effects with an evaluation of a common method of treatment were considered to outweigh this risk. The original copies consisting of study material will be safely housed according to every institutional research requirement, law and regulation.

The WMA Declaration of Helsinki states that all research including human participants requires informed consent. Though, §32 declare “there may be exceptional situations where consent would be impossible or impracticable to obtain for such research” (55). These words can, according to the investigators, be applied on this current study. This because of the great amount of patients included in this study whose medical records require a manually analysis for finding and including suitable subjects. Several of these candidates had already passed away making an informed consent impossible. In excess of this decision regarding the Helsinki declaration the “Lagen om etikprovning och forskning som avser människor” state in §20 and §21 that research is allowed without informed consent in some conditions, which corresponds well with this current study.

Results

The data collection resulted in 213 patients included in the case group with a total of 284 ulcers treated with punch-graft. The gender distribution was around 60 % women (129 of 213) and around 40 % (84 of 213) men. The mean age was 73.2 (\pm 13.6) years and the number of patients with an age of 65 or older was almost 80 % (169 of 213) (Table 3).

Table 3. Characteristics of the patients treated with punch-graft

		Case -subjects n=213 N (%) or Mean \pm SD
<i>Sex</i>		
	<i>Female</i>	129 (60.6%)
	<i>Male</i>	84 (39.4%)
<i>Age (years)</i>		
	<i>Mean</i>	73.2 \pm 13.6
	<i>Range</i>	23-96
	≥ 65	169 (79.3 %)

17.8 % of the patients (38 of 213) had diabetes, either type I or II and 15.0 % (32 of 213) had a history of deep venous thrombosis in the punch-grafted extremity. Furthermore, almost 30 % of the patients had previously undergone either arterial, venous or both types of surgery in the affected limb (Table 4). Data regarding cardiovascular comorbidity is also presented in Table 4. In summary almost 60 % (124 of 213) of all cases had some kind of cardiovascular disease, hypertension being the most common (47.9 %) followed by myocardial infarction (16.0 %).

Table 4. Comorbidity of the 213 case-subjects treated with punch-graft

		Case-subjects n=213
		N (%)
<i>Cardiovascular disease</i>		124 (58.2 %)
	<i>Myocardial infarction (MI)</i>	34 (16.0%)
	<i>Stroke</i>	23 (10.8%)
	<i>Angina</i>	23 (10.9%)
	<i>Hypertension</i>	102 (47.9%)
<i>Diabetes</i>		38 (17.8%)
	<i>Type I</i>	6 (2.8%)
	<i>Type II</i>	32 (15.0%)
<i>Previous Deep Venous Thrombosis (DVT)</i>		32 (15.0%)
<i>Previous Vascular surgery</i>		62 (29.1%)
	<i>Arterial</i>	28 (13.2%)
	<i>Venous</i>	40 (18.8%)

Table 5 presents all data collected at baseline for all of the patients included in this study. For example the mean number of outpatient visits was 6.5 (± 9.7) during the first year from baseline meaning the frequency of visits, either to a physician or nurse, at the Department of Dermatology due to the specific ulcer. Moreover the mean number of hospitalizations because of the punch-grafted ulcers was 1.7 (± 1.5) times during a period of 12 months from baseline. When calculating the mean number of analgesics at baseline each patient had approximately 5 pills consisting of paracetamol/NSAID, non-narcotic prescriptions, narcotic prescriptions, transdermal analgesic patches or combinations of these. 14 patients (6.6 %) had vascular surgery (arterial or venous) within 3-12 months after baseline.

