

Topical Wound Oxygen Therapy in the Treatment of Severe Diabetic Foot Ulcers: A Prospective Controlled Study

Eric Blackman, MD, FRCS(C), FAADEP; Candice Moore, RN; John Hyatt, MD, FRCS(C); Richard Railton, MD, FRCS(C), FACS; and Christian Frye, MD, MPH

Abstract

Diabetic foot ulcers (DFU) are common, difficult-to-treat, and prone to complications. A prospective, controlled study was conducted to: 1) examine the clinical efficacy of a pressurized topical oxygen therapy (TWO₂) device in outpatients (N = 28) with severe DFU referred for care to a community wound care clinic and 2) assess ulcer reoccurrence rates after 24 months. Seventeen (17) patients received TWO₂ five times per week (60-minute treatment, pressure cycles between 5 and 50 mb) and 11 selected a silver-containing dressing changed at least twice per week (control). Patient demographics did not differ between treatment groups but wounds in the treatment group were more severe, perhaps as a result of selection bias. Ulcer duration was longer in the treatment (mean 6.1 months, SD 5.8) than in the control group (mean 3.2 months, SD 0.4) and mean baseline wound area was 4.1 cm² (SD 4.3) in the treatment and 1.4 cm² (SD 0.6) in the control group ($P = 0.02$). Fourteen (14) of 17 ulcers (82.4%) in the treatment group and five of 11 ulcers (45.5%) in the control group healed after a median of 56 and 93 days, respectively ($P = 0.04$). No adverse events were observed and there was no reoccurrence at the ulcer site after 24 months' follow-up in either group. Although the absence of randomization and blinding may have under- or overestimated the treatment effect of either group, the significant differences in treatment outcomes confirm the potential benefits of TWO₂ in the management of difficult-to-heal DFUs. Clinical efficacy and cost-effectiveness studies as well as studies to elucidate the mechanisms of action of TWO₂ are warranted.

Key Words: controlled prospective study, outpatients, diabetic foot ulcer, topical oxygen therapy, silver dressing

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Foot disorders such as ulceration, infection, and gangrene, along with subsequent amputation, are significant complications of diabetes, the leading causes for diabetes-related hospitalization, and estimated to cost billions of dollars each year.^{1,2} Diabetic peripheral wounds are a major risk factor for lower extremity amputation.³ Approximately 40% to 70% of all lower extremity amputations are performed in patients with diabetes; approximately 100,000 nontraumatic lower-limb amputations were performed in the US among persons with diabetes in 2008.⁴ Even superficial diabetic wounds are often difficult to treat and show high rates of complications.⁵

Oxygen (O₂) is essential to wound healing. Local tissue hypoxia, caused by disrupted or compromised vasculature, is a key factor that limits wound healing.^{6,7} It is well established that O₂ is vital in the synthesis of collagen, enhancement of fibroblasts, angiogenesis, and leukocyte function.⁸⁻¹⁰ O₂ also has key functions in energy metabolism^{11,12} and in the inhibition of microbial growth.¹³

Clinical use of O₂ to promote wound healing began in the 1960s with the administration of systemic full body hyperbaric oxygen therapy (HBO) to treat wounds.¹³ Today, HBO is usually administered in single- or multiplace chambers utilizing pressures of 2,500 mb and higher. HBO is reimbursed

Dr. Eric Blackman is an orthopedic surgeon; Ms. Moore is a registered nurse and advanced wound specialist; Dr. Hyatt is a vascular surgeon; and Dr. Railton is a general surgeon, St. Catharines Wound Clinic, St. Catharines, Ontario, Canada. Dr. Frye is a consultant for AOTI, Ltd., Galway, Ireland. Please address correspondence to: Dr. Christian Frye, Pittinger Platz 17, 82008 Unterhaching, Germany; email: christian.frye@online.de.

by the Center for Medicare and Medicaid Services in the US to treat certain wounds, including diabetic foot ulcers (DFUs) that have failed to heal using standard care. A Cochrane review by Kranke et al¹⁴ demonstrated that in people with foot ulcers due to diabetes, HBO significantly reduced the risk of major amputation and may improve the chance of healing at 1 year. The availability of HBO facilities, contraindications, the need to transfer the patients to the HBO facilities, and the risks of undesired systemic side effects such as barotraumas of the ear or confinement anxiety limit the widespread use of HBO to treat diabetic ulcers on a global basis.¹⁵

In an effort to address some of these drawbacks, the principle of topical pressurized oxygen administration or topical wound oxygen therapy (TWO₂) was introduced in the late 1960s.¹⁶ The approach of topically oxygenating the wound is quite different from HBO. TWO₂ does not involve pressures as high as in HBO. Additionally, TWO₂ is portable and can be administered in varied care sites, including in the patient's home. A number of published studies,¹⁶⁻²¹ including smaller random controlled trials (RCTs) and case series involving patients with diabetic ulcers, venous ulcers, pressure ulcers, and other wounds demonstrates positive outcomes with TWO₂, but the medical community is not commonly familiar with the principle.

The purpose of this prospective, controlled study was to: 1) compare healing rates of chronic DFUs treated with TWO₂ versus DFUs treated with advanced moist dressing therapy and 2) compare DFU recurrence rates after 24 months in both treatment groups.

Methods

Study design, setting, and population. A prospective, controlled study was conducted at a single center, St. Catharines Wound Clinic, St. Catharines, Ontario, Canada. One trained research nurse in this outpatient wound care center screened patients referred for wound care for study eligibility. Because all devices and dressings are registered products in Canada, no IRB approval was obtained. Informed consent of the participating patients was obtained, including the option to opt out at any time. Patients were considered eligible for participation if they met the following criteria: provision of informed consent, at least 18 years of age, an ankle-brachial index (ABI) of at least 0.5 in the affected limb, and diagnosis of a DFU with a grade 2-A or worse according to the University of Texas (UT) Wound Classification System.²² Patients were ineligible to participate if they had a chronic wound of nondiabetic origin, deep vein thrombosis (DVT), were pregnant or lactating, were receiving palliative care, were known to be nonadherent with therapy, or had a HbA1c above 10%.

The manufacturer of the topical wound oxygen devices, AOTI Ltd (Galway, Ireland), supported the study by providing the medical devices and the oxygen for use during the study.

Study protocol. After obtaining informed consent, a patient history and baseline assessment were obtained by the

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Key Points

- A prospective controlled study involving 28 outpatients was conducted to compare outcomes of diabetic foot ulcer treatments.
- The proportion of wounds healed and time to healing was good in both treatment groups but significantly better in the topical oxygen (TWO₂) than in the silver dressing group.
- Research to elucidate the mechanisms of action of TWO₂ and randomized controlled clinical efficacy and cost-effectiveness studies are warranted.

study nurse. Variables assessed included: ABI; wound duration and location, and size; loss of protective sensation (determined by 10-g monofilament); and HbA1c. All wounds were classified according to the UT classification for diabetic wounds by an advanced wound specialist based on clinical and laboratory data. All wounds were surgically debrided to a bleeding base; the number of debridements was not limited but usually debridements were performed once a week before treatment commenced. All wounds were offloaded with the Active Offloading Walker (Royce Medical, Camarillo, CA).

If a TWO₂ device was available after the initial assessment (there were a total of four devices), the patient was asked to be in the TWO₂ arm. If all TWO₂ devices were occupied at the first visit of the study participant, or the patient refused daily TWO₂ therapies, the patient was assigned to the control group (see Figure 1) and provided an advanced moist wound therapy (AMWT) using a silver-based dressing (Silvercel™, Johnson and Johnson Inc., Somerville, NJ), which is licensed for the treatment of DFUs by Health Canada.

Hyper-Box Topical Wound Oxygen Therapy Systems (AOTI Ltd., Galway, Ireland) were provided by the Canadian distributor (Therapeutic Surface Solutions Inc., Hamilton, Ontario, Canada) for use in the trial. This system is a class II medical device licensed for the treatment of DFUs as well as other wound types by Health Canada. The device also has US Food and Drug Administration (FDA) 510(k) clearance and CE-Mark approval for the same indications. It delivers humidified medical grade O₂ into an extremity chamber in a cyclical manner. This cycle consists of pressurizing the chamber to 50 mb and then venting the O₂ out of the chamber, allowing pressure to reduce toward ambient pressure (5 mb) before re-pressurizing. Treatment consisted of daily 60-minute TWO₂ treatments, conducted Monday through Friday. Saline-soaked gauze dressings, applied following treatment, remained in place until the next scheduled treatment. Both groups received treatment based on current best practice guidelines, as decided in consultation with three participating surgeons. Dressing changes in the control group also were performed in the study center according to the physicians' recommendation

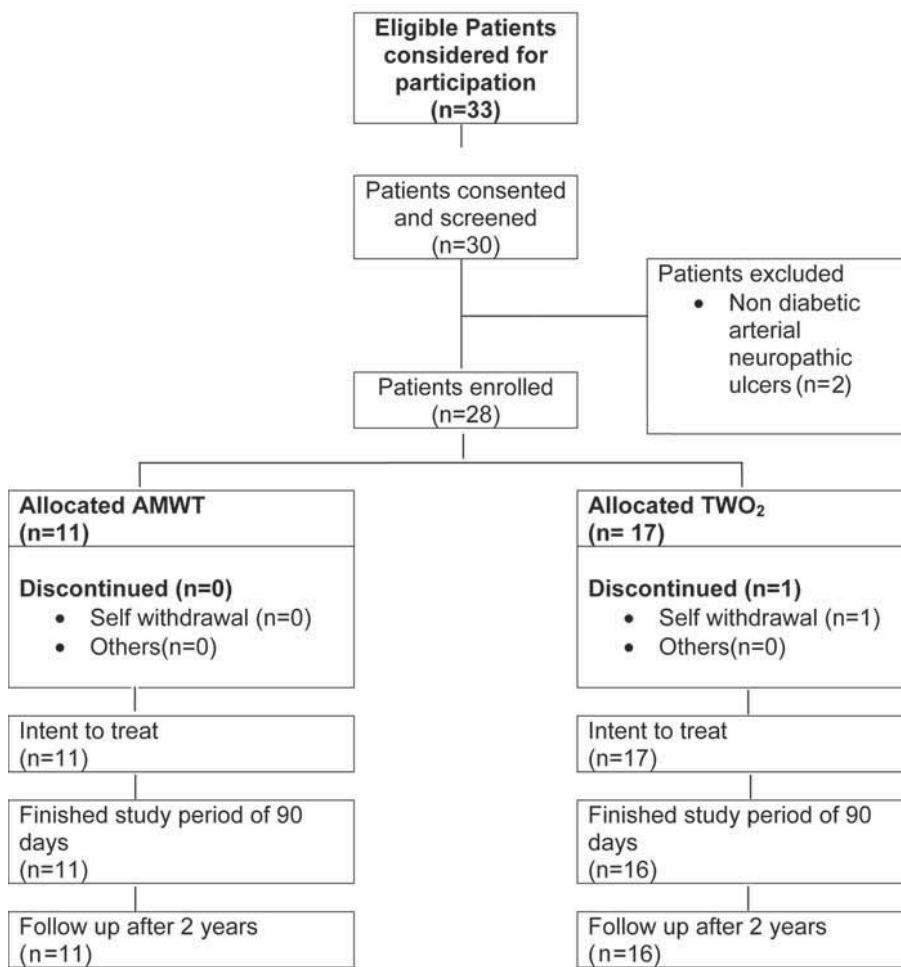


Figure 1. Study population.

at a minimum of twice a week. Each participant’s wound was assessed weekly and debrided if necessary. All patients were followed for 90 days in the active treatment phase (ATP) until the wound healed; all patients were monitored monthly for 24 months in the follow-up phases (PUP) to determine if the wound recurred.

The primary study outcome was wound closure, defined as complete epithelialization of the wound with the absence of drainage. The secondary endpoint was reoccurrence rate after 24 months.

Statistical analysis. Data entry was performed twice and computations were performed using the statistical package SAS for Windows version 9.1 (SAS Institute, Cary, NC). Wound area was calculated using length and width measured with a digital caliper. Data from all patients enrolled in the study were analyzed (intent to treat) mainly using a time-to-event strategy with Kaplan-Meier estimates, followed by a log rank test. This statistical procedure provides a comparison of the distribution of events between the two treatment groups. In addition to the event rates, mean and median time to 100% closure were calculated, as well as the proportion of patients

with healed ulcers within the active treatment phase. Continuous demographic variables, such as the patient’s age at enrollment, were summarized using descriptive statistics and between-group differences were compared with a two-sample *t*-test. Categorical demographic variables such as gender were summarized and compared using a two-tailed chi-square statistic. Comorbidity risk factors were summarized by treatment assignment and according to the type of variable (categorical, continuous) and compared between groups.

Results

In the first week of January 2007, 33 eligible patients were asked to participate in the trial; of these, 30 agreed. Two patients had to be excluded after signing informed consent because they had nondiabetic arterial neuropathic ulcers, leaving a total sample size of 28 patients for follow-up and data analysis. Of those, 27 were followed-up until December 31, 2008 to document DFU reoccurrence in healed wounds. One patient in the TWO₂ group withdrew from the study after 81 days and missing >50% of treatments (see Figure 1).

The TWO₂ and AMWT groups were similar with respect to age, gender distribution, HbA1c, and ABI. Baseline wound area was significantly larger in the TWO₂ than in the control group (mean 4.1 cm² [SD 4.3] versus 1.4 cm² [SD 0.6]; *P* = 0.02). Wound duration was longer in the TWO₂ group (6.1 months [SD 5.8] versus 3.2 months [SD 0.4] for control) but the difference was not statistically significant. All patients had plantar wounds and peripheral neuropathy as indicated by a loss of protective sensation. No toe or heel ulcers were noted in the study population. Except for one mid-foot ulcer in the TWO₂ group, all ulcers were located at the first, third, and fifth metatarsal (see Table 1).

The proportion of ulcers with complete healing was significantly greater in the TWO₂ than in the AMWT group (*P* = 0.013) (see Figure 2). Fourteen (14) out of 17 (82.4%) versus five (5) out of 11 (45.5%), respectively, showed complete epithelialization of the wound (*P* = 0.04). Median time to closure was 56 days (interquartile range [IQR] 39–81 days) in the TWO₂ group and 93 days [IQR: 62–127]) in the control group. In the follow-up phase of up to 24 months, there were no reoccurrences at the healed ulcer site in either the TWO₂ therapy or control group.

No treatment-related adverse events were documented in either group.

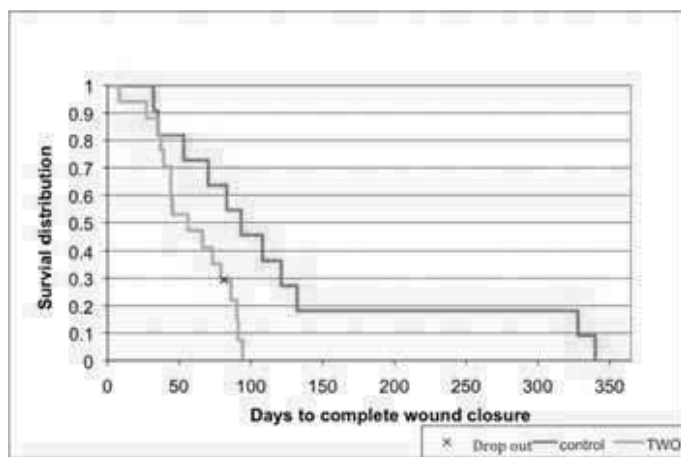


Figure 2. Kaplan-Meier estimate for time to complete wound closure.

Time to complete closure TWO_2 group = 94 days; time to complete closure control group = 340 days ($P = 0.013$)

Discussion

Overall study results. Wounds in patients treated with TWO_2 in this study were significantly more likely to heal and during a shorter period of time than wounds in patients receiving AMWT. These results must be interpreted within the context of the study design. There was no formal randomization and in the vast majority of cases the secretary of the wound care center assigned the groups based on equipment availability and patient preference without knowledge about wound severity. Nevertheless, all staff members were aware of group assignments and it seems likely that more serious wounds were assigned to the TWO_2 group after noting positive results in a pre-study phase before this study commenced in January 2007. This selection bias helps explain why wounds in the TWO_2 group had a larger surface area, UT classification as more severe, and longer wound duration before enrolling into the study than wounds in the control group. In this respect, the results of this trial may underestimate the potential benefits of TWO_2 compared to AMWT.

On the other hand, it is also possible that a “self-selection” of patients took place in favor of AMWT treatment for persons with less interest in following the protocol of care and visiting the center five times a week. According to the study protocol, patients were given the option not to go into the treatment group but no patient “randomized” by the secretary refused to go into the treatment group.

Patient adherence to protocol (particularly with offloading) in a study of neuropathic DFU is an important factor in healing. All patients received offloading but it is possible that poor adherence is at least partly responsible for the outcome differences observed. An additional potential bias is the positive reinforcement of daily 1- to 2-hour visits for the treatment group versus twice-per-week visits for the control group. Positive reinforcement of weight-bearing limitation is likely to

occur during these visits. However, the magnitude of the differences observed is unlikely to have occurred as a result of these potential differences only.

Previous studies²³⁻²⁷ conducted on DFUs that compare AMWT to other adjunctive modalities have shown proportions of wounds healed ranging from 26% to 46.2% following 12 weeks of care in their control groups. The best results (46.2% healed after 12 weeks) were reported in a prospective, randomized, multicenter study²⁷ of UT grade 1 or 2 DFUs ($n = 86$) that investigated healing time between patients receiving a cellular matrix and standard care. The high proportion of wounds healed in the more severe wounds enrolled in the control group of the current study, 45.5% of UT grade 2 and 3 wounds, suggests that the standard of care provided in control group in this wound clinic was good.

The role of oxygen. Although questions about the mechanism of action of TWO_2 remain, evidence suggests that TWO_2 plays a key role in achieving the needed oxygen balance in the wound bed required for wound healing to progress, as suggested by Sibbald and Woo.²⁸

It is well established that oxygen is vital in collagen synthesis, fibroblast enhancement, angiogenesis and leukocyte function.⁸⁻¹⁰ Hypoxia caused by disrupted vasculature is a key factor that has been found to limit wound healing.^{6,7} The partial pressure of oxygen (pO_2) in the wound is lower than in healthy tissue; in dermal wounds, pO_2 ranges from 0 to 10 mm Hg in the center of the wound to 60 mm Hg at the periphery.⁶ In contrast, the pO_2 in arterial blood is approximately 100 mm Hg.

Oxygen needed for collagen synthesis proceeds in direct proportion to pO_2 across the entire physiologic range, from 0 to hundreds of mm Hg. Collagen synthesis requires several enzymes. A measure to characterize an enzyme is the substrate concentration at which the reaction rate reaches half of its maximum value ($V_{max}/2$). This concentration can be shown to be equal to the Michaelis constant (KM). The KM of O_2 in collagen synthesis has been determined to occur at a pO_2 of 20 to 25 mm Hg. V_{max} is approximately 250 mm Hg, suggesting that new vessels cannot approach their greatest possible rate of growth unless the wound tissue pO_2 is as high as 66.²⁹ Consequently, *in vivo* and human studies have shown that hypoxic wounds deposit collagen poorly and are more likely to become infected.³⁰

Recent research has focused on oxygen and infection. In a wound bed, large amounts of molecular oxygen are partially reduced to form reactive oxygen species (ROS). Leading researchers view the NADP(H)-linked oxygenase as a key factor. *In vitro* studies have shown that this enzyme increases leukocytic oxygen consumption by as much as 50-fold and subsequently uses most of the oxygen delivered to wounds.³¹ The NADPH oxidase catalyzes the production of ROS by phagocyte cells such as neutrophilic and eosinophilic granulocytes, monocytes, and macrophages. Exposing these phagocytes to an infectious stimulus activates a “respiratory burst” caused

Table 1. Baseline patient and wound characteristics

Characteristics	Control group N = 11	TWO ₂ group N = 17
Age (years)	63.4 (9.6)	62.4 (9.7)
Gender (male)	8 (72.7%)	12 (70.6%)
HbA1c (%)	7.4% (1.2%)	7.3 (1.2)
Current smoker	0 (0%)	2 (11.8%)
Ankle-brachial systolic pressure index (mm Hg)	1 (0.18)	0.9 (0.21)
Wound duration before therapy (months)	3.2 (0.4)	6.1 (5.8)
Wound area (cm ²)	1.4 (0.6) ^a	4.1 (4.3) ^a
Wound stage		
C II	0 (0%)	0 (0%)
C III	0 (0%)	1 (5.9%)
D II	7 (63.6%)	5 (29.4%)
D III	4 (36.4%)	11 (64.7%)
Received offloading therapy	11 (100%)	17 (100%)
Plantar location of wound	11 (100%)	17 (100%)
1st metatarsal	10 (91%)	4 (22%)
3rd metatarsal	1 (10%)	1 (6%)
5th metatarsal	--	11 (61%)
Midfoot	--	1 (5%)
Loss of protective sensation	11 (100%)	17 (100%)
History of plantar ulceration	10 (90%)	15 (88%)
Charcot foot		1 (5.9%)

Data are mean (SD) or number of patients (%)

^aP = 0.05

by activation of the plasma membrane-bound NADPH oxidase. Research presented by Hunt¹³ has shown that approximately 98% of the oxygen consumed by wound neutrophils is utilized for respiratory burst. In simpler terms, the majority of oxygen in infected chronic wounds is probably used to fight infection via the ROS-system, leaving almost no oxygen for wound healing.

The ROS includes oxygen-free radicals such as the superoxide anion (O₂⁻) as well as hydrogen peroxide (H₂O₂). The superoxide anion also drives endothelial cell signaling required during angiogenesis. Endogenous hydrogen peroxide drives redox signaling, a molecular network of signal propagation that supports key aspects of wound healing such as cell migration, proliferation, and angiogenesis.³²

In summary, the dilemma in wound healing is that the oxygen supply is limited while oxygen demand increases significantly. Three major factors are responsible for wound tissue hypoxia: peripheral vascular diseases (PVDs) limiting the blood supply and thus the needed oxygen; increased oxygen demand of the healing tissue needed for collagen synthesis and angiogenesis; and the generation of ROS needed for infection control (respiratory burst) and redox signaling

Topical oxygen therapy. The big question is whether topical oxygen can penetrate the wound surface to increase the pO₂ in the wound tissue. Fries et al¹⁸ studied the efficacy of topical oxygen in an experimental setting using a pre-clinical model involving excisional dermal wounds in pigs. Exposing open dermal wounds to topical oxygen treatment increased superficial wound tissue pO₂. Fries et al used a probe designed to measure superficial pO₂ at 2 mm depth at the center of the wound bed and saw an increase of pO₂ from the baseline of 5 to 7 mm Hg to 40 mm Hg in as little as 4 minutes. More indirect evidence of the oxygen penetration into the tissue with topical oxygen devices comes from Scott and Reeves³³ uncontrolled experiments on three patients with plantar diabetic wounds. Using multiplex ELISA assays of growth factor cytokines, the authors quantified levels of total proteins detectable in fluids collected twice weekly from wounds after exposure to topical oxygen. TWO₂ was shown to increase the levels of a variety of angiogenesis-related growth factors (BFGF, HB-EGF, KGF and VEG-F) in chronic wounds. In chronic DFUs treated with TWO₂, the most crucial angiogenesis-related growth factor, VEG-F, increased as much as 20-fold.³⁴

Gordillo et al³² analyzed data from two simultaneous nonrandomized studies to test the effects of HBO and topical oxygen therapy. In total, 1,854 patients were screened in outpatient wound clinics for non-randomized enrollments into the HBO (n = 32; 31% were persons with diabetes) and TWO₂ (n = 25; 52% were persons with diabetes) studies. HBO did not result in statistically significant improvements in wound size or significant changes in the expression levels of any of the genes studied. Topical oxygen treatment significantly reduced wound size and was associated with higher VEGF165 expression in healing wounds.

After an initial prospective case series study by Fisher¹⁶ in 1969, only in the last 5 to 10 years has there been new interest in topical approaches to oxygenate cutaneous wounds.^{18-21,28-36} The results obtained in this trial confirm previously published results of using TWO₂ in chronic wounds. In a prospective case series, Fisher¹⁶ treated 52 patients with venous ulcers (n = 16), pressure ulcers (n = 26), and DFUs (n = 2) with topical oxygen that had failed to heal from several months to several years without improvement. The diabetic ulcers were superficial and had been present for 4 and 5 months. With topical oxygen treatment, the two diabetic ulcers healed within 6 and 9 days, failing in six of the 52 cases. In four of these failures, an underlying osteomyelitic process, unknown at the start of therapy, was noted. In the same study, six patients had almost identical lesions on both lower extremities and hips. One lesion was treated conventionally and the contralateral lesion was treated with topical oxygen. Two of six control-treated

wounds showed mild improvement; all TWO₂ treated wounds healed within 7 weeks.

Heng et al²⁰ conducted a prospective randomized controlled study utilizing TWO₂. Participants included 40 inpatients with 79 necrotic/gangrenous ulcers assigned to TWO₂ or control treatment. The ulcers were of mixed etiology — 39 were diabetic ulcers, 23 of which were located on the foot. Control group patients received standard wound care including sharp debridement as needed and wet-to-dry or hydrocolloid dressings were changed one to three times daily. TWO₂ consisted of topical oxygen delivered at 1.03 to 1.04 atmospheres, with treatment set at 4 hours per day, 4 days per week, for a maximum treatment time of 4 weeks. In the TWO₂ group, 90% of ulcers healed compared with 22% in the control group.

Heng et al²¹ also conducted a 3-month prospective cohort study to assess the healing rate and cost-effectiveness of TWO₂ in healing necrotic/gangrenous wounds in patients with and without diabetes. Necrotic tissue was debrided by sharp debridement and infected ulcers were treated with oral or intravenous antibiotics. Gangrenous digits or forefeet were treated by partial amputation with subsequent treatment of the skin defect with TWO₂. Fifteen (15) patients had 24 wounds, out of which 22 healed in 24 weeks.

Tawfik et al³⁶ recently published the results of an 83-patient parallel observational study comparing TWO₂ and conventional compression therapy used in venous ulcer management. After 12 weeks, 80% of TWO₂-managed ulcers were completely healed (median 45 days) compared to 35% of the control group ulcers (median 182 days) ($P < 0.0001$). Pain scores in TWO₂-managed patients improved and nine of the 19 methicillin-resistant *Staphylococcus aureus* (MRSA)-positive ulcers in the TWO₂ group were MRSA-negative after 5 weeks of treatment regardless of ulcer closure compared to none of the 17 MRSA-positive ulcers in the control group.

Implications for practice. The diabetes epidemic is a worldwide problem. In the most recent national cross-sectional study³⁷ from the year 2000 of coronary risk factors in Saudi Arabia (the CADIS study), 23.7% of adults over 40 years of age had diabetes. The sample included 16,806 adults and the final response rate was 93%. In 2007, more than 100,000 patients with diabetes in the US had a foot amputation.⁴ The mortality rate after a diabetes-related lower leg amputation is high. A retrospective database query and medical record review for January 1, 1990, to December 31, 2001 by Aulivola et al³⁸ reported survival rates after major amputation of patients with diabetes of 69.7% and 34.7% at 1 and 5 years, respectively. In the current study, the attending orthopedic and vascular surgeons estimated that 25% of the TWO₂ group patients faced imminent risk of amputation had the treatment regimen not been successful.

The financial burden of DFUs is also considerable. An uncomplicated DFU is estimated to cost \$8,000 to treat, an infected ulcer can cost \$17,000 and the cost of amputation can

reach \$45,000.^{39,40} Considering the results obtained in this and other studies, TWO₂ has the potential to provide substantial cost savings.

Conclusion

A significant difference in the proportion of DFUs healed was observed between daily TWO₂-treated wounds and those managed with advanced wound dressings. TWO₂ is a simple-to-apply, noninvasive therapy. No adverse events were observed in this or previously published studies. During the 24-month follow-up, no reoccurrence of healed ulcers was observed in either treatment group. Well-designed RCTs to confirm the efficacy and evaluate the cost-effectiveness of TWO₂ are needed. ■

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