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ARTICLE

Intermittent mild negative pressure applied to the lower limb in patients with spinal cord injury and chronic lower limb ulcers: a crossover pilot study

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Abstract

Study design Randomized, assessor-blinded crossover pilot study.

Objectives To explore the use of an intermittent negative pressure (INP) device for home use in addition to standard wound care (SWC) for patients with spinal cord injury (SCI) and chronic leg and foot ulcers before conducting a superiority trial.

Setting Patient homes and outpatient clinic.

Methods A 16-week crossover trial on 9 SCI patients (median age: 57 years, interquartile range [IQR] 52–66), with leg ulcers for 52 of weeks (IQR: 12–82) duration. At baseline, patients were allocated to treatment with INP + SWC or SWC alone. After 8 weeks, the ulcers were evaluated. To assess protocol adherence, the patients were then crossed over to the other group and were evaluated again after another 8 weeks. Lower limb INP treatment consisted of an airtight pressure chamber connected to an INP generator (alternating 10 s −40mmHg/7 s atmospheric pressure) used 2 h/day at home. Ulcer healing was assessed using a photographic wound assessment tool (PWAT) and by measuring changes in wound surface area (WSA).

Results Seven of nine recruited patients adhered to a median of 90% (IQR: 80–96) of the prescribed 8-week INP-protocol, and completed the study without side effects. PWAT improvement was observed in 4/4 patients for INP + SWC vs. 2/5 patients for SWC alone ($P = 0.13$). WSA improved in 3/4 patients allocated to INP + SWC vs. 3/5 patients in SWC alone ($P = 0.72$).

Conclusions INP can be used as a home-based treatment for patients with SCI, and its efficacy should be tested in an adequately sized, preferably multicenter randomized trial.

Introduction

Skin breakdown of the lower limbs resulting in chronic ulceration is a common complication of spinal cord injury (SCI) [1]. In those with longstanding SCI, loss of supraspinal sympathetic nervous system control is often accompanied by multiple cardiovascular sequelae and physiological changes below the level of lesion [2]. It has been speculated that people with SCI also show impaired ulcer healing due to poor peripheral circulation in the lower limbs compared to able-bodied individuals [3]. It is well established that optimal tissue perfusion is vital for the ulcer healing cascade, which involves cell proliferation, angiogenesis, collagen synthesis, epithelialization, and defense against infections [4].

Reduced blood flow in the lower limbs after SCI is partly due to extreme lower limb inactivity, leading to muscle and vascular atrophy, and consequently, increased vascular resistance [5–7].

There is, however, growing evidence that interventions may help to reverse poor circulation. For example, 6 weeks of electrically stimulated leg training has shown to reverse increased vascular resistance in people with SCI [5, 8]. Additional clinical studies indicate that intermittent negative pressure (INP) applied to the lower limb for 6–8 weeks induces beneficial clinical effects on ulcer healing, peak walking distance, and resting blood flow in patients with severe peripheral arterial disease [9–11]. In previous studies, we have demonstrated that application of INP to the

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lower leg and foot increased macro- and microcirculatory flow pulsatility in the treated extremity. INP treatment resulted in increased foot macro- and microcirculation in healthy volunteers [12],in patients with peripheral arterial disease [13], and also in people with SCI [14].

INP-induced increases in pulsatile flow may potentially cause beneficial vascular adaptions that facilitate the ulcer healing process. The proposed theoretical foundation for using INP to facilitate ulcer healing is that INP improves blood flow in the small blood vessels of the cutaneous tissue through abrupt changes in perfusion pressure during the onset of negative pressure [9, 15] and by circumventing the venoarteriolar reflex [16], thereby increasing the delivery of oxygen and nutrients to the cells.

The long-term effects and the feasibility of using a home-based INP device in people with SCI and chronic ulcers are unknown. Due to the lack of previous clinical reports on home-based INP treatment in patients with chronic ulcers, we performed a pilot study where we focused on the methods, procedures and outcomes to be used in designing a larger scale superiority trial. We explored the feasibility outcomes like recruitment rate, protocol adherence, safety and adverse events, identified necessary modifications, and explored the potential clinical effects as reported in previous INP studies [9, 11].

Methods

Aims

The aims of this pilot study were to explore the following feasibility outcomes (i) recruitment and retention rate, ii) compliance with the prescribed home-based INP device (FlowOx™, Oslo, Norway), and (iii) safety and potential adverse events from INP treatment, in addition to exploring outcomes related to evaluate effects of INP on ulcer healing laying the groundwork for a planned full-size clinical trial.

Experimental design

The experiment was a prospective, single-center, randomized, controlled, single assessor-blinded, 16-week, crossover pilot study. In order to effectively evaluate feasibility outcomes, a crossover design was chosen to allow all of the recruited patients to be exposed to both treatments. Due to potential carryover effects, only the first 8-week period was chosen to explore the effect of adding INP to standard wound care (SWC). In our power calculations, we needed to include 13 patients in each group. We based this assumption on a statistical power of 80%, with a dichotomous outcome of ±15% healing, and assuming 80% healing in the INP + SWC group and 20% healing in the SWC only group.

Sampling and recruitment

Eligible patients with chronic SCI and leg and foot ulcer were recruited from Sunnaas Rehabilitation Hospital and from the Norwegian Spinal Cord Injury Association. The recruitment period was between June 2016 and December 2016. The Regional Committee for Medical and Health Research Ethics (REK) in Norway approved the experimental protocols (protocol number: 2015/1318). The study was performed in accordance with the Declaration of Helsinki. The clinical trial was registered August 2016 at ClinicalTrials.gov, identification number: NCT02866708. Written and oral informed consent was obtained from all participants.

Outcomes

The feasibility outcomes of the study were recruitment and retention rates, adherence to the prescribed 120 min per day INP protocol, and to explore safety and potential adverse events. We explored ulcer healing measured as changes in the Photographic Wound Assessment Tool (PWAT) [17] and wound surface area (WSA) over time, comparing the treatment groups INP + SWC and SWC alone.

Patients and eligibility criteria

Chronic ulcers were defined as ulcers that had been present and showed no progress towards healing for a minimum of 6 weeks prior to study inclusion.

Inclusion criteria were: (i) chronic SCI, including paraplegic and tetraplegic individuals (≥6 months); (ii) non-healing leg or foot ulcer ≥ 6 weeks; and (iii) older than 18 years of age. Exclusion criteria were: (i) previous surgery or endovascular treatment for peripheral arterial disease on the affected leg; (ii) history of inflammatory phlebitis or pulmonary embolism; (iii) diagnosed or suspected deep vein thrombosis; and (iv) limbs with uncontrolled infection. Informed written consent was obtained from all patients before the study began. SCI levels were classified as complete or incomplete according to the American Spinal Injury Association classification system [18]. Baseline medical history, medication data, and tobacco use, was collected by the wound physician from the patient record and verbally from the patient prior to treatment allocation.

Randomization

Prior to the start of the study, our statistician randomized patients to study groups based on a computer-generated block randomized list (block sized 2–4), using Stata's ralloc command (Stata, version 11, StataCorp LP, College Station, TX). The patients were allocated to treatment groups at visit

one. Allocations were made using sequentially numbered, opaque sealed envelopes [19]. A third party not affiliated with the study prepared the envelopes in advance. After 8 weeks, the patients were re-examined and crossed over to the other treatment group.

Measurements of peripheral circulation at baseline

Patients rested in a supine position for about 5 min in a room with a temperature of 20–22 °C. With patients in a supine position, we measured the ankle-brachial pressure index (ABPI) (Macrolab, STR Teknikk, Aalesund, Norway). The ABPI for each limb was calculated as the higher of the dorsal or tibial pedis arteries' systolic pressures divided by the higher brachial artery systolic blood pressure [20]. Thereafter, lower limb pulse-volume recordings were measured in the supine position using an air-plethysmography cuff placed on each participant's ankle, connected to a plethysmograph (MacroLab, STR Teknikk, Aalesund, Norway) [21]. The same clinician carried out all vascular measurements.

Intermittent negative pressure

INP treatment was performed at home with an INP device (FlowOx™, Otivio AS, Oslo, Norway) (Fig. 1). Patients randomized to the INP + SWC treatment group were trained on the application and INP protocol before treatment started. The INP device consisted of a rigid, molded polyethylene subatmospheric pressure chamber coupled to a pressure control system that records and stores its use on a USB memory stick to document compliance (FlowOx™; Otivio AS, Oslo, Norway). The INP device generates −40 mmHg negative pressure, alternating 10 s negative pressure and 7 s atmospheric pressure (~3 INP cycles per minute). Internal padding in the pressure chamber allows for insertion of the leg, preventing pressure points on the skin of the leg and foot, with only the posterior part of the lower leg in contact with the internal padding. The pressure chamber was sealed by the patient just below the knee with a thermoplastic elastomer (TPS-SEBS) (Fig. 1). In the INP-intervention group, patients were asked to use the INP device daily for a total of 2 h, divided into two 1 h-sections. Patients were instructed to use the device seated in their wheelchair or in a regular chair (as shown in Fig. 1.) To adhere to the protocol, a minimum of 1 h use per day was required. Patients who were dependent on a personal care assistant or next of kin to perform daily living activities, were instructed together with their caregiver to preferably use the device 60 min in the morning and 60 min in the evening, respectively, or whenever suitable throughout the day. Each patient received about 20 min of instructions at baseline, which included a description of the treatment protocol along with a tutorial on how to operate the device.

Fig. 1 Illustration of the home-based INP device. The participant's lower leg and foot with the ulcer was placed in a subatmospheric pressure chamber interfaced with the pressure control system delivering INP. See text for further explanation. Illustration: Øystein H. Horgmo, University of Oslo

Standard wound care

A certified wound nurse and a physician with more than 10 years of experience with wound care performed SWC according to departmental guidelines [22–24]. At each visit, ulcers were cleansed and all non-viable tissue was debrided, before administering inflammation control and ulcer dressing. To ensure follow-up outside the lab visits, all patients were followed weekly during the course of the study using video consulting via Norwegian Health Network encrypted software from the wound care unit. All communication took place in real time via Norwegian Health Network encrypted software.

Figure 2 depicts a flow chart of the study design. Patients were randomly allocated to one of two treatment sequences at the start of the study: (A) start INP + SWC or (B) start SWC only.

Ulcer measurements

After registering the patients' medical history, performing an examination, ulcer measurements were performed. If osteomyelitis was suspected, patients would routinely have been sent to an magnetic resonance imaging. The researchers placed a 15 cm disposable ruler with millimeter divisions and the patients' identification code next to the ulcers. The ulcers were then photographed perpendicularly at a fixed distance (27 cm) with a 12-megapixel camera. The camera was equipped to automatically allow variations in lighting to permit close-up photos of the ulcers. Photos taken at each measurement time were then projected to actual size and given a number code before analysis, using digital planimetric

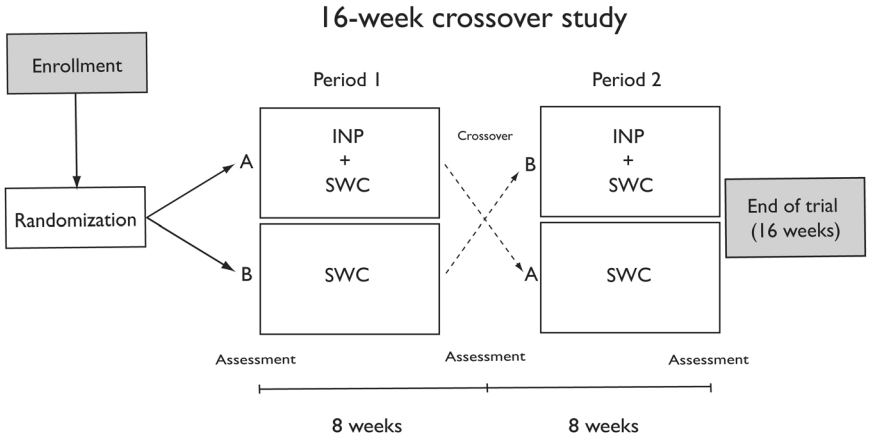

Fig. 2 Flow chart of the intervention wound study. Patients were allocated to treatment arm (**a**) (INP + SWC) or (**b**) (SWC alone) at baseline (period 1). After 8 weeks, treatments were crossed, amounting to a total study duration of 16 weeks. *INP* intermittent negative pressure, *SWC* standard wound care

software (ImageJ, National Institute of Health, USA) [25]. For each photographic image, the caliper was calibrated using the millimeter ruler captured in the image. The WSA in squared centimeters was calculated by manually tracing the ulcer edges with a digital tracer, using the software's freehand selection tool [25]. The procedure was repeated twice by each assessor, and the measurements were averaged. WSA was assessed by one of the authors (HH) and also by a wound nurse not participating in the study. The WSA assessor was blinded to the patients' group assignments using randomized, number-coded photos. Percentage changes in WSA after 8 weeks of treatment compared to baseline were used in the calculations.

In cases where the patient had more than one ulcer, the most severe (highest stage) was chosen as the index ulcer at the first visit and used to evaluate healing rates in subsequent analyses.

Photographic wound assessment tool

All ulcer images were assessed using the PWAT [17]. The PWAT consists of eight domains, where each domain is given a score between 0 and 4 to calculate a final score between 0 and 32. A completely healed ulcer receives a score of 0 [17]. The PWAT has been shown to be a valid and reliable tool to examine ulcers of different etiologies [17]. Two blinded assessors (HH and II), both with more than 10 years of clinical experience scored the PWAT individually. For each patient, the photos were assessed randomly, using an Excel-based random number generator.

Statistical analysis

Descriptive data are presented with median and interquartile range [IQR] (25th and 75th percentile difference), or mean with 95% confidence intervals [CI] when group differences are of interest. A statistical significance level of 5% was used throughout the study. All analysis regarding the effect of INP on ulcer healing was performed in the statistical package R version 3.3.3 (R Foundation for Statistical Computing, Vienna, Austria). Reproducibility data were tested using the intra-class correlation coefficient (ICC) for ulcer size (cm^2) and PWAT scores with Statistical Package for Social Sciences (SPSS Inc., version 22.0, Chicago, IL) with an F-test.

Effect of INP on ulcer healing

To avoid biases by selective dropout, patients were retained in their randomized treatment groups independently of the received treatment. To explore the effect of INP on ulcer healing, WSA and PWAT scores between baseline and after the 8 week period, we used a chi-square test with Agresti-Caffo correction and Wilcoxon rank sum test. Since a normal distribution could not be assumed, confidence intervals for estimated group differences were calculated using 100,000 bootstrapping replications (percentile bootstrapping). We calculated the group differences in PWAT and WSA outcomes as the additional reduction of INP-treatment compared to SWC alone, as a percentage of the expected level after SWC alone (e.g., the average outcome with INP treatment minus the average outcome in SWC alone, divided by the average before treatment minus the average reduction in SWC alone).

Results

All of the approached patients were included in the study, but the inclusion period was slow and recruitment was terminated after the inclusion of only nine patients (all Caucasian: eight males, one female). Seven out of nine patients (six males, one female) completed the 16-week

study protocol, and we had two dropouts. All patients included in the study were wheelchair users.

The median time with ulcers for all nine patients was 52 weeks (IQR: 12 to 82) at inclusion (Table 1). At baseline in period 1, patients allocated to SWC had a median (IQR) WSA of 1.81 cm^2 (Wilcoxon rank sum test, 1.61–3.76) compared to 0.62 cm^2 (0.49–0.76) for those allocated to INP + WSA (Wilcoxon rank sum test, $P = 0.19$). At baseline in period 1, patients allocated to SWC had a median (IQR) PWAT score of 10 (8.0–11.0) compared to a score of 4.5 (3.8–6.0) for those allocated to INP + WSA ($P = 0.29$) (Fig. 3, panels a and b). From the clinical examination, the medical history of the ulcers, and the electronic patient journal, we considered some of the ulcers to be of mixed origin, involving pressure origin, diabetic origin but also mixed arterial or venous pathologies based on the circulatory measurement we performed. Two patients had diabetes mellitus type I (patient 2A and patient 7B, Table 1), and one had diabetes mellitus type II (patient 9B, Table 1). One patient was a smoker. None of the patients suffered from known cardiovascular disease or had been treated with any prior arterial reconstruction. All except two patients used vasoactive substances (spasmolytic drugs), and one patient had a history of asthma, using a beta-2-agonist (Salbutamol). Five patients used analgesics regularly, and one used acetylsalicylic acid. Three patients were depended on a personal care assistant to administer the INP device. The treatment was administered whenever suitable throughout the day. Figure 3 presents the results comparing the study groups' WSA and PWAT scores during period 1 (week 8) and during period 2 (week 16) of the study.

Compliance

The seven patients who completed the 16-week study used the INP device for a median of 90% (IQR: 80–96) of the prescheduled time (120 min per day for 56 days). The median days of INP use was 56 days (IQR: 53–56, range: 49–56). All patients reported that they were able to use the INP device at home, either independently or with the help of a personal assistant or next of kin. Three of the patients had a personal assistant.

Effects of INP on ulcer healing

The patients allocated to start with INP + SWC demonstrated improved ulcer healing during the treatment period. One patient withdrew from treatment after one session, but was allocated to INP + SWC for analysis. Thus, improvement in WSA (binary outcome) was observed in 3 of 4 patients for INP + SWC vs. 3 of 5 patients for SWC alone ($P = 0.72$). The observed difference in WSA after INP + SWC compared to SWC alone was 42% (95% CI −168 to 73%) in favor of the INP + SWC group. PWAT improvement (binary outcome) was observed in 4 of 4 patients for INP + SWC vs. 2 of 5 patients for SWC alone ($P = 0.13$) (Fig. 3). The observed difference in PWAT after INP + SWC compared to SWC alone was 11% (95% CI −151 to 45%) in favor of the INP + SWC group.

The second period was not included in the statistical analysis due to potential crossover effects. Two patients still had open ulcers after 8 weeks of SWC alone, both completed 8 weeks with INP + SWC and reduced their WSA during the treatment period (Fig. 3). Among those allocated

Table 1 Patients' characteristics

Patient	Age	Height (cm)	Weight (kg)	Lesion level	ASIA Class[a]	Lesion duration (yr)	Time with wound (wk)	ABPI[b]	Type of wound and (pressure ulcer categories [39])
1B	48	184	83	C1-C4	A	29	60	0.73	Pressure ulcer (IV)[d]
2A	68	189	70	T1-S5	A	12	222	1.15	Pressure ulcer (III)
3B	57	190	90	T1-S5	A	6	100	0.73	Pressure ulcer (IV)[d]
4A	74	170	66	C5-C8	A	41	17	0.50	Pressure ulcer (IV)[d]
5A	66	178	75	T1-S5	A	30	82	1.00	Pressure ulcer (III)
6B	52	170	85	T1-S5	A	2	12	1.00	Pressure ulcer (III)
7B	53	198	80	C1-C4	A	26	9	1.43[c]	Pressure ulcer (III)[e]
8A	41	196	80	T1-S5	A	16	8	1.00	Pressure ulcer (III)
9B	57	185	100	C1-C4	C	8	52	1.03	Pressure ulcer (III)

A, start INP + standard wound care period 1; B, start standard wound care period 1

[a]American Spinal Injury Association (ASIA score): A, sensory and motor complete; B, sensory incomplete but motor complete; C, sensory and motor incomplete but no functional motor activity

[b]*ABPI* ankle brachial pressure index

[c]Incompressible vessels

[d]Arterial insufficiency and mixed etiology

[e]Diabetic ulcer/small vessel disease

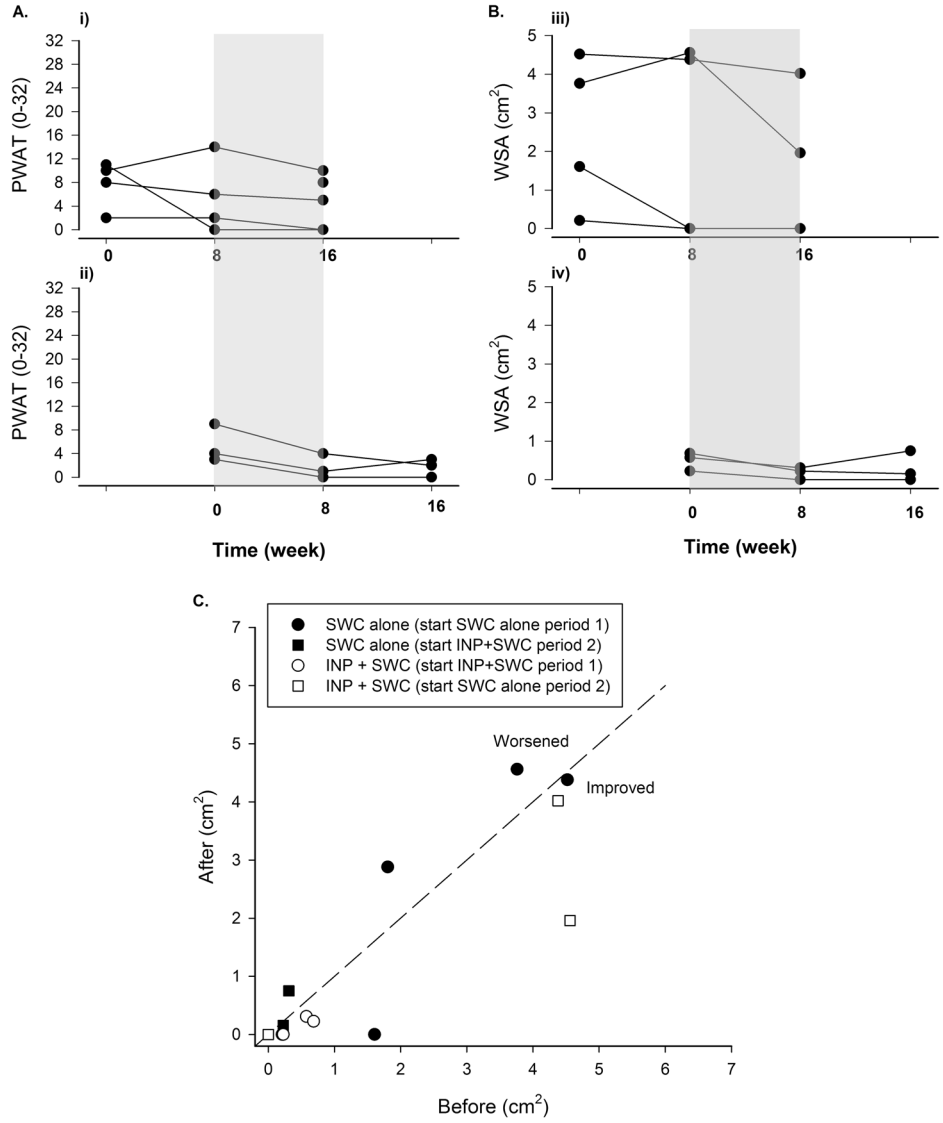

Fig. 3 **a** Photographic Wound Assessment Tool (PWAT) score for each patient ($n = 7$) during the different study phases (control, intervention and/or post-control). Gray background indicates an INP + SWC study phase. **b** Absolute change (cm^2) in wound surface area (WSA) during the period 1 and period 2. **c** The graph shows WSA in the two study groups during both the first and second study period. Circles denote period 1 and squares denote period 2. Black circles denote observations in the SWC-alone treatment group, and open circles denote observations in the INP + SWC treatment group. Black squares denote observations in SWC-alone treatment group, and open squares denote obervations in INP + SWC treatment group. Values above the reference line indicate ulcer worsening, while values below the reference line indicate ulcer improvement

to SWC only at baseline, two patients' ulcers completely or almost completely epithelized during the first control period (Fig. 3). Three patients did not have a fully epithelialized ulcers after period 1 (SWC alone treatment). All three patients' improved their PWAT scores after completing Period 2 with INP + SWC treatment (Fig. 3).

ICC of ulcer measurement data

Reproducibility data were tested using the ICC for ulcer size (cm^2) and PWAT scores with Statistical Package for Social Sciences (SPSS Inc., version 22.0, Chicago, IL) using a *F*-test. A specialized wound nurse (HH) with more than 10 years of experience working with wounds and a wound nurse not affiliated with the study evaluated the ulcers for inter-observer variability by comparing two blinded, independent ulcer tracings of the same ulcer photos in the digital planimetry software (ImageJ). Two of the authors also scored the PWAT separately to obtain the ICC for inter-rater reliability for the PWAT scores, one in a blinded (HH) and the other in a non-blinded fashion (II). ICC for WSA inter-observer variability was 0.96 (95% CI 0.90 to 0.98, $p < 0.001$).

The ICC for inter-observer variability for PWAT scores was 0.75 (95% CI 0.44 to 0.89, $p < 0.001$).

Adverse events

Two patients did not complete the study protocol. One patient (Patient ID 9B in Table 1) acquired an erysipelas infection during the end of period 1 (SWC) of the study and the INP treatment was discontinued. The infections was

later diagnosed as erysipelas with staphylococcus aureus. The other patient (patient 2A in Table 1) withdrew from the INP + SWC group after one INP session due to bleeding from the ulcer.

Discussion

In this pilot study on SCI patients with chronic leg and foot ulcers, a total of nine patients were recruited during the allocated recruitment period. All nine patients recruited were included into the study. Seven patients completed the study protocol of 120 min per day for 8 weeks. This pilot study showed that INP may be used as a home-based intervention for people with SCI and lower limb ulcers. An encouraging aspect of the present study was that all seven patients who completed the study protocol demonstrated improved ulcer healing, compared to 2–3 patients out of five in the SWC alone treatment group, depending on the chosen outcome measurement. However, the recruitment rate was slow, resulting in a sample size that was too low to draw reliable conclusions about potential effects on ulcer healing.

Among the seven patients who completed the treatment protocol, the study obtained about 90% median compliance. Of the nine patient recruited, two patients dropped out of the 16-week study due to bleeding and infection, respectively. The bleeding was not considered a serious event by the medical doctor and the wound nurse. Bleeding may indicate increased ulcer perfusion, and all patients were informed before the start of the pilot study that this could occur during and after the first weeks of INP treatment. The other patient who withdrew from the study (Patient ID 9B in Table 1) acquired an erysipelas infection in the foot during the first period. Treatment was ended, when this was detected. In total, this patient had used the INP device for 178 min before withdrawal.

Currently, the use of focal subatmospheric pressure applied to the wound area of acute and chronic wounds has been extensively investigated using negative pressure wound therapy devices (NPWT) [26]. NPWT is different from the INP method in several ways: (i) It applies subatmospheric pressure to the wound area only, and not to a larger part of the extremity; (ii) it applies higher subatmospheric pressures (typically −125 mmHg); and (iii) it applies constant or intermittent (5 min on and 2 min off) subatmospheric pressure [27]. Consistent with previous research [28, 29], we demonstrated that more than 2 min constant subatmospheric pressure applied to a limited part or a whole limb resulted in reduced blood flow [12], possibly due to venoarteriolar and myogenic vasoconstrictor responses [29]. By contrast, with NPWT, subatmospheric pressure is thought to remove edema, provide mechanical forces and increase blood flow, thereby facilitating healing of acute and chronic wounds [30, 31]. Despite its popularity, there is conflicting evidence of whether NPWT improves perfusion and healing of chronic wounds compared to SWC [26].

In addition to WSA, we also evaluated ulcer healing using PWAT. PWAT has been validated previously [17, 32] and has shown ability to detect changes in ulcer status when used in clinical trials [33, 34]. PWAT has demonstrated moderate to excellent intra and interrater correlation coefficients [ICC] when used to assess the appearance of chronic leg ulcers among trained and experienced health care professionals [17, 32]. This is also consistent with the results in the present study. Improved ulcer healing in all of the patients in the INP + SWC group is consistent with the findings of previous studies, examining the acute effects of INP on macro- and microcirculatory effects of the foot in healthy volunteers [12], in patients with lower extremity peripheral arterial disease [13], and in people with SCI [14]. Due to the large uncertainty in our small dataset, combined with the fact that an ulcer can become infinitely worse, but never can heal more than 100%, confidence intervals are in practice strongly asymmetric on the percentage scale in the present pilot study.

Preserving blood flow is critical for vital skin and underlying tissues, as it provides nourishment, removes waste products in the cells, and protects against infections. It is therefore also essential for the prevention and healing of leg ulcers [35]. A recent case study from our lab on patients with severe peripheral arterial disease and hard-to-heal leg and foot ulcers showed increased foot perfusion and ulcer healing after 8 weeks of INP for 1–2 h per day [11]. Similar findings have also been reported for the use of intermittent negative and positive pressure devices in randomized trials [36, 37]. To the best of our knowledge, this is the first randomized controlled study to examine home-based use of INP applied to the lower leg and foot on ulcer healing in a patient population. In the late 1960s, Smyth [9] published a small prospective study on the application of INP (−150 mmHg) to the lower leg and foot in 46 patients with peripheral vascular disease. Smyth [9] reported that three patients with ulcers all experienced complete ulcer healing after 6 weeks of treatment. These findings, together with the findings in the present study, suggest a possible clinical effect from INP treatment.

Future investigations need to carefully distinguish between the potential additive effect of INP + SWC compared to SWC alone. The fact that a treatment protocol prescribes extensive follow-up for both treatment groups may lead to improved ulcer healing for SWC alone compared to standard outcomes for SWC outside the study context. From our results, we suggest using an estimate of 50% improvement in SWC alone group vs. 95% in INP + SWC group with 90% power, giving a required total of 42

patients in future trials (giving a statistical power of 91% using Fisher's exact two-sample proportions test with 21 patients in each group).

Whether combined INP and SWC may have an additive effect on ulcer healing compared to SWC alone should be investigated in adequately powered randomized controlled trials. Based on the observations in this pilot study, we have several recommendation for the design of a future full-size randomized controlled trial. Given a similar recruitment environment, the recruitment period should be much longer than the 7 months used in the present study, as we observed lower than expected number of recruited patients per month. In addition, we learned that improvement in the SWC group might be quite frequent given the extensive follow-up prescribed in the treatment protocol. Thus, a higher healing rate in the SWC group should be assumed in future power calculations.

Methodological considerations

Several limitations need to be considered when interpreting the results of this pilot study. We explored outcomes, laying the foundation to perform a full scale randomized controlled trial. Our estimates on ulcer healing in the present pilot study are quite crude owing to the small sample sizes. By chance, patients randomized to start in the INP + SWC group had smaller ulcers than did patients allocated to SWC alone. It was not feasible to perform a double-blinded study with a sham intervention in this trial. The clinicians performing the screening and follow-up were not blinded to the patients' treatment allocation. However, ulcer sizes were evaluated by a wound nurse using randomized, number-coded photos. To allow reliable and valid PWAT assessment, two of the pilot study's experience wound clinicians who followed the patients throughout the study also performed the PWAT assessments with number-coded photos. Although we calculated PWAT scores in a blinded fashion, it is possible that the assessor might have recognized the ulcers during patient follow-up.

Since a normal distribution could not be assumed, we performed bootstrapping techniques to evaluate ulcer data over time. Due to the large uncertainty in our small dataset, combined with the fact that an ulcer can become infinitely worse, but never can heal more than 100%, confidence intervals are in practice strongly asymmetric on the percentage scale.

The pilot study's crossover design allowed us to evaluate the feasibility of the INP device and study procedures with a larger number of patients than would be the case with a parallel design. For effect estimation, however, only the first period could be used, due to possible carryover effects. It is possible that 8 weeks was too short to achieve complete closure of chronic ulcers. It is therefore unclear whether the observed ulcer healing can be sustained long-term.

Another potential limitation of the present pilot study is that the ulcers were of a very diverse nature. A more uniform ulcer etiology might have generated more consistent results. However, many chronic ulcers patients have multiple comorbidities, which often exclude them from randomized controlled trials [38]. As a result, the inclusion of diverse ulcer etiologies may strengthen the generalizability of the present pilot study's results. Lastly, while patient recruitment during the short time period available was first priority in this pilot study, larger trials of longer duration could stratify patients between groups to improve comparability with respect to variables such as age and gender.

Conclusions

This pilot study showed that INP may be used as a home-based intervention among people with SCI who have lower limb ulcers. Our clinical pilot study was too small to produce reliable statistical estimates of the effect of INP on ulcer healing, but the results were promising, with ulcer healing in all patients completing the INP treatment. Based on the observations in this pilot study, we recommend a longer recruitment period, given a similar recruitment environment. A higher healing rate in the SWC group should be assumed in future power calculations. In order to reliably determine the potential clinical impacts of the INP method on ulcer healing in patients with SCI, larger and preferably multi-center, prospective randomized controlled studies with a longer follow-up period and parallel design are warranted.

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Author contributions ØHS, LØH, and HW-F conceived of and designed the study. ØHS, II, HH, EL performed the experiments. ØHS, LØ, HW-F, JH, II, and HH, analyzed the data; ØHS, LØH, JH, and II interpreted the results of the experiments; ØHS, LØH, and JH prepared the figures; ØHS wrote the manuscript. All authors edited and critical reviewed the manuscript, and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest The Research Council of Norway provided funding to Otivio (NFR grant no: 241589) for this study as part of an industrial PhD project. ØHS is a PhD student at the University of Oslo.

ØHS is also employed at and is a shareholder in Otivio AS. Otivio AS owns and has the commercial rights to the intermittent negative pressure (INP) technology used in the study. IM is the Chief Scientific Officer and co-founder of Otivio AS, and is a shareholder in the company. None of the other authors have any personal conflicts of interest—financial or otherwise. The authors alone are responsible for the content and writing of the paper. Otivio has not had any role related to the design of the study, collection and analysis of data, or the decision to publish the results.

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