Table 5. Data at baseline for the 213 case-subjects

	Case-subjects n=213
	N (%) or Mean \pmSD
<i>Age</i>	73.2 \pm 13.6
<i>Systolic blood pressure (mmHg)</i>	139.8 \pm 19.1
<i>Ankle pressure (mmHg)</i>	134.0 \pm 40.9
<i>ABPI (Ankle Brachial Pressure Index)</i>	0.9 \pm 0.3
<i>Length (cm)</i>	169.8 \pm 9.6
<i>Weight (kg)</i>	77.6 \pm 24.5
<i>BMI (Body Mass Index)</i>	27.2 \pm 7.2
<i>Fasting blood sugar (mmol/L)</i>	5.8 \pm 1.5
<i>HbA1c (mmol/mol)</i>	53.1 \pm 15.3
<i>Toe pressure (mmHg)</i>	73.9 \pm 33.6
<i>Number of outpatient visits</i>	6.5 \pm 9.7
<i>Number of hospitalizations</i>	1.7 \pm 1.5
<i>Antibiotic prescription within 3 months</i>	0.7 \pm 0.9
<i>Number of prescriptions</i>	
<i>1</i>	59 (27.7%)
<i>2</i>	24 (11.3%)
<i>3</i>	11 (5.2%)
<i>4</i>	3 (1.4%)
<i>On-going antibiotic treatment at baseline</i>	100 (46.9%)
<i>Initiation of antibiotic treatment at baseline</i>	3 (1.4%)
<i>Baseline frequency of analgesics</i>	5.1 \pm 4.6
<i>Type of analgesics</i>	
<i>Paracetamol</i>	125 (59.2%)
<i>Prescription non-narcotic</i>	24 (11.3%)
<i>Narcotic prescriptions</i>	84 (39.6%)
<i>Transdermal analgesic patches</i>	9 (4.2%)
<i>3 month frequency of analgesics</i>	6.0 \pm 4.7
<i>Type of analgesics</i>	
<i>Paracetamol</i>	20 (71.4%)
<i>Prescription non-narcotic</i>	4 (4.0%)
<i>Narcotic prescriptions</i>	21 (21.2%)
<i>Transdermal analgesic patches</i>	0
<i>Vascular surgery within 3-12 months</i>	14 (6.6%)
<i>Arterial surgery</i>	10 (4.7%)
<i>Venous surgery</i>	4 (1.9%)

About 10 % (31 of 284) of the ulcers were located to the foot and 90 % (253 of 284) to the leg. The distribution of ulcer type is presented in Table 6 and reveal a venous aethiology in almost 45 % (124 of 284) of the ulcers followed by approximately 25 % (68 of 284) mixed ulcers, about 20 % (56 of 284) ulcers with an "other" classification, 10 % (32 of 284) arterial ulcers, 1 % (4 of 284) diabetic ulcers but no pressure ulcers. The mean duration of all ulcers at baseline was 24.9 (± 33.8) months and the mean value for each ulcer is presented in Table 6, venous ulcers having the longest duration before punch-graft. Overall, almost 70 % (193 of 284) of the ulcers, regardless of aethiology, had existed for 6 months or more.

Table 6. Characteristics for the 284 ulcers treated with punch-graft

	Specific ulcers n=284
	N (%) or Mean \pmSD
<i>Ulcers of the foot</i>	31 (10.9%)
<i>Ulcers of the leg</i>	253 (89.1%)
<i>Venous ulcers</i>	124 (43.7%)
<i>Arterial ulcers</i>	32 (11.3%)
<i>Diabetic ulcers</i>	4 (1.4%)
<i>Pressure ulcers</i>	0
<i>Mixed ulcers</i>	68 (23.9%)
<i>Other ulcers</i>	56 (19.7%)
<i>Mean duration of ulcer presence at baseline (months)</i>	24.9 \pm 33.8
	<i>Venous ulcers</i>
	30.9 \pm 37.9
	<i>Arterial ulcers</i>
	20.6 \pm 23.2
	<i>Diabetic ulcers</i>
	21.0 \pm 17.2
	<i>Mixed ulcers</i>
	22.2 \pm 23.5
	<i>Other ulcers</i>
	18.7 \pm 39.6
<i>Duration of ulcer \geq6 months</i>	193 (68.0%)

When analysing data regarding healing rate at 3 and 12 months after the punch-graft, data was found for 97.9 % (278 of 284) and 87.3 % (249 of 284) of the subjects. When investigating the outcome at 3 months after baseline almost 20 % (52 of 284) of all ulcers were healed and at 12 months almost 50 % (130 of 284) had healed. The mean time to healing was 136.2 (± 89.6) days regardless aethiology. Numbers for each ulcer type are presented in Table 7. For more details regarding patient age and number of healed ulcers within 3 months, see Table 8 and 9.

When applying a Cox proportional hazards survival model there was no significant correlation between: ulcer duration before punch-graft and time to healing ($p=0.285$), age of the patient and time to healing ($p=0.478$) or location of the ulcer and time to healing ($p=0.4776$). The same statistical method was also used to investigate if there was any difference in time to healing between all the various types of ulcers. A significant result was only found for the ulcers classified as arterial ($p=0.0486$) and “other” ($p=0.0005$).

With arterial ulcers having a mean time to healing of 151.4 ± 85.4 days (95 % Confidence interval 107.5-195.3) they had the second longest time to healing and a higher mean value than the group of all ulcers regardless type (136.2 ± 89.6 days). The group with ”other” ulcers had a mean time to healing of 93.8 ± 78.9 days (95 % Confidence interval 65.8-121.8) meaning they had the shortest time to healing of all ulcer types and also a faster healing rate than the group consisting of all ulcers.

Table 7. Ulcer healing with aetiological classification of the 284 ulcers

		Specific ulcers n=284 N (%) or Mean \pm SD
<i>Healing rate at 3 months</i>		52 (18.7%)
	<i>Venous ulcers</i>	20 (16.3%)
	<i>Arterial ulcers</i>	4 (13.8%)
	<i>Diabetic ulcers</i>	1 (25.0%)
	<i>Mixed ulcers</i>	5 (7.4%)
	<i>Other ulcers</i>	22 (40.7%)
<i>Healing rate at 12 months</i>		130 (52.2%)
	<i>Venous ulcers</i>	56 (49.1%)
	<i>Arterial ulcers</i>	17 (73.9%)
	<i>Diabetic ulcers</i>	2 (50.0%)
	<i>Mixed ulcers</i>	22 (36.1%)
	<i>Other ulcers</i>	33 (70.2%)
<i>Mean time to healing (days)</i>		136.2 \pm 89.6
	<i>Venous ulcers</i>	144.4 \pm 92.6
	<i>Arterial ulcers</i>	151.4 \pm 85.4
	<i>Diabetic ulcers</i>	116.5 \pm 85.6
	<i>Mixed ulcers</i>	169.3 \pm 84.1
	<i>Other ulcers</i>	93.8 \pm 78.9

Table 8. Ulcer healing with age of the patients

Age	Ulcers treated with punch-graft	Healed ulcers at 3 months, N (%)
20-29	2	1 (50.0%)
30-39	8	2 (25.0%)
40-49	9	1 (11.1%)
50-59	17	7 (41.2%)
60-69	50	14 (28.0%)
70-79	86	15 (17.4%)
80-89	90	11 (12.2%)
90-99	16	1 (6.3%)

Table 9. Ulcer healing with age of the patients

Age (years)	Ulcers treated with punch-graft	Healed ulcers at 3 months, N (%)
<60	36	11 (30.6%)
≥60	242	41 (16.9%)
<70	86	25 (29.1%)
≥70	192	27 (14.1%)

Complications within 3 months from baseline were found in 59 patients whereof 9 patients had 2 complications during this period of time. In Table 10 the different types of complications are listed, the most common was infection involving the specific ulcer. Some patients underwent punch-graft more than once for the same ulcer during 12 months after baseline, also presented in Table 10.

Table 10. Complications and adverse events for the 284 ulcers

	N (%)
<i>Patients with complications within 3 months</i>	59 (27.7%)
<i>Type of complication</i>	
<i>Infection</i>	55 (80.9%)
<i>Hard to heal ulcer at donor site</i>	2 (2.9%)
<i>Infection at donor site</i>	3 (4.4%)
<i>Other</i>	8 (11.8%)
<i>Ulcers with repeated punch-graft treatment within 1 year</i>	81 (28.5%)
<i>Treatment repeated once</i>	62 (76.5%)
<i>Treatment repeated twice</i>	14 (17.3%)
<i>Treatment repeated thrice</i>	5 (6.2%)

Discussion

When evaluating the clinical outcome of the punch-graft treatment given by the Department of Dermatology at Sahlgrenska University Hospital between January 2004 and September 2013 by means of this study the results show a healing rate of almost 20 % at 3 months and 50 % at 12 months. This was slightly less than the results made by Öien *et al.* having a healing rate of 40 % 3 months after pinch-graft (45). The mean time to healing for all ulcers was 136.2 (± 89.6) days with "other" ulcers having the shortest mean time (about 94 days) meanwhile mixed ulcers had the longest time (about 169 days). In the study made by Nordström *et al.* 50 % of the ulcers were healed in a mean time of 76 days (52). An explanation to this high rate could be the low number of patients, with only 22 patients included in the study. Taking a closer look to the group of diabetic ulcers they also had a high healing rate (50% at 12 months). However, the number of ulcers must be taken into consideration and this group consisted of only 4 out of 284 ulcers, therefore these results cannot be considered trustworthy.

The distribution of ulcer localisation was that leg ulcers being nine times more common than the foot ulcers. Venous ulcers dominated and made up for about 40 % of all ulcers followed by the mixed ulcers (almost 25 %). Arterial aethiology was not that common, contributing to about 11 % of the ulcers. When comparing these results it corresponds with other studies showing that venous aethiology is the most common (8, 17). One possible explanation for mixed ulcers being this common in our study could be the insecurity when the physician is diagnosing the patient. Since many ulcers do not have typical signs of strictly venous or arterial disease but instead a mix of both it might be impossible deciding which aethiology is the primary cause and the diagnose mixed ulcer is used. Another explanation could be that

this type of ulcer is harder to heal compared with other aetiologies and therefore, punch-graft is more often used as a treatment for mixed ulcers.

The unequal sex distribution with about 60 % women and 40 % men indicate that more women than men are treated with punch-graft. An explanation to this could be that the total population suffering from leg ulcers constitutes of more women than men, as showed in previous studies (26). This in turn allows us to believe that it has not been any selection regarding sex to the punch-graft treatment. Instead it is an average of the total leg ulcer population and this correspond with the conclusion that no directives are used when deciding who shall receive the punch-graft treatment. Furthermore the age distribution with a mean age of 73.2 (± 13.6) and approximately 80 % of the punch-grafted population being 65 years or older indicates that punch-graft is more common in the elderly population which correspond with other studies showing that leg ulcers are most frequent in older people (7). For this study it means that probably no selection has been done regarding age and punch-graft treatment, instead it is a mean of the total leg ulcer population contributing to the conclusion that there is a lack of directives when determine about punch-graft treatment in every single patient case.

When investigating the mean duration of all ulcers at baseline the time was about 25 months and as many as almost 70 % of the ulcers had existed for more than 6 months. Further investigation of each ulcer type showed that the venous ulcers had the longest mean duration with almost 30 months until they were punch-grafted. The remaining ulcer types had almost the same mean value with about 20 months until they were punched. Taking this into consideration it is possible that ulcers chosen for the punch-graft treatment are the ones which have failed to heal during a long time and were most of other options such as conservative

treatment have shown unsatisfactory results. This could mean that the population treated with punch-graft include the most slow-healing ulcers with a poor healing potential from the start affecting the punch-graft result. With this in mind we made a statistical analysis investigating if there was any correlation between the ulcer duration before the punch-graft treatment and the time to healing. However the result was not significant and no conclusions could be made. Therefore it might be of interest investigating this relationship, if there is any, further.

Methodological considerations

One of the findings in this study was the shortage of patient information in some medical records making the quantity and quality of the data for each patient a lot varied. Taking this into consideration there is a possibility that data regarding healing rate might be inaccurate in some subjects reducing the strength of this study's result. As an example for the sometimes parsimonious information in the records the data regarding healing rates at 12 months from baseline was found for 87.7 % (249 out of 284) ulcers. One way to avoid this problem could be an implementation of directives regarding follow-up for these patients making this work a lot easier for all involved in this type of health care.

Almost 30 % of the patients had some kind of complication during the first 3 months. However this number is not completely representative since almost all of this data was obtained from medical records from the primary care or other health care providers, not including Sahlgrenska University Hospital, and many of these records did not arrive to the investigator in time. It is hard suggesting a solution to this problem since it is a retrospective study depending on the help and participation of other care units providing with their medical

records. The ultimate system from a research perspective would be a medical record system available for all medical units where access to each valid patient was possible.

Ulcers that was punch-grafted more than once within one year from baseline was about 30 %. Remarkably there was no guidelines or criteria to follow when deciding if a new punch-graft should be made. In fact, there were no guidelines found for punch-graft at all. Instead it seemed like it was up to each physician to decide if and when the treatment should be given. This might need to be taken into consideration in the future, making the given health care more equal and easier to evaluate.

This study has some weaknesses. Since it is a retrospective case-series study no control-subjects are included meaning there is nothing to make comparisons with. Therefore the conclusions that can be made are limited to a defined population, in this case the punch-grafted population at the Department of Dermatology at Sahlgrenska during a certain period of time. It would be of greater value having a control-group with another treatment method for comparison of the outcome and value the effectiveness of punch-graft. The data was manually collected meaning there is risks of the human error were data could be missed etc. Furthermore data based only on medical charts depends on quality of the content and this has varied greatly with some charts including almost all the data and some very few. Another weakness is the dependence of charts being sent from the primary care and other health care providers, which in this case has not been complete.

A possible strength is the number of persons collecting the data. Only 2 persons have been involved in this process minimizing the risk of different methods regarding data collection. Furthermore there is only one care unit, the Department of Dermatology at Sahlgrenska University Hospital that has performed the punch-graft in all these patients hopefully using almost the same method. However this can also mean a selection of patients since many of the chronic wound ulcer patients are treated in the primary care and therefore not included in a study like this. Instead only the more complicated cases are treated at Sahlgrenska University Hospital resulting in ulcers that might be harder to heal from the beginning, which might result in a lower healing frequency regardless treatment method.

This study does not answer for a complete evaluation of the punch-graft method and therefore further research is required. However this study could serve as inspiration for future research. The optimal evaluation would be a randomized controlled trial consisting of punch-grafted patients in a case-group and patients treated with standard of care or pinch-graft in the control-group.

Conclusions and Implications

In this study about 20 % of all included ulcers healed within 3 months and 50 % within one year after the punch-graft treatment, numbers that are somewhat lower than those in previous studies made on pinch-and punch-graft. Furthermore, there was no statistically significant evidence showing any correlation between the time of ulcer duration before punch-graft and the time to healing. However, ulcers categorized as "others" had the shortest time to healing, a statistically significant result meaning that these might be the most suitable ulcers for treatment with punch-graft. Since this was a case-series study meaning no control-subjects were participating it is hard making any conclusions regarding these results. Instead this study can serve as groundwork for upcoming research when determining the future of this treatment method for hard to heal ulcers.

Populärvetenskaplig sammanfattning

Bensår av kronisk karaktär är något som framförallt drabbar den äldre befolkningen och som ger upphov till både fysiskt och psykiskt lidande, framförallt är smärta ett stort problem. Med tanke på den ökande medellivslängden och det ökande antalet diabetiker finns en risk att även antalet bensår ökar framöver. Därför är det av stor vikt att fokusera på behandlingsmetoder för detta tillstånd för att kunna undvika onödigt lidande och stoppa utvecklingsprocessen.

På Hudkliniken, Sahlgrenska, har man sedan mitten av 90-talet använt sig av en behandlingsmetod för kroniska bensår kallad punchgraft. Denna metod går ut på att man flyttar små hudbitar, ca 4mm i diameter, från patientens hud på låret till det aktuella såret. Trots denna långvariga användningsperiod på hudkliniken har man inte gjort någon ordentlig utredning av behandlingsmetoden och därför kan man inte säga om det verkligen fungerar eller inte i jämförelse med andra behandlingsalternativ (såromläggning mm.). Därför genomfördes denna studie där fokus var att undersöka hur många sår som hade läkt 3 respektive 12 månader efter själva punchgraft-behandlingen. Man undersökte även hur länge såret hade funnits innan det behandlades samt ifall det fanns något samband mellan hur lång tid såret hade funnits innan behandlingen och tiden det tog för såret att läka.

Genom att gå igenom journaler för de patienter som fått denna behandling under januari 2004 till och med september 2013 kunde ovanstående frågor besvaras. Sammanfattningsvis hade ca 20 % av alla sår läkt efter 3 månader, ca 50 % efter 12 månader och såren hade i genomsnitt funnits i ca 25 månader innan behandlingen. 25 månader är en lång tid och man skulle kunna tolka det som att såren man behandlar med punchgraft är de sår som under en lång period har behandlats med andra alternativ utan ett tillfredsställande resultat. Dessa sår skulle alltså kunna vara svårare att behandla redan från början. Vid undersökning av ett eventuellt

samband mellan sårets duration och läkningstiden så kunde inget sådant samband statistiskt säkerställas.

Med dessa resultat i handen så har man inte blivit särskilt mycket klokare över behandlingen. Istället indikerar denna studie att vidare forskning krävs där man använder sig av ett annat tillvägagångssätt för att skaffa sig en tydlig bild av behandlingens effekt och om den har bättre resultat i jämförelse med andra behandlingsmetoder. Dock skulle denna studie kunna användas som underlag till kommande forskning. Under studiens gång insåg man även att riktlinjer för denna behandlingsmetod inte fanns tydligt dokumenterat någonstans samtidigt som en stor andel av de anteckningar som var gjorda i patientjournalerna var bristfälliga. En lärdom av detta skulle därför vara att nya riktlinjer för metoden krävs för ett konsekvent användande av denna tillsammans med förbättrad dokumentation av sjukdomsförlopp osv. för att framförallt underlätta för personalen men på sikt även se ett bättre patientomhändertagande.

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