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Topical Wound Oxygen Therapy in the Treatment of Severe Diabetic Foot Ulcers:

A Prospective Controlled Study

Dr. Eric Blackman, MD, FRCS(C), FAADEP[1]

Ms. Candice Moore, RN[1]

Dr. John Hyatt, MD, FRCS(C) [1]

Dr. Richard Railton, MD, FRCS(C), FACS[1]

Dr. Christian Frye, MD, MPH[2]*

***Correspondence to:**

Dr. Christian Frye

2131 Palomar Airport Road

Suite 350

Carlsbad, Ca 92011

USA

Phone: +49 1721596431

Fax: +1 760 683 3063

E-mail: christian.frye@online.de

1. St. Catharines Wound Clinic, Ontario, Canada
2. AOTI Ltd., Galway, Ireland

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Abstract

Diabetic foot ulcers (DFU) are frequent, difficult to treat and show high rates of complications. We examined the clinical efficacy of a unique pressurized topical oxygen therapy (TWO_2) device in an outpatient setting in patients with severe diabetic foot ulcers (DFU). Patients visiting a community wound care clinic for treatment of severe DFU's were offered TWO_2 or advanced moist wound treatment (AMWT). TWO_2 patients were treated daily for 60-minutes 5 times a week. The device delivered humidified medical grade oxygen with pressure cycles between 5 and 50 mb. The primary endpoint was complete ulcer closure after 90 days. 28 patients were included into the study. The TWO_2 treatment group recruited more severe wounds. The TWO_2 treatment group had significantly more complete ulcer closures after 90 days than the AMWT group (14/17, 82.4%, median 56 days vs. 5/11, 45.5%, median 93 days; (p=0.04)). There was no reoccurrence at the ulcer site after 24 months follow up in either group. Conclusion: Patients with severe DFU's treated with TWO_2 demonstrated significantly higher and faster healing rates with no ulcer reoccurrence after two years compared to AMWT.

MESH: Diabetic Foot; Topical Oxygen, Ulcer, Prospective Study

Introduction

Foot disorders, such as ulceration, infection and gangrene are a major source of morbidity and a leading cause of hospitalization for persons with diabetes.[1] Ulceration, infection, gangrene, and amputation are significant complications of the disease, estimated to cost billions of dollars each year.[2] Foot disorders are also the leading causes of hospitalization in patients with diabetes mellitus.[2] Diabetic peripheral wounds are a major risk factor for lower extremity amputation.[3] Approximately 40-70% of all lower extremity amputations are performed in patients with diabetes, and some 100,000 non-traumatic lower-limb amputations were performed among U.S. diabetics in 2008 alone.[4] Even superficial diabetic wounds are often difficult to treat and show high rates of complications.[5]

Oxygen (O_2) is essential to wound healing. Local tissue hypoxia, caused by disrupted or compromised vasculature, is one key factor that limits wound healing.[6,7] It is well established that O_2 is vital in the synthesis of collagen, enhancement of fibroblasts, angiogenesis and leukocyte function.[8-10] O_2 also has key functions in energy metabolism[11,12] and in the inhibition of microbial growth.[13]

Clinical use of O_2 to promote wound healing began in the 1960's with the administration of systemic full body Hyperbaric Oxygen Therapy (HBO) to treat wounds.[13] Today HBO is usually administered in single or multi place chambers utilizing pressures of 2,500 mb and higher. HBO is reimbursed by the Center for Medicare and Medicaid Services in the US to treat certain wounds, including DFU's. A Cochrane review by Kranke et al. 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 one year.[14] The availability of HBO facilities,

contraindications, the need to transfer the patients to the HBO facilities and the risks of undesired systemic side effects, limits the widespread use of HBO to treat diabetic ulcers on a global basis.

In an effort to address some of these drawbacks, the principle of topical pressurized oxygen administration or topical wound oxygen therapy (TWO_2) was introduced in the late 60'.[15] The approach of topically oxygenating the wound is quite different from HBO. TWO_2 does not involve pressures anywhere near as high as in HBO. Additionally, TWO_2 is portable and can be administered in varied care sites, including in the patient's home. There have been a number of studies, including smaller RCTs and Case Series etc., published demonstrating positive outcomes with TWO_2 [15-20] but still the principle seems to be quite unknown to the medical community as a whole.

In this study the efficacy and potential economic benefits of TWO_2 in the treatment of chronic diabetic foot ulcers is assessed.

METHODS

Study Design, Setting and Population

The study was conducted as a single center prospective, controlled study at the St. Catharines Wound Clinic, Ontario, Canada. A single, trained research nurse in an outpatient wound care centre in Toronto, Canada screened referred patients for study eligibility. Informed consent of the participating patients was obtained including the option to opt out at any time. Patients considered eligible for entry fulfilled 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, diagnosis of a diabetic foot ulcer with a grade II-A or worse according to the University of Texas Wound Classification System.[21] Patients considered ineligible for entry included those with a chronic wound of non-diabetic origin, those with known deep vein thrombosis (DVT), pregnant or lactating females, those receiving palliative care, patients known to be non-adherent with therapy, and those with 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

Each consenting patient received a baseline wound assessment conducted by the study nurse – this included the ankle-brachial systolic pressure index, duration of the wound, measurement of the wound size, location, loss of protective sensation (10-g monofilament), and HbA1c. All wounds were classified according to the University of Texas classification for Diabetic wounds by a very experienced nurse based on clinical and laboratory data. All wounds were surgically debrided to a bleeding base prior to commencing treatment and on a weekly basis as needed. All patients were off- loaded with the Active Off-loading Walker (Royce Medical, Camarillo, California).

The study was designed as a prospective, controlled study. If the TWO_2 device was available after the initial assessment, the patient was asked to be in the TWO_2 arm. If all TWO_2 devices were occupied at the first visit of the study participant, or the patient refused daily TWO_2 therapies they were assigned to the control group (Figure 1).

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 diabetic foot ulcers as well as other wound types by Health Canada. The device also has 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 consisted of pressurizing the chamber to 50 mb, and then venting the O2 out of the chamber, allowing pressure to reduce towards ambient pressure (5 mb), before then re-pressurizing again. Treatment consisted of daily 60-minute TWO_2 treatments, conducted Monday to Friday. During the week, saline soaked gauze dressings were applied following each TWO_2 treatment. Both groups received treatment based on current best practice guidelines, as decided in consultation with the three participating surgeons. Dressing changes in the control group were performed in the study center according to the physicians' recommendation but at a minimum of twice a week. As AMWT dressing, the control group used a silver based dressing (Silvercel™, Johnson and Johnson Inc. a silver based dressing (Silvercel™, Johnson and Johnson Inc. which is licensed for the treatment of diabetic foot ulcers by Health Canada) covered by a gauze dressing. On Fridays the study nurse applied a silver based dressing which remained in place until Monday on control patients and patients in the TWO_2 group. Each participant's wound underwent a weekly standardized wound assessment and debridement if necessary. The wound was measured using a digital caliper. Patients were followed for a period of 90 days in the active treatment phase (ATP), until the wound healed and followed up for 24 month in total in the follow up phase (PUP) to determine if there was a recurrence of the wound.

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

Statistical Analysis

Analysis was by intention to treat. We analyzed data 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, we also calculated the mean and median time to 100% closure as well as the proportion of patients with healed ulcers within active treatment phase. Continuous demographic variables, such as the patient's age at enrolment, were summarized for the population with descriptive statistics and compared between groups with a two-sample t test. Categorical demographic variables, such as sex, were summarized as a proportion of the intention-to-treat population and compared between treatments by use of a two-tailed Chi-square statistic. Co-morbidity risk factors were summarized for the intention-to-treat population by treatment assignment and according to the type of variable (categorical, continuous) and compared between groups.

RESULTS

Figure 1 describes the patient flow through each stage of the study. Starting in the first week of January 2007 thirty three (33) eligible patients were asked to participate in the trial. 30 patients consented to participate. Two patients had to be excluded after their consent as they had non-diabetic arterial neuropathic ulcers. 28 patients started the active treatment phase of the study. One patient in the TWO_2 group, who was noncompliant to the protocol, was excluded after 81 days of treatment. The patient had missed more than 50% of the daily treatment to this point and was not willing to be more compliant. All patient data collected during the active treatment phase of 90 days were included in the intention to treat analyses. All Patients were followed up until the 31st of Dec. 2008 to measure the reoccurrence of DFU in healed wounds.

Table 1 summarizes the baseline characteristics of the two study groups. The groups were quite similar with respect to age, gender distribution, HbA1C, and ABI. Baseline wound area was significantly larger in the TWO_2 group vs. control group (mean 4.1cm^2 SD 4.3 vs. 1.4 cm^2 SD 0.6; p=0.02), and wound duration (6.1 months SD 5.8 vs. 3.2 months SD 0.4) of the ulcer was longer in the TWO_2 group but not statistically significant. All patients had plantar wounds. There was neither a toe ulcer nor a heel ulcer in the population. Except for one midfoot ulcer in the TWO_2 group all ulcers were located at the first, third and fifth metatarsal. All patients received off-loading therapy and had peripheral neuropathy as indicated by a loss of protective sensation.

Complete ulcer closure was defined as skin closure (100% re-epithelization) without drainage or dressing requirements. The TWO_2 group proportion was significantly (p=0.04) greater for complete ulcer closure than the AMWT group (14/17, 82.4% vs. 5/11, 45.5%). Median time to closure was 56 days [IQR 39–81 days] in the TWO_2 group and 93 days [IQR: 62–127]) in the control group. In the follow up phase of up to 24 month we saw no reoccurrences at the healed ulcer site in either the TWO_2 therapy or control group.

Figure 2 shows the number of days until patients had complete wound closure. Patients assigned to receive TWO_2 had complete wound closure in a significantly shorter time than control patients (p=0.013). In assessing safety, no patient had a treatment-related adverse event.

DISCUSSION

Patients treated with TWO_2 in this study were significantly more likely to have their wounds progress to closure, and remain closed, when compared with patients receiving AMWT.

These results must be interpreted within the context of the study design. There was no formal randomization. In the vast majority of the cases the secretary of the wound care center chose the groups without knowing about the severity of the wound. Nevertheless it seems likely that secretary, treating physicians and the study nurse were aware of group assignment and tended to assign more serious wounds into the TWO_2 group after having experienced positive results in a pre-study phase before this study commenced in January 2007. This is supported by the fact that the patients in the TWO_2 group clearly had far more severe wounds, evidenced by a significantly larger surface area, a more severe University of Texas (UT) classification and longer wound duration prior to enrolling into the study, then those in the control group. In this

respect the results of this trial would underestimate the true potential benefits of TWO_2 compared to AMWT.

On the other hand the therapy had to be conducted in the wound care center 5 times a week. Potentially selection bias in a sense that patients that are less compliant managed to self-select themselves into the control group. We believe that it is unlikely that our results are caused by this 'self-selection' of less compliant patients into the control group. In the study protocol there was an option not to go into the treatment group but as it turned out no patient that was 'randomized' by the secretary refused to go into the treatment group.

Compliance in a study of neuropathic DFU is an important factor in healing, via adherence with off-loading. All patients received off-loading but it is possible that poor compliance is at least partly responsible for the worse outcome in the control group. An additional "Exposure" bias is the additional positive reinforcement of daily 1-2 hour visits for the treatment group, versus twice per week visits by the control group. Positive reinforcement of weight bearing limitation is likely to take place during these visits. As the closure rates in the control group was one of highest recorded in the literature, we believe that this effect was probably minimal.

Previous studies conducted on DFU's comparing AMWT to other adjunctive modalities have shown closure rates from 26% to 46.2% in their control groups,[22-26] of which the best healing results were demonstrated in a prospective, randomized, multicenter study of Reyzelman et al. who investigated healing time between patients receiving a cellular matrix and standard of care in a randomized multi-center study (n=86).[26] Reyzelman et al. treated wounds UT grade 1 or 2 which were less severe than the ulcers treated in our study (UT grade 2 and 3). The closure rate of the control group in our study of 45.5% indicates the excellent standard of care provided in the wound clinic.

Although not all of the precise mechanisms of TWO_2 have been elucidated, there is evidence to suggest 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 the Sibbald and Woo.[27]

Hypoxia caused by disrupted vasculature is a key factor that limits wound healing.[6,7] The partial pressure of oxygen (pO_2) in the wound is lower than in healthy tissue. The pO_2 in dermal wounds ranges from 0 to 10 mmHg in the center of the wound to 60 mmHg at the periphery.[6] In contrast, the pO_2 in the arterial blood is approximately 100 mmHg. It is well established that oxygen is vital in the synthesis of collagen, enhancement of fibroblasts, angiogenesis and leukocyte function.[8-10] Oxygen is also needed for collagen synthesis which proceeds in direct proportion to pO_2 across the entire physiologic range, from 0 to hundreds of mmHg. It is worthwhile to have a more detailed look into the enzyme kinetics. The KM is the substrate concentration at which the reaction rate reaches half of its maximum value (Vmax/2). Collagen synthesis is half maximal (KM) at a pO_2 of 20-25 mmHg. Vmax is approximately 250 mmHg, suggesting that new vessels cannot even approach their greatest possible rate of growth unless the wound tissue pO_2 is as high as 66.[28] Consequently hypoxic wounds deposit collagen poorly and are more likely to become infected.[29]

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. This enzyme increases leukocytic oxygen consumption by as much as 50-fold and thus uses most of the oxygen that is delivered to wounds.[13] The NADPH oxidase, catalyzes the production of ROS by phagocyte cells like neutrophilic and eosinophilic granulocytes, monocytes, and macrophages. Exposure of these phagocytes to an infectious stimulus activates a ‘‘respiratory burst,’’ caused by an activation of the plasma membrane-bound NADPH oxidase. Approximately 98% of the oxygen consumed by wound neutrophils is utilized for respiratory burst.[30] 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 the healing.

The ROS includes oxygen free radicals such as the superoxide anion (O2-) as well as hydrogen peroxide (H_2O_2). 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.[28]

In summary, the dilemma in wound healing is that the oxygen supply is limited while oxygen demand increases significantly. There are three major factors responsible for the 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 syntheses, angiogeneses, and
- the generation of reactive oxygen species (ROS) needed for infection control (respiratory burst) and redox signaling

The big question is whether topical oxygen can penetrate the wound surface to increase the pO_2 in the wound tissue. Fries et al studied the efficacy of topical oxygen in an experimental setting using the pre-clinical model involving excisional dermal wounds in pigs. The exposure of open dermal wounds to topical oxygen treatment increased tissue pO_2 of superficial wound tissue.[17] Fries et al used a probe designed to measure superficial pO_2 at 2 mm depth at the center of the wound bed and saw an increase of pO_2 from the baseline of 5-7 mmHg to 40 mmHg in as little as 4 minutes. More indirect evidence of the penetration of oxygen into the tissue with topical oxygen devices comes from the uncontrolled experiments on three patients with plantar diabetic wounds from Scott and Reeves.[31] By multiplex ELISA assays of growth factor cytokines, Scott and Reeves quantified levels of total proteins detectable in fluids collected twice weekly from wounds after exposure to topical oxygen. TWO_2 was shown to increase the levels of a variety of angiogenesis related growth factors (BFGF, HB-EGF, KGF and VEG-F) in chronic wounds. The most crucial angiogenesis related growth factor, VEG-F, was increased by as much as twenty-fold in chronic diabetic foot ulcers treated with TWO_2.[32] Gordillo et al. analyzed data from two simultaneous non-randomized studies to test the effects of HBO and topical oxygen therapy. In total, 1854 patients were screened in outpatient wound clinics for non-randomized enrolments into the HBO (n = 32; 31% diabetic) and TWO_2 (n = 25; 52% diabetic) 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.[32]

After an initial prospective case series study by Fisher[15] published in the Lancet (1969) it's only been in the last 5 to 10 years that a new interest in topical approaches to oxygenate cutaneous wounds arose.[17-20; 27-34] The results obtained in this trial fit well with the findings obtained in other published trials examining the use of TWO_2 in chronic wounds. Fisher treated 52 patients in a prospective case series successfully with topical oxygen.[15] All of the patients had been treated from several months to several years without improvement. The wounds were of mixed etiology: 2 diabetic ulcers, 16 venous ulcers, and 26 pressure ulcers. The diabetic ulcers were superficial and had been present for four and five months. The lesions healed within six and nine days. Topical oxygen treatment failed in 6 of the 52 cases. In 4 of these failures there were was an underlying osteomyelitic process, unknown at the start of therapy. However, in 6 other patients, with almost identical lesions on both lower extremities and hips, one side was treated conventionally while the contralateral lesion was exposed to topical oxygen. All wounds in the TWO_2 group healed within 7 weeks, while only two out of 6 control wounds showed mild improvement. When the TWO_2 treated wound had healed, the device was then applied to the control wounds. All control wounds subsequently healed when treated with the TWO_2 device, with healing times remarkably similar to those of the originally treated wounds.

A prospective randomized controlled study utilizing TWO_2 was conducted by Heng et al and published in 2000.[19] Participants included 40 inpatients with 79 necrotic/gangrenous ulcers assigned to treatment in the TWO_2 or control group. The ulcers were of mixed etiology. 39 of the ulcers were diabetic of which 23 were located on the foot. Control group patients received standard wound care. Sharp debridement was performed on necrotic tissue to produce active bleeding of the wound bed. Patients with osteomyelitis or persistent necrotic tissue were treated with intravenous antibiotics. Patients with digital gangraene and/or life threatening osteomylitis received digital or forefoot amputations whenever appropriate. Wet to dry dressings or hydrocolloid dressings were changed 1 to 3 times daily as needed. TWO_2 consisted of topical oxygen delivered at 1.03-1.04 atmospheres, with treatment set at 4 hours per day, 4 days per week for a period of 4 weeks (or less if the wound healed). Total healing rates of 90% were demonstrated in the TWO_2 group, compared with 22% in the control group. It was also demonstrated that TWO_2 resulted in quicker healing times, with 18 of the TWO_2 treated ulcers healing within 4 weeks compared with 2 ulcers healed in the control group during the same period. Further, all Stage II and III wounds in the TWO_2 group healed within 6 to 10 weeks, and 4 out of 7 Stage IV ulcers within 16 weeks.

Heng et al. also conducted a 3-month prospective cohort study to assess the healing rate and cost-effectiveness of TWO_2 in the healing of necrotic/gangreanus wound in diabetic and non diabetic patints.[20] Necrotic tissue was debrided by sharp debridement. Infected ulcers were treated with oral or intravenous antibiotics. Gangrenous digits or forefoot were treated by partial amputation with subsequent treatment of the skin defect with TWO_2. Wet to dry saline dressings and hydrocolloid dressings were changed 1-3 times a day. The results were then compared to outcomes from similar patients who did not receive TWO_2. None of the control group ulcers

healed during the 4-week observation period compared with 100% (6/6), 90% (9/10) and 88% (7/8) of Level 2, 3 and 4 topical oxygen treated ulcers healed, respectively.

Tawfick et al. recently published the results of an eighty three patient parallel observational comparative study on TWO_2 effectiveness in venous ulcers, showing significant benefits of TWO_2 over conventional compression therapy. At 12 weeks 80% of TWO_2 managed ulcers were completely healed, compared to 35% of the control group ulcers ($p < 0.0001$). Median time to full healing was 45 vs. 182 days. The pain score threshold in TWO_2 managed patients improved from 8 to 3 by day 13. 9 of the 19 MRSA positive ulcers in the TWO_2 therapy group were MRSA negative after 5 weeks of treatment regardless of closure of the ulcer, compared to none of the 17 MRSA positive ulcers in the control group.[34]

The diabetic 'epidemic' is a worldwide problem. In Saudi Arabia, the incidence level of diabetes has been reported at 27% and is expected to increase to as high as 50%.[35, 36] In 2007 more than 100,000 diabetic patients in the US had a foot amputation.[4] The mortality rate after a diabetes-related lower leg amputation is high. Aulivola et al. reported in a retrospective database query and medical record review for January 1, 1990, to December 31, 2001 a survival of diabetic patients after major amputation of 69.7% and 34.7% at one and 5 years, respectively.[37] In our study, the attending orthopedic and vascular surgeons estimated that 25% of the TWO_2 group patients faced imminent risk of amputation had the treatment regimen not been successful. The DFU is a major cost driver. An uncomplicated diabetic foot ulcer is estimated to cost $8,000 to treat. An infected ulcer increases the costs to $17,000. If an amputation is required the costs go up to $45,000.[38,39] Economically, TWO_2 has the potential to provide substantial cost savings to the global health care system, the scale of which has to be investigated in future studies.

Conclusions

This trial in patients with severe DFU's demonstrated significantly better healing rates of patients treated with TWO_2 vs AMWT. TWO_2 is a simple to apply non-invasive therapy that presents no known clinical risks. There is a need for further well designed randomized controlled trials to verify these advantages compared to the existing standards of care, as well as the potential positive economic implications.

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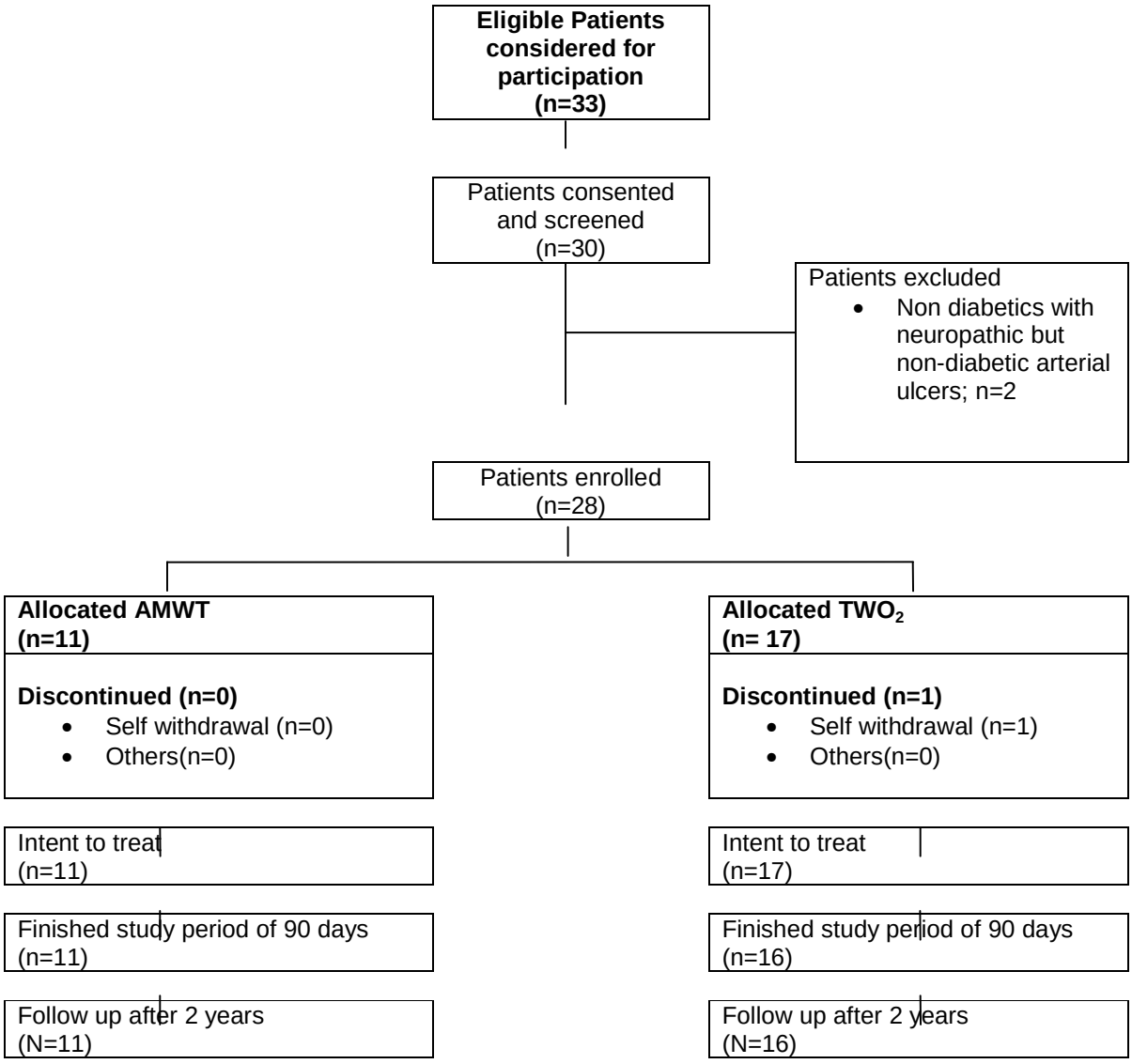

Figure 1: Study population

Table 1: Baseline Patient Characteristics

Characteristics	Control group n=11		TWO_2 group n=17	
Age (years)	63.4	(9.6)	62.4	(9.7)
Sex (male)	8	(72.7%)	12	(70.6%)
HbA_{1c} (%)	7.4%	(1.2%)	7.3	(1.2)
Current smoker	0		2	(11.8%)
Ankle-brachial systolic pressure index (mm Hg)	1	(0,18)	0,9	(0,21)
Wound duration prior to start (months)	3,2	(0.4)	6.1	(5.8)
Wound area (cm^2)*	1.4	(0.6)	4.1	(4,3)
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 off-loading therapy	11	(100%)	17	(100%)
Plantar location of wound	11	(100%)	17	(100%)
1^{st} metatarsal	10	(91%)	4	(22%)
3^{rd} metatarsal	1	(10%)	1	(6%)
5^{th} metatarsal	-	-	11	(61%)
Midfoot	-	-	1	(5%)
Loss of protective sensation	11	(100%)	17	(100%)
History of plantar ulceration	10	(90%)	15	(88%)
Charcot Foot				
Charcot foot			1	(5.9%)

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

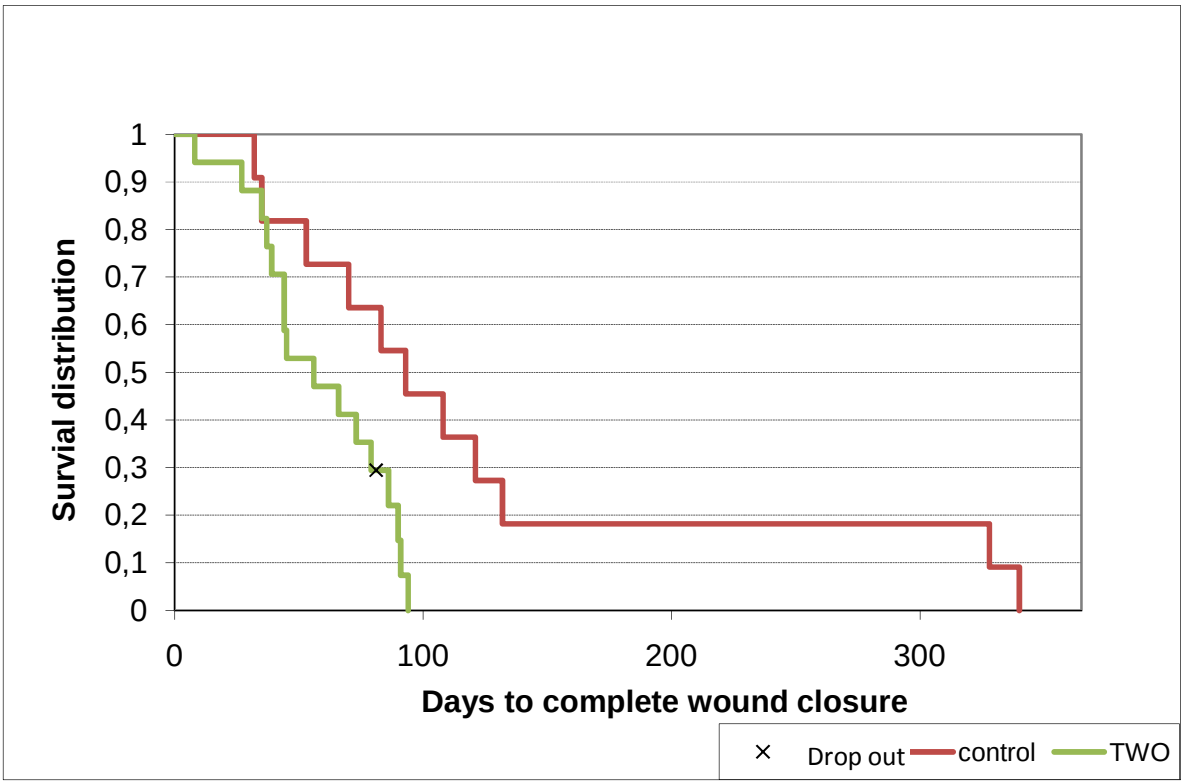

Figure 2: Kaplan-Meier estimated for time to complete wound closure

Blackman E, Moore C, Hyatt J, Railton R and Frye C. Topical Wound Oxygen Therapy in the Treatment of Severe Diabetic Foot Ulcers: A Prospective Controlled Study. Ostomy Wound Manage. 2010;56. In press.