A Multinational, Multicenter, Randomized, Double-Blinded, Placebo-Controlled Trial to Evaluate the Efficacy of Cyclical Topical Wound Oxygen (TWO2) Therapy in the Treatment of Chronic Diabetic Foot Ulcers: The TWO2 Study

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OBJECTIVE

Topical oxygen has been used for the treatment of chronic wounds for more than 50 years. Its effectiveness remains disputed due to the limited number of robust high-quality investigations. The aim of this study was to assess the efficacy of multimodality cyclical pressure Topical Wound Oxygen (TWO2) home care therapy in healing refractory diabetic foot ulcers (DFUs) that had failed to heal with standard of care (SOC) alone.

RESEARCH DESIGN AND METHODS

Patients with diabetes and chronic DFUs were randomized (double-blind) to either active TWO2 therapy or sham control therapy—both in addition to optimal SOC. The primary outcome was the percentage of ulcers in each group achieving 100% healing at 12 weeks. A group sequential design was used for the study with three predetermined analyses and hard stopping rules once 73, 146, and ultimately 220 patients completed the 12-week treatment phase.

RESULTS

At the first analysis point, the active TWO2 arm was found to be superior to the sham arm, with a closure rate of 41.7% compared with 13.5%. This difference in outcome produced an odds ratio (OR) of 4.57 (97.8% CI 1.19, 17.57), $P = 0.010$. After adjustment for University of Texas Classification (UTC) ulcer grade, the OR increased to 6.00 (97.8% CI 1.44, 24.93), $P = 0.004$. Cox proportional hazards modeling, also after adjustment for UTC grade, demonstrated >4.5 times the likelihood to heal DFUs over 12 weeks compared with the sham arm with a hazard ratio of 4.66 (97.8% CI 1.36, 15.98), $P = 0.004$. At 12 months postenrollment, 56% of active arm ulcers were closed compared with 27% of the sham arm ulcers ($P = 0.013$).

CONCLUSIONS

This sham-controlled, double-blind randomized controlled trial demonstrates that, at both 12 weeks and 12 months, adjunctive cyclical pressurized TWO2 therapy was superior in healing chronic DFUs compared with optimal SOC alone.

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**A complete list of the TWO2 Study Group collaborators, Steering Committee, and Data Monitoring Committee can be found in the Supplementary Data online.*

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See accompanying article, p. 515.

With the growing worldwide prevalence of diabetes there has been a resultant increase in the incidence of diabetic foot ulcerations (DFUs) with attendant morbidity, mortality, and health care costs (1–3). Common diabetes comorbidities including peripheral neuropathy, deformity, and peripheral arterial disease (PAD) are among a number of well-established risk factors for DFUs (2,4). These person-level conditions when combined with numerous underlying cellular or metabolic and ulcer-related factors (hypoxia, inflammation, bioburden, etc.) will quite frequently lead to impaired wound healing and to possible amputation (5,6).

Over the last decade it has become clear that basic standards of care for DFUs mandate rigorous attention to proper debridement and off-loading (7–9). While a number of new adjunctive therapies have become available, including growth factors, cellular and acellular tissues, topical negative pressure, oxygen therapies, etc., most therapies suffer from inadequately designed or nongeneralizable studies that cannot attest to their efficacy, safety, and cost-benefit (1,10,11).

Oxygen is an essential component in the wound-healing cascade. Energy metabolism (ATP synthesis), reactive oxygen species generation, redox signaling, H_2O_2 production, antioxidant generation, collagen synthesis, deposition of extracellular matrix, VEGF gene expression, and angiogenesis are among processes dependent on a sufficient supply of oxygen for their activities (12–15).

Hyperbaric oxygen therapy (HBOT) has been studied extensively for its efficacy in healing DFUs and amputation prevention, but despite several recent randomized clinical trials, the results remain inconsistent regarding its effectiveness in healing DFUs (10,16–19). Topical oxygen therapies (TOTs), used in clinical practice for >50 years, supply oxygen directly to the hypoxic wound surface without the potential complications posed by HBOT (13,15,20,21). Despite long-standing clinical evidence supporting the effectiveness of topically applied oxygen for chronic wounds, hyperbaric oxygen proponents have raised concerns about such benefits without systemic hyperoxygenation (22).

To study the effect of topically administered oxygen on cutaneous wounds, Fries et al. (23) conducted a controlled porcine dermal wound-healing experiment. They found that topical oxygen increased the wound tissue partial pressure of oxygen (PO_2) levels 10-fold after 4 min and that repeated treatments accelerated wound closure compared with control (air-exposed) wounds. Histological examination showed a stronger presence of VEGF, signs of improved angiogenesis, and more advanced remodeling with better quality collagen. Their findings suggest several biological mechanisms for the enhanced healing found in other topical oxygen studies. While numerous reports have similarly suggested the potential benefits of topical oxygen in healing chronic wounds, its effectiveness in healing DFUs remains disputed due to a combination of poorly designed studies, inconsistent results, and the paucity of robust investigations through randomized controlled clinical trials (RCTs) (15,24–26).

In recognition of the need for more rigorous studies of this therapy, a randomized, double-blinded, sham-controlled clinical trial was designed to explore the efficacy of cyclical pressurized Topical Wound Oxygen (TWO2) therapy in healing refractory DFUs that had failed to heal with optimal standard of care (SOC) alone. We herein present the results of the TWO2 diabetic foot ulcer study.

RESEARCH DESIGN AND METHODS

Study Design

The TWO2 study was designed as a prospective, multinational, multicenter, double-blinded, placebo-controlled, randomized clinical trial with 17 diabetic foot centers participating across the U.S., U.K., France, Germany, and Luxembourg. The protocol was approved by the governing institutional review or local ethics board of each of the participating centers throughout the U.S. and Europe. The study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines of the International Conference on Harmonization. Written informed consent was provided by all participants prior to performance of study procedures. An independent data monitoring committee and a study steering committee were established to monitor the conduct and analysis of the study.

Sample Size and Design Rationale

Limited information was available on RCTs looking at the efficacy of cyclical pressurized topical oxygen for healing DFUs. Aburto and Frye (27), in a randomized study of topical oxygen, demonstrated better healing in DFU patients after 90 days (90% vs. 40%) compared with the control group. Blackman et al. (20) enrolled 28 patients with DFUs and obtained a similar result (82.4% vs. 45.5%). In combining the results of these two studies, the control group achieved a healing rate of 9 of 21 (42.8%), and in the active group healing occurred in 23 of 27 (85.2%). Using these figures, we would anticipate a tentative expected control rate of 43%, and it was proposed that a conservative estimate of difference between groups would be half that experienced in these trials at 21%. In order to address the unknown outcomes, we used a group sequential design with three predetermined analysis points. With three analyses, the level of significance needed to be adjusted to maintain the integrity of the analysis. The Pocock stopping boundary method requires a more stringent P value threshold ($P < 0.022$) at each of the three analysis points to achieve an overall probability of $P < 0.05$ at the final evaluation. For achievement of a minimal level of significance between study arms, it was calculated that 110 patients would be required in each study arm ($n = 220$). The resultant analyses would therefore be performed after one-third (73), two-thirds (146), and finally all (220) enrolled patients completed the active phase of the study. Since analysis would be exclusively of the intention-to-treat (ITT) cohort, all patients would be analyzed as per the 12-week primary end point (healed vs. unhealed). Furthermore, no up-rating of this sample size was made to take into consideration patients lost to follow-up.

Patients

Inclusion criteria for participation in the trial were as follows: patients with type 1 or 2 diabetes with nonhealing, full-thickness, University of Texas Classification (UTC) grade 1 or 2 DFU measuring ≥ 1 cm^2 and <20 cm^2 post-debridement. All ulcers included were to be between 4 weeks and 1 year in duration and to have been receiving standard care for at least 4 weeks. Patients with modest limb ischemia were permitted with an ankle brachial index (ABI) >0.7. To account for falsely elevated ABI measurements (7), we performed

a secondary confirmatory measurement of distal perfusion adjacent to or distal to the index ulcer in all patients, including a transcutaneous oxygen pressure ($TcPO_2$) >30 mmHg, skin perfusion pressure >30 mmHg, toe pressure >30 mmHg, or a Duplex ultrasound showing biphasic waveforms below the knee. Detailed study enrollment criteria can be found in Table 1.

Randomization

Patients were randomly assigned in a 1:1 ratio double blinded to either the SOC plus sham therapy (SC+Sham) arm or to an SOC plus active TWO2 therapy (SC+TWO2) arm. The randomization list of 220 codes in A or B format was generated by the blinded statistician using a random permuted block design, with blocks of 2, 4, 6, and 8. Study arm allocation was randomly assigned by a centralized study coordinator for each patient at the randomization visit.

Interventions

All patients were recruited as outpatients in participating wound care centers. At the screening visit and after obtaining informed consent, the patient's wound was sharply debrided and digitally photographed. All patients were then provided with the same study foam dressings and hydrogel (Kendall; Covidien), instructions, and the study off-loading device (Optima Diab; Salvatelli srl, Civitanova Marche, Italy). After a run-in period of 2 weeks, patients returned for their randomization visit. Only if the wound area reduction was <30% were patients subsequently randomized double-blind into either the active (SC+TWO2) or sham (SC+Sham) study arm.

The U.S. Food and Drug Administration–cleared, CE-marked TWO2 therapy device (HyperBox; AOTI Ltd., Galway, Ireland) operates by inflation of a single-use extremity chamber over the patient's limb; then, humidified oxygen is cycled between 10 mb and 50 mb within the chamber. A 10 liters per minute oxygen concentrator was used to provide the oxygen supply rather than oxygen cylinders.

Both the active and sham devices looked and operated identically. However, the sham device did not deliver pressurized oxygen into the extremity chamber, even though values displayed on the device controls looked as if this was being performed. The sham treatment therefore consisted only of unrestricted nonpressurized ambient room air in the nonocclusive extremity chamber.

Delivery, installation, and training on the use of the blinded study device was performed by blinded home equipment providers. No study-related procedures or treatments were provided by these representatives. Patients treated themselves at home for 90 min daily five times per week with either the allocated TWO2 or sham therapy. Dressing changes were performed at home by either the patient or their personal caregiver. No study therapy was done at the study centers.

Patients visited a local study center weekly for the duration of the study for wound assessment, debridement, and digital wound photographs. Patients recorded therapy and off-loading compliance daily on diary cards that were verified at each study visit. Additionally, therapy hours were verified by the TWO2 device itself. The active treatment phase was continued until the ulcer healed or for a maximum of 12 weeks.

Data Collection and Outcome Measures

The treatment phase of the study was 12 weeks. The randomization visit measurement after debridement served as the index (baseline) measurement. If multiple ulcers were present, the largest area ulcer at the baseline visit was designated the index ulcer. Weekly digital wound images were transmitted electronically and were assessed for area changes and closure confirmation by a single blinded central assessor using automated CE-marked

Table 1—Inclusion/exclusion criteria

Inclusion criteria	Exclusion criteria
Males and females aged between 18 and 89 years	Evidence of gangrene on any part of affected limb
Documented diagnosis of type 1 or 2 diabetes	Documented evidence of osteomyelitis on any part of affected limb
Foot ulcer at or below ankle with duration >4 weeks to <1 year • If the index ulcer is postamputation, date of surgery must be >30 days • If >1 ulcer is present, largest is considered as the study index ulcer • Index ulcer must be ≥1 cm from any other ulcers present on the foot	Index ulcer has exposed bone Active Charcot foot on the study limb Uncontrolled diabetes: HbA_{1c} >12% (108 mmol/mol) Renal dialysis or creatinine >2.5 mg/dL (221 μmol/L)
Ulcer size ≥1 and ≤20 cm^2 after debridement at start of run-in period	Known immune insufficiency
Ulcer of UTC grade 1A, 1B, 1C, 1D, 2A, 2B, 2C, or 2D	Active treatment for malignancy (not specific to study limb)
ABI >0.7 with a $TcPO_2$ >30 mmHg, skin perfusion >30 mmHg, toe pressure >30 mmHg, or Duplex ultrasound with biphasic waveforms below the knee	Chronic steroid use or immunosuppressive agents within the last 3 months or anticipated to require them during the duration of the study
No planned revascularization procedure or vascular surgery within the last or next 30 days	Subject participated in another investigational device, drug, or biological trial within last 30 days
Subject and caregiver willing and able to comply with all specified care and visit requirements	Index ulcer exhibits signs of severe clinical infection that requires hospitalization or immediate surgical intervention
Subject has a reasonable expectation of completing the study	Subject is pregnant at the time of screening
Subject completed 2-week run-in period with <30% wound size reduction	Subject has had a deep vein thrombosis within the last 30 days Subject has received growth factor therapy, autologous platelet-rich plasma gel, bilayered cell therapy, dermal substitute, extracellular matrix, etc., within the screening period

wound measurement software (MOWA; Healthpath srl, Rome, Italy).

Once a wound was initially determined to be closed by the blinded study site investigator, that visit served as the first of two confirmatory visits. Wound closure (complete epithelialization) was confirmed at the second closure visit 2 weeks later (28). Upon completion of the 12-week treatment phase, patients entered the posttreatment follow-up period for an additional 38 weeks, whereby they returned for wound closure assessment and quality of life (QOL) questionnaires.

The maximum duration for participation in the study was 54 weeks. During the follow-up phase, patients without healed ulcers received standard care according to their clinician's recommendation and were asked not to participate in another wound care trial.

The primary study end point was the percentage of ulcers in each group achieving 100% healing at 12 weeks. Secondary end points included wound area reduction, 12-month incidence of both recurrence and complete healing, incidence of amputation, Cardiff Wound Impact Schedule (CWIS) QOL assessment, and adverse events (1,28,29).

Statistical Analysis

All analyses were performed solely on the ITT study population using Stata 12 (StataCorp, College Station, TX). Results are reported to one decimal place; *P* values and SDs have been reported to two significant figures. For the primary end point of ulcers achieving 100% healing at 12 weeks, statistical significance was assessed at the Pocock 2.2% level ($P < 0.022$). Logistic regression analysis was used to determine the influence of possible confounding variables. Model diagnostics were used to check regression model assumptions and transformations if they did not hold. For this analysis, a backward elimination process was used incorporating the following variables: age, sex, ulcer area, ulcer duration, presence of neuropathy, UTC grade, and HbA_{1c} (%). The same potential confounders were examined within the Cox proportional hazards model. Confounders were included in both models if they changed the odds ratio (OR) or hazard ratio (HR) by >10%. The final logistic regression model and longitudinal hazard models included 97.8% CIs. For all other analyses, statistical significance was assessed at the two-sided 5% level ($P < 0.05$) with 95% CIs provided as appropriate. The statistician conducting all analyses was blinded to treatment allocation (with groups identified as A and B) until results had been finalized.

RESULTS

Between November 2014 and December 2017, 136 patients were screened for the study. Of these, 63 patients (46%) were excluded from randomization for not meeting the inclusion criteria. Thirty-four patients (25%) returned from the 2-week run-in with wound size reductions ≥30%, 10 (7%) had ABI values or second vascular assessments out of range, and 19 (14%) either were not willing to comply fully with the protocol or had other laboratory values out of range. Therefore, 73 patients were randomized into the active phase of the study (see Fig. 1).

At baseline, 65 patients (89%) had type 2 diabetes and 8 patients (11%) had type 1 diabetes. Fourteen index ulcers (39%) in the active arm, compared with six index ulcers (16%) in the sham arm, were assessed to be UTC grade 2 (penetrating to tendon or capsule). Conversely, 22 ulcers (61%) in the active arm, compared with 31 ulcers (84%) in the sham arm, were assessed to be UTC grade 1 wounds ($P = 0.038$). Additionally, 10 patients (28%) in the active arm, compared with 4 patients (11%) in the sham arm, had a previous diagnosis of PAD ($P = 0.066$). Seventeen patients (47%) in the active arm had a history of prior amputations on the index limb in contrast to eight (22%) in the sham arm ($P = 0.018$) (see Table 2).

Primary Outcome

At the first ITT analysis point of 73 patients, the independent data monitoring committee recommended that enrollment should conclude per the predetermined stopping rules, as the active arm

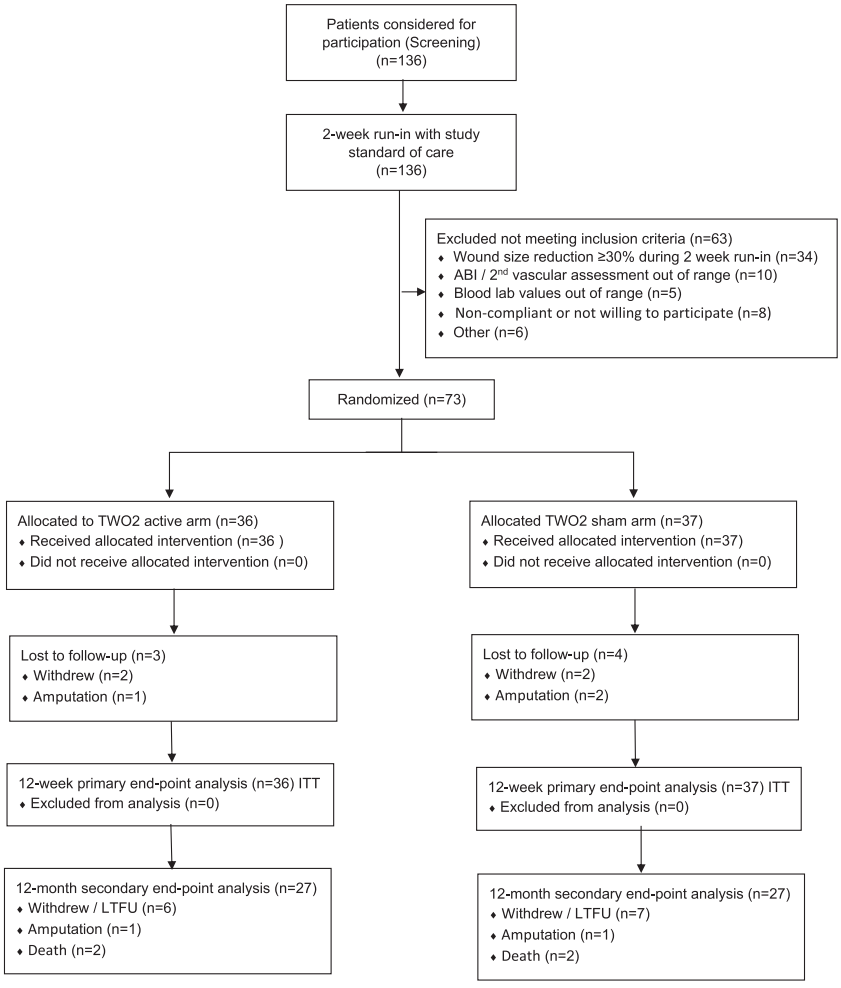

Figure 1—CONSORT diagram of study flow. lab, laboratory; LTFU, lost to follow-up.

Table 2—Baseline characteristics

	Sham TWO2 (n = 37)	Active TWO2 (n = 36)	Total (n = 73)	P
Age, years, mean (SD)	61.9 (9.5)	64.6 (10.3)	63.3 (9.9)	0.21
Sex, male, *n* (%)	31 (84)	32 (89)	63 (86)	0.53
Race, *n* (%)				
White/Hispanic	24 (65)	26 (72)	50 (68.5)	0.90*
Black	5 (14)	5 (14)	10 (14)	
Asian	1 (2.7)	2 (5.6)	3 (4.1)	
American Indian	1 (2.7)	0 (0)	1 (1.4)	
Not reported	6 (16.2)	3 (8.3)	9 (12.3)	
Type 2 diabetes, *n* (%)	33 (89)	32 (89)	65 (89)	0.97
BMI (kg/m^2), mean (SD)	31.2 (7.6)	30.8 (5.9)	31 (6.8)	0.85
Wound area (cm^2), mean (SD)	3.22 (2.54)	3.02 (2.66)	3.13 (2.57)	0.74
Wound perimeter (cm), mean (SD)	6.85 (4.18)	6.22 (2.85)	6.54 (3.55)	0.45
Ulcer duration (days), mean (SD)	174.6 (94)	160.3 (96)	166.4 (95)	0.53
Wound classification, *n* (%)				
UTC grade 1A	27 (73)	20 (56)	47 (64)	
UTC grade 1B	2 (5.4)	1 (2.8)	3 (4.1)	
UTC grade 1C	2 (5.4)	1 (2.8)	3 (4.1)	
UTC grade 2A	4 (10.8)	9 (25)	13 (17.8)	**0.04****
UTC grade 2B	0 (0)	1 (2.8)	1 (1.4)	
UTC grade 2C	2 (5.4)	4 (11.1)	6 (8.2)	
Neuropathic foot, *n* (%)	29 (78)	28 (78)	57 (78)	0.95
Charcot deformity, *n* (%)	3 (8.1)	1 (2.8)	4 (5.4)	0.32
Ulcer location, *n* (%)				0.32
Dorsal foot	5 (13.5)	8 (22.2)	13 (17.8)	
Leg below malleoli	4 (10.8)	1 (2.8)	5 (6.8)	
Pedal foot	22 (59.5)	18 (50)	40 (54.8)	
Toe	6 (16.2)	9 (25)	15 (20.5)	
Previous history of lower-extremity amputation, *n* (%)	8 (21.6)	17 (47.2)	25 (34.3)	**0.02**
Comorbidities, *n* (%)				
Hypertension	30 (81)	28 (78)	58 (79)	0.73
Cardiovascular disease	9 (24.3)	13 (36.1)	22 (30.1)	0.27
PAD	4 (10.8)	10 (27.8)	14 (19.2)	0.07
Venous disease	1 (2.7)	2 (5.6)	3 (4.1)	0.54
Renal disease	6 (16.2)	10 (27.8)	16 (21.9)	0.23
Neurologic disease	31 (83.8)	28 (77.8)	59 (80.8)	0.52
Peripheral edema	1 (2.7)	3 (8.3)	4 (5.4)	0.29
Hyperlipidemia	25 (67.6)	23 (63.9)	48 (65.8)	0.74
Smoker, *n* (%)	10 (27)	13 (36)	23 (31.5)	0.41
Peripheral arterial circulation parameters				
Mean ABI (SD)	1.00 (0.23)	1.07 (0.23)	1.03 (0.23)	0.20
Mean toe systolic blood pressure (SD), mmHg	83.00 (32.75)	84.50 (30.55)	83.77 (30.63)	0.84
Blood work values, mean (SD)				
Prealbumin, μmol/L	4.29 (1.45)	4.44 (0.93)	4.36 (1.18)	0.61
CRP, nmol/L	140 (173)	65.7 (96.2)	99.6 (139)	**0.05**
Creatinine, μmol/L	105.2 (30.1)	113.2 (81.3)	108.7 (61)	0.57
HbA_{1c}, %	8.14 (1.49)	8.43 (1.75)	8.25 (1.64)	0.46
HbA_{1c}, mmol/mol	65 (16.3)	69 (19.1)	67 (17.9)	0.46

All comparisons are nonsignificant except for values in boldface type. *Due to low frequency in each cell, white race was compared with all other races combined. **Due to low frequency in UTC categories, UTC I was compared with UTC II.

was shown to be superior to the sham arm for the primary outcome. In the active arm 15 wounds (41.7%) completely healed versus 5 wounds (13.5%) in the sham arm at 12 weeks [Pearson $\chi^2 = 7.27$ (1 df), $P = 0.007$]. The difference in outcome produced an OR of 4.57 (97.8% CI 1.19, 17.57), $P = 0.010$. Examination of the potential confounding by other baseline variables revealed that UTC ulcer grade substantially changed the OR in favor of the TWO2 group (OR = 6.00 [97.8% CI 1.44, 24.93], $P = 0.004$). The active TWO2 arm showed >3.5 times the likelihood to completely heal over 12 weeks compared with the sham arm with an HR of 3.64 (97.8% CI 1.11, 11.94), $P = 0.013$. With inclusion of the UTC ulcer grade into the model, the HR increased to 4.66 (97.8% CI 1.36, 15.98), $P = 0.004$. The Kaplan-Meier curve shown in Fig. 2 clearly shows the separation between groups throughout the active phase of the study. The patients then entered into the follow-up phase of the study where they were assessed for index ulcer recurrence, healing, and QOL changes for 12 months postenrollment (see Table 3).

Secondary Outcome Measures

Ulcer Recurrence

At 12 months postenrollment, only 1 of 15 healed ulcers (6.7%) in the active arm recurred, compared with 2 of 5 healed ulcers (40%) in the sham arm, falling just short of statistical significance ($P = 0.070$). In total, 20 (56%) active arm (SC+TWO2) ulcers were closed at 12 months postenrollment compared with 10 (27%) of the sham arm (SC+SHAM) ulcers [χ^2 (1 df) = 6.13, $P = 0.013$].

Wound Area Reduction

Of the patients with open ulcers at the end of the 12-week active phase, the mean (SD) absolute reduction in ulcer area from baseline was 1.97 (2.75) cm^2 for the active arm compared with 0.40 (1.75) cm^2 for the sham arm [t (df) = 2.12 (35), $P = 0.041$].

For the patients with larger open ulcers >4 cm^2 at the end of the active phase, the mean (SD) absolute reduction in ulcer area from baseline was 4.12 (1.51) cm^2 for the active arm compared with a 1.34 (1.18) cm^2 increase for the sham arm [t (df) = 2.85 (8), $P = 0.021$].

QOL

The wound care–focused CWIS QOL index improved during the study for patients whose ulcers healed across all functional domains. This positive increase was observed in both full and partial responders. The greatest improvement was seen for the well-being

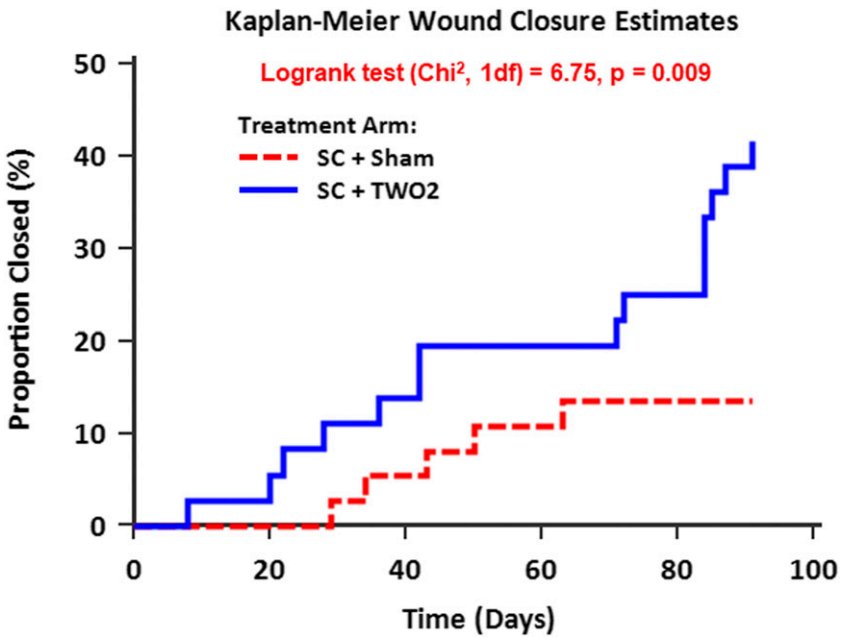

Figure 2—Kaplan-Meier curve showing the separation between study groups throughout the 12-week trial.

component, with mean (SD) score difference between baseline and the end of 12-week treatment in the active arm of 9.1 (13.9) compared with −0.1 (16.9) in the sham arm [t (df) = 2.18 (53), $P = 0.033$].

TWO2 Therapy and Off-loading Compliance

Therapy compliance in both the active and sham arms was high, with 94% and 96% completing treatments, respectively. Off-loading device compliance in both the active and sham arms was also high, with 97% and 99% using the off-loading >75% of the time.

Adverse Events

During the study, there were equal numbers of serious adverse events (10) and adverse events (8) experienced in both study arms. There were no TWO2 device–related adverse events reported. Two index limb amputations (5%) occurred in the active arm compared with three index limb amputations (8%) in the sham arm.

CONCLUSIONS

TOT has been reported to improve healing of DFUs in several earlier prospective randomized studies (20,27,30,31). However, these studies suffered from methodological weaknesses, such as a lack of blinding, uncontrolled SOC, or inappropriate analyses of the ITT populations. The present TWO2 study has demonstrated, in a randomized, sham-controlled trial, that cyclical pressurized TOT adjunctive to optimal SOC is significantly superior to standard care alone in healing recalcitrant DFUs within a 12-week home-based treatment period. To this end, trial enrollment was terminated at the first predetermined analysis point, since the primary end point had been achieved after the initial 73 randomized patients had completed their 12-week treatment phase.

Despite the loss of 25% of patients in the 2-week run-in period prior to randomization, a four-and-a-half–fold increased likelihood of healing was achieved at 12 weeks in patients allocated to the active TWO2 therapy. With adjustment for UTC ulcer grade, this effect increased even further. A very high degree of compliance with treatment and off-loading was demonstrated in both groups. Clinically, the durability of healing as measured by index ulcer recurrence at 12 months was sixfold better than that in the sham group and that seen in other studies (2). Of interest, and distinct from other topical oxygen studies, this RCT allowed for patients with up to UTC grade 2 ulcers with modest degrees of ischemia. Although not statistically significant, nearly 28% of patients randomized to the active therapy had a prior history of PAD compared with just 10% in the control group. However, despite double-blinded randomization, a significant 47% of active therapy patients had a history of lower-extremity amputations compared with just 22% in the sham arm.

This study is consistent with results reported in several previous studies using topical oxygen in DFU (20,30–32) and venous leg ulcers (33,34), as well as animal studies (23). Several other reviews of this approach have also suggested mechanisms of action and putative benefits of topically applied oxygen in the management of chronic wounds (13,15,24,26). Blackman et al. (20), in a prospective open-label study, examined the clinical efficacy of TWO2 therapy in healing DFU patients in a community wound care clinic. Patients were allocated to topical oxygen or otherwise treated with advanced moist wound therapy. At 12 weeks, 82.4% of the ulcers in the TWO2 therapy arm and 45.5% in the control arm healed completely ($P = 0.04$). Median time to complete healing was 56 days in the active and 93 days in the control arm ($P = 0.013$). Another unblinded comparative study investigated the benefits of continuous diffusion of oxygen compared with variable standard care for DFUs (31). Notwithstanding methodological weaknesses, they found significantly faster rates of healing in the topical oxygen group compared with the standard care group and most notably in deeper ulcers. A more recent randomized placebo-controlled trial using a continuous diffusion of oxygen device for only UTC grade 1A ulcers reported a higher proportion of healed DFUs (32.4% vs. 16.7%, $P = 0.033$) and a faster time to closure ($P = 0.015$) in the active group at 12 weeks (30). This study was also planned with a group sequential design; however, their interim analysis end point was not met, and their ITT analysis did not include 35% of randomized patients who were subsequently removed from the trial.

Strengths and Limitations

This TWO2 study followed the guidance for wound-healing therapies put forth by the U.S. Food and Drug Administration (28) as well as subsequent publications from leading authorities calling for more robustly designed sham-controlled RCTs

Table 3—Summary of the results: ITT analysis

	Sham TWO2 (n = 37)	Active TWO2 (n = 36)	Pearson χ^2 or OR or HR (97.8% CI), P value
Primary outcome			
Ulcers completely healed at 12 weeks, n (%)	5 (13.5)	15 (41.7)	**χ^2 7.27 (1 df), P = 0.007**
By randomized treatment group, univariate			**OR 4.57 (1.19, 17.57), P = 0.010**
			HR 3.64 (1.11, 11.94), P = 0.013
After adjustment for UT grade			**OR 6.00 (1.44, 24.93), P = 0.004**
			HR 4.66 (1.36, 15.98), P = 0.004
Margin of effect/relative performance	**68%/309%**		
Secondary outcomes			
Healing durability			
Ulcer recurrence at 12 months, n (%)	2 (40.0)	1 (6.7)	P = 0.070
Ulcers closed at 12 months, n (%)	10 (27)	20 (56)	**P = 0.013**
Margin of effect/relative performance	**52%/207%**		
Healing trajectories			
Absolute change in ulcer area over 12 weeks, cm^2	0.40 (1.75)	1.97 (2.75)	**P = 0.041**
Absolute change in ulcer area in ulcers >4 cm^2 over 12 weeks, cm^2	−1.34 (1.18)	4.12 (1.51)	**P = 0.021**
Time to complete wound closure, weeks	6.3 (1.9)	8.2 (4.2)	P = 0.350
QOL			
CWIS well-being improvement between baseline and week 12	−0.1 (16.9)	9.1 (13.9)	**P = 0.033**
CWIS social life improvement between baseline and week 12	4.1 (12.4)	7.9 (16.9)	P = 0.340
CWIS physical symptom improvement between baseline and week 12	4.6 (11.8)	12.1 (23.2)	P = 0.130
Index limb amputations, n (%)	3 (8)	2 (5)	P = 0.668
TWO2 therapy and off-loading compliance			
Used TWO2 therapy device 5 days/week, 90 min/day, n (%)	35 (96)	34 (94)	P = 0.978
Used off-loading device >75% of the time, n (%)	36 (99)	35 (97)	P = 0.984
Safety analysis			
Incidence of serious adverse events, n	10	10	P = 0.943
Wound infection	2	3	
Osteomyelitis	5	2	
Hypoglycemic event	1	0	
Urinary tract infection	0	2	
Significant necrotic tissue	1	0	
Cardiovascular event	0	1	
UTC grade 2 ulceration	0	1	
Severe maceration/dermatitis	1	0	
Pneumonia	0	1	
Incidence of adverse events, n	8	8	P = 0.950
UTC grade 1 ulceration	0	3	
Ulcer decline	0	2	
Minor infection	1	1	
Minor osteomyelitis	0	1	
Minor necrotic tissue	1	0	
Cellulitis	1	0	
Swelling/edema	1	1	
Maceration	2	0	
Dermatitis	1	0	
Contusion	1	0	
Incidence of adverse device events	0	0	

Data are means (SD) unless otherwise indicated. Boldface type indicates significant differences.

(1,29,35). Nonetheless, and despite randomization of known and unknown potential confounders between groups, it does have limitations. One is the relatively small number of patients included in the primary end point analysis of our ITT population, although the group was similar in size to those of other wound care RCTs (2,36). In a group sequential design study, predetermined hard stopping rules are put in place that in our case were met at the first analysis point of 73 patients. At that point, the primary outcome was achieved by finding significantly more patients in the active group had healed compared with the sham-treated group (41.7% vs. 13.5%, P = 0.007). This approach is used when the magnitude of the treatment effect is uncertain, as it allows for stopping a trial once a wide treatment effect is proven. This also ethically ensures that patients are not further randomized to an inferior arm. In our study, a large margin of effect (68%) and relative performance ratio (309%) were achieved.

The quality of DFU studies is often measured by the results obtained in the control groups. In our sham-treated control group, 13.5% of patients achieved complete ulcer healing within the 12-week outcome period. This rate is similar to that of some studies and lower than

others (17,30,37,38). Interestingly, a recent topical oxygen RCT reported an active group healing rate lower than ours at 32.4% and a similar control healing rate (30). For the more chronic ulcers, their placebo arm healing rate dropped to 13.2%. Despite the large margin of effect between our active and sham groups, we attribute our ostensibly low sham healing rate to the chronicity of the ulcers, complexity of the patients, and the control of, rather than a failure of, SOC treatment. In this regard, the average duration of ulcers enrolled in the trial was >5 months, with a nonsignificant 14-day longer duration in the control group. After the 2-week run-in period, 25% of enrolled patients were excluded from randomization due to a reduction in wound area ≥30%. The study off-loading device, itself proven to be as efficacious as gold standard total contact casting (39), may have enabled progress toward healing that excluded patients likely to heal with such standard care alone. This allowed only patients with wounds more difficult to heal (true SOC failures) to be randomized into this trial. Since there was a very high degree of compliance with both blinded treatments and off-loading throughout the study, we have no reason to believe that the control group healing result was due to any shortcoming in the SOC protocol.

Our sham therapy itself provided nothing more than nonpressurized room air that was free to circulate within the extremity chamber. Room air cannot conceivably be detrimental to the control patients or have a negative impact on ability to heal. Even at the 12-month follow-up evaluation point, long after the active therapy had ended, there was still a clear separation between study groups, with the sham control patients achieving a healing rate of only 27%. Analysis for predictors of healing at 12 weeks resulted only in the treatment effect and UTC ulcer grade being significant. Furthermore, we found no difference in compliance with the therapy or off-loading between study groups. In the absence of otherwise explanatory data to account for the control healing rate, we are left with our presumption that those randomized into the study had ulcers that were truly hard to heal and that the difference in healing rates between active and sham groups was indeed a treatment effect.

The mean age of our study population was ~63 years old, which mirrors that seen in other DFU studies. Eighty-six percent of our study patients were men, likely resulting somewhat from the fact that one-half of the U.S. study sites were Veterans Affairs wound care clinics. Multiple studies have shown DFUs to be more prevalent in men than women to a degree similar to that seen in this RCT (4,10,38). With no significant differences in covariates seen between the two study groups, our findings support the premise that these results are generalizable to similarly afflicted patient populations.

Conclusion

The results of the TWO2 study demonstrate that cyclical pressurized TOT in conjunction with both optimal off-loading and good standard wound care can heal significantly more DFUs at 12 weeks compared with optimal SOC alone. In fact, we found a >4.5-fold increased likelihood of healing within this time period for our actively treated patients. This therapy was safe, without complications, and provided more durable healing for those who had wound closure during active treatment. Uniquely, the therapy has additional benefit in that it can be administered by the patient at home without the expense and difficulties of daily travel to a specialized center. In contrast to recently reported systemic HBOT studies (16,18,40), this robust double-blinded, sham-controlled trial provides evidence to support use of this adjunctive cyclical pressurized TOT for chronic DFUs.

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Aside from delivery and home setup of study devices, no sponsor employee or agent participated in any aspect of patient care or study treatments.

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