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An Observational Clinical Trial Examining the Effect of Topical Oxygen Therapy (Natrox™) on the Rates of Healing of Chronic DiAbetic Foot Ulcers (OTONAL Trial)

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Abstract

Natrox™ topical oxygen therapy (TOT) (*Inotec AMD Ltd*, Hertfordshire, UK) employs a small battery-powered "oxygen generator" to concentrate atmospheric oxygen and feeds pure, moist, oxygen through a fine, soft tube to a dressing-like "oxygen distribution system", which is placed over the wound and is held in place by a conventional dressing. The aim was to determine the effectiveness of Natrox™ for non-healing diabetic foot ulcers (DFU) over a 3-month period.Longitudinal, single-arm, open prospective registry study using 12 weeks of TOT using a 4 week run-in period. 20 patients recruited to *OTONAL* had chronic DFU greater than 3 months duration or minor amputation sites with less than 50% healing in 4 weeks.There were 13 (65%) males and the mean age was 65.7 (±11.6) years. The mean glycated haemoglobin (HbA1c) was 6.9 (±1.3) mmol mol^{-1} and mean wound duration before TOT was 114 (±79.1) days. 18/20 (90.0%) patients had concomitant lower limb revascularization angioplasty for chronic limb threatening ischaemia. The mean size of the foot ulcer at baseline was 11.3 ± 14.8 cm^2 and mean transcutaneous oxygen measurement value was 34.1 (±19.6) mm Hg. Wound closure of >75% was observed in 14/20 (70.0%) patients. There was a 91.3% (±14.9%) wound area reduction by 3 months (P = .001) and mean time for 100% closure was 77.6 ± 32.5 days. Mean pain scores reduced from 2.4 (±1.8) at baseline to .5 (±1.0) at 3 months (P = .008). All patients were very satisfied using the ambulatory device. Use of TOT in chronic diabetic foot wounds stimulates a healing state, underpinning the concept that oxygen plays a central role in wound healing. Our results are more compelling if you consider they started with relatively large-sized DFUs and majority of patients were frail with underlying peripheral artery disease. (NCT03863054)

Keywords

topical oxygen therapy, wound, healing, diabetic foot ulcer

Introduction

Diabetic foot ulcer (DFU) prevalence is estimated at 6.3% worldwide and the lifetime risk of developing a DFU is approximately 15%–25%.[1,2] Furthermore, the burden of DFU has a significant impact on mortality, with only a 50% five-year survival rate after developing a foot ulcer.[3] In Singapore, the prevalence of diabetes has increased from 8.2% in 2004 to 11.3% in 2010 and is projected to increase to 15% by 2050.[4] The rate of all diabetes-related

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major lower extremity amputation (LEA) has increased from 11.0/100 000 population in 2008 to 13.3/100 000 in 2013 and according to the latest *Organization for Economic Co-operation and Development (OECD) Report*,[5,6] major LEA rates in Singapore are 2 to 3 fold higher than that in western countries – the highest in the world. The magnitude of the disease burden from DFU places considerable demands on the local healthcare settings. This was highlighted in a recent Asian study, evaluating the clinical and economic burden of wound care in the tropics over a 5-year period.[7] This showed a high wound per patent ratio and escalating healthcare costs corresponding to a more proximal level of amputation. Diabetic neuroischaemic ulcers accounted for 97.2% of inpatient episodes with a mean length of hospital stay of 15 days. The data showed that the study population had poor glycaemic control (median HbA1c 9.9%) and DFU recurrence was high, with each patient having an average of 6.1 wounds within the 5 year period.

Oxygen is critical to many of the processes required in wound healing including the production of energy to fuel cell function and metabolism, angiogenesis, collagen synthesis, epithelization and resistance to infection.[8] Wound hypoxia is an important initiator of wound healing but once healing has started, adequate levels of oxygen are required for healing to progress and for full re-epithelization.[9] Persistent wound hypoxia as a consequence of peripheral arterial disease can have a multitude of deleterious effects that delay healing and promote wound chronicity. Many aspects of wound healing can be significantly increased when higher than normal levels of oxygen are present. Fibroblast proliferation and protein production are maximal at oxygen levels of 160 mmHg.[10] Chronic wounds have an oxygen level of 5 to 20 mmHg, which can go down to 0 to 5 mmHg in devascularized areas. Wound cells convert to an anaerobic state at oxygen levels below 20 mmHg, slowing the wound healing process.[10] The concept of increasing the oxygen concentration in healing wounds developed originally with hyperbaric oxygen therapy (HBOT). Poor tissue oxygenation, as is often seen in DFU, is a significant impediment to cellular activity and is, therefore, very likely to impair wound healing. HBOT has shown only limited success because it is only possible to use it for very short periods of the week (<5%), limiting its efficacy in raising oxygen levels in wounds for a prolonged period.[11] For DFUs, 20 to 40 sessions of HBOT are required to show any significant change. In addition to potential adverse effects arising from barotrauma and oxygen toxicity, access is difficult in many areas; it is also expensive and requires a considerable time commitment from the patient.[12]

An alternative solution to increase oxygen levels may be an exciting disruptive technology in this expanding market. The Natrox™ topical oxygen therapy (TOT) system (*Inotec AMD Limited*, Hertfordshire, UK) employs a small battery-powered electrochemical "oxygen generator" that electrolyses atmospheric water vapour to produce pure humidified oxygen. The oxygen is passed down a fine, soft, tube to a dressing-like "oxygen distribution system" that is placed over the wound and is held in place by a conventional dressing. This allows a high concentration of oxygen to build up under the dressing and allows the oxygen to pass along a diffusion gradient into the hypoxic wound. The large real-world observational study of TOT device has been shown to stimulate wound healing in chronic wounds and to help wound closure in a randomized controlled studies of non-healing DFUs.[13–15] The systematic review and meta-analysis suggests that TOT improves the likelihood of DFU healing; however, its effect on amputation and cost-effectiveness remain unclear.[15]

This pilot study aims to examine the efficacy and safety of Natrox™ for non-healing or slow healing DFU over a 3-month period from a cohort of multi-ethnic Asian patients, with the majority having previously undergone endovascular revascularisation for chronic limb threatening ischaemia (CLTI) from Singapore.

Methods

Study Design

OTONAL was an **O**bservational clinical **T**rial examining the effect of topical **O**xygen therapy (**N**atrox™) on the rates of healing of chronic di**A**betic foot u**l**cers. This was a longitudinal, single-arm, open prospective registry study using 12 weeks of Natrox™ therapy. 20 non-consecutive diabetic subjects who had chronic DFU greater than 3 months or minor amputation sites with less than 50% healing in 4 weeks were recruited between August 2018 and April 2021. See Figure 1 for study flow diagram.

The study was conducted in accordance with good clinical practice standards and the ethical principles of the *Declaration of Helsinki* and its amendments. The local Institutional Review Board approved this study (CIRB number: 2018/2355) and the trial was registered at *Clinicaltrials.gov* (NCT03863054). Several inclusion and exclusion criteria and secondary outcome measures were changed subsequently because of logistic and practical reasons and are highlighted below. Informed consent was obtained in accordance to institutional protocol from each patient prior to enrolment. Data were collected prospectively onto a secure computer database using a dedicated proforma, with a telephone questionnaire conducted if required for subsequent clinic follow-up non-attendance.

Inclusion Criteria

1. A DFU greater than 12 weeks and less than 18 months in duration

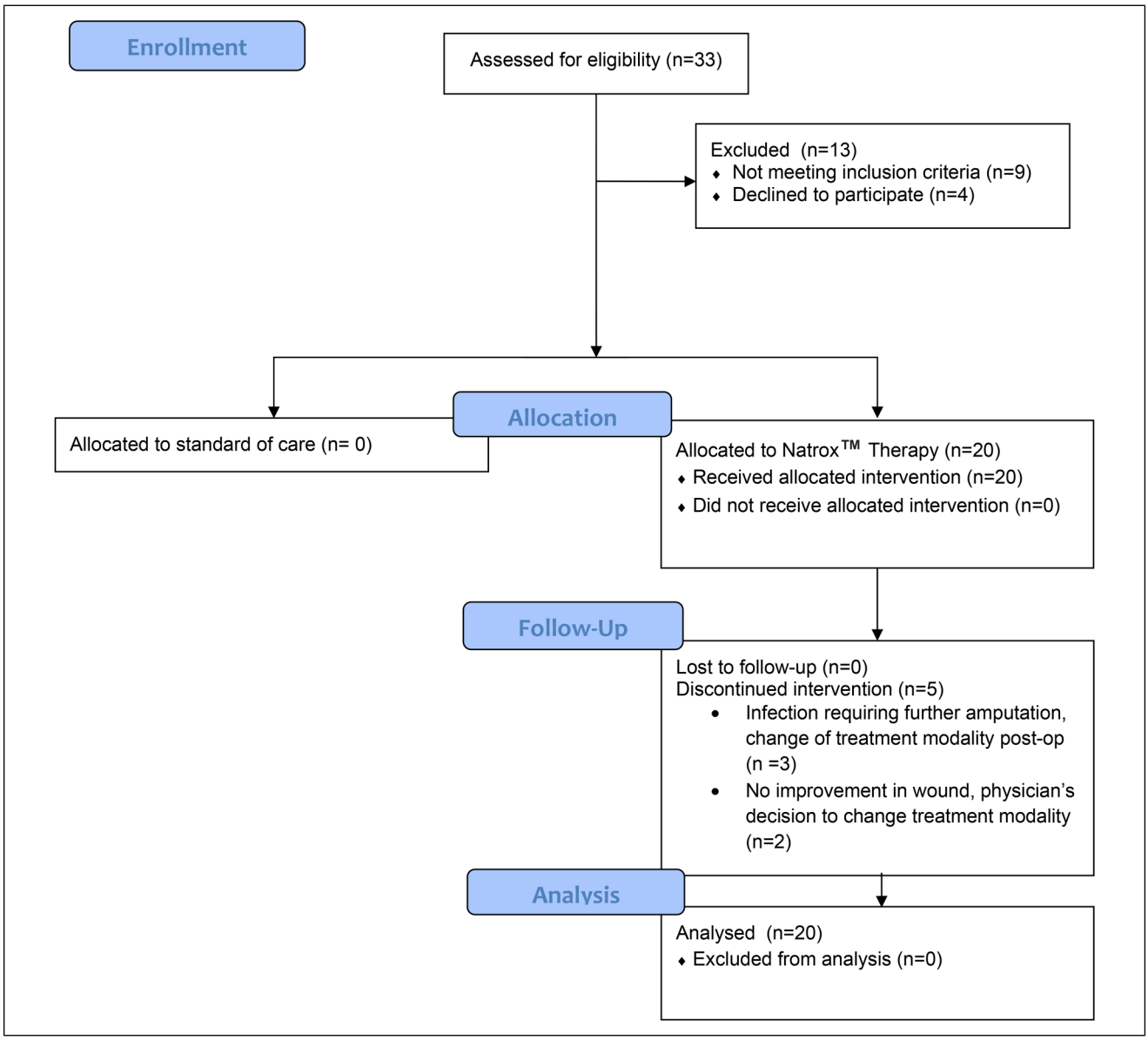

Figure 1. Study flow chart.

2. Minor amputation sites with <50% healed in 4 weeks (the use of negative pressure wound therapy to optimise wound bed was allowed);
3. 4 weeks of standard of care at the hospital-based diabetic foot clinic prior to entry into the open registry;
4. No planned future revascularisation (endovascular or open surgery) within 4 weeks following revascularization being performed
5. Ongoing active chemical or sharp wound debridement prior to, and during, the application of Natrox™;
6. No limit on level of ischaemia, either high or low. The extent of arterial disease was documented by angiogram or Duplex ultrasound and toe blood pressure.
7. The patient was willing to complete >75% of follow-up evaluations required by the study protocol
8. The patient was 21 to 90 years of age
9. The patient was able to abstain from any other treatment of the ulcer for the duration of the study, which would fall outside the normal standard care for a DFU, unless medically necessary;
10. The patient agreed to abstain from enrolment into any other clinical trial for the duration of the study;

Exclusion Criteria

1. Inability to comply with dressing regime or manage the Natrox™ device;
2. Absolute need for a total contact cast;
3. Disseminated malignancy;
4. Subjects with a life expectancy <1 year;
5. Subjects with an ulcer which was <.5 cm^2 or >50 cm^2;
6. DFU was connected to a sinus wound
7. The subject had an invasive soft tissue infection at the time of baseline assessment, requiring oral or intravenous antibiotic therapy;
8. Exposed bone without soft tissue or granulation tissue across the surface;
9. Acute osteomyelitis (stable, chronic osteomyelitis, including those maintained on oral antibiotics, was allowable as long as there was no planned surgical intervention);
10. Subject being treated with immunosuppressive medication greater than 7.5 mg prednisolone daily;
11. Pregnant/lactating females (self-reported or tested, per institutional requirements);
12. Glycated haemoglobin HbA1C of >12 mmol mol^{-1};
13. Subjects who had evidence of connective tissue disorders (eg vasculitis or rheumatoid arthritis) under active treatment;
14. Subjects who is dialysis dependent for less than 1 year (ie subject is eligible for study if has CKD/ESRF and is on dialysis for >1 year);
15. The subject has other concurrent conditions that in the opinion of the primary investigator may compromise subject safety;
16. The subject was unable to follow the protocol or provide consent

Study Protocol

A longitudinal study, utilizing standard of care through a 4-week run in period and followed by 12 weeks of Natrox™ therapy was adhered to. The wounds were type I or II diabetic with full thickness University of Texas Classification (UTC) grade I, II and III DFU measuring > than .5 cm^2 and < 50cm^2 post debridement. Subjects were screened and inclusion and exclusion criteria checked and a microbiology swab taken. Following screening, subjects attended clinic for the baseline visit (Visit 0). The tissue oxygenation was measured at baseline using a standard transcutaneous oxygen (TcPO2) device – Periflux 5000 (Perimed AB, Jakobsberg, Sweden). Following screening (Visit 0), subjects signed a consent form and were enrolled into the study. The wound size was measured following wound debridement. The wounds were dressed 2 or 3 times a week according to standard best practice by the hospital podiatrist. The subject returned for weekly clinic visits to have the wound size measured and to review safety for the first 4 weeks (V1 to V4). At V4, the wound area following debridement was compared to that at V1. Those patients exhibiting a greater than 40% area reduction continued with standard of care (SOC). Those patients with less than a 40% wound area reduction were transferred across to Natrox™ therapy. See Figure 2 for study schedule. Prior to having the device fitted, wound size was measured following wound debridement. Subjects pain scores were recorded and their quality of life assessed using the Diabetic Foot Ulcer Scale – Short Form (DFU-SF).[17] Subjects had the Natrox™ device fitted and were then allowed home. The wound was dressed twice a week with podiatry until healed. The subject returned for weekly clinic visits to have the wound size measured and to review safety for the 12 weeks (V0 through to V12), their quality of life was assessed every 4 weeks. Data from the pilot study in the UK showed that there was a separation of the groups responding to, or not responding to, Natrox™ at 8 weeks and analysis of these data showed this distinction should be clear by 12 weeks. Therefore, it was felt that it would be unreasonable to continue to treat a DFU with potentially an ineffective product for much longer than 12 weeks based on current knowledge.[18] Post visit 12, subjects visited clinic every two weeks (V12 through to V16), completing the same assessments until week 16 or until the ulcer has remained healed for 4 weeks.

Primary Endpoint

- The change in ulcer size after 12 weeks of Natrox™ therapy relative to baseline measurement

Secondary Endpoints

- Absolute closure numbers
- Number of infection episodes
- Quality of life improvement
- Pain as reported via a visual analogue score
- Safety and adverse events

Study Device

The Natrox™ topical oxygen therapy system employs a small battery-powered electrochemical "oxygen generator" to concentrate atmospheric oxygen and feed pure, moist, oxygen at a rate of around 15 mL/hour through a fine, soft tube to a dressing-like "oxygen distribution system" (ODS) that is placed over the wound and is held in place by a conventional dressing. The oxygen generator is worn

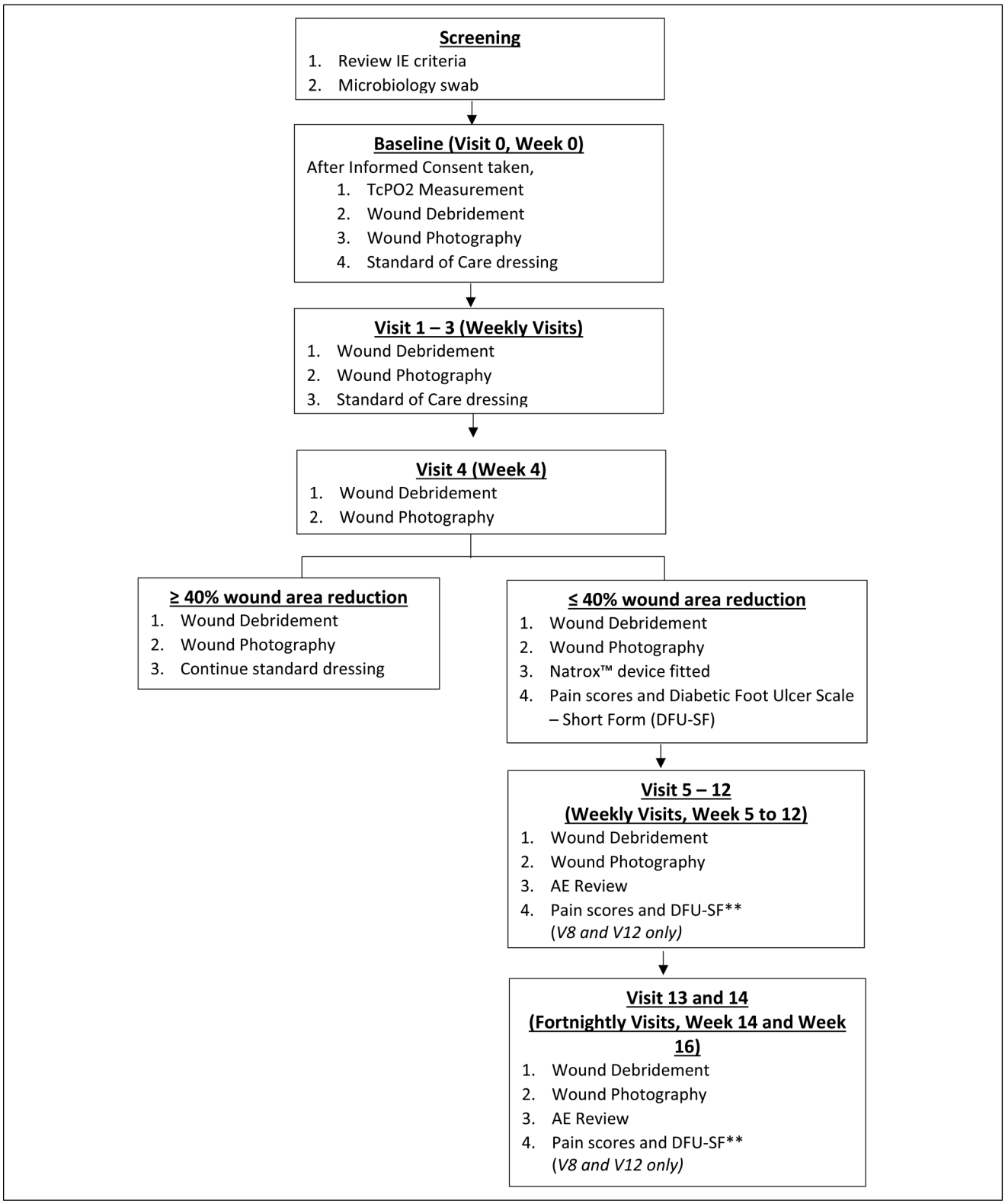

Figure 2. Study schedule.

in a holster on the waist or above the calf or is placed in a trouser pocket, thus enabling the patient to enjoy normal mobility (hence "ambulatory") while receiving continuous oxygen treatment. Each battery has a 30-h rechargeable life span and the patient is provided with 2 interchangeable batteries.

During the application of the TOT device, the ODS was positioned at the centre of the wound to allow equal distribution of oxygen over the entire wound bed, while the other end was connected to the device unit. Melgisorb® Ag and Mepilex® XT foam dressings (Molnlycke Health care; Gothenburg, Sweden) were placed over the wound bed. These were chosen as the primary dressings for two reasons: firstly, being an alginate dressing, it helped to optimize moisture absorbency, and secondly, silver in the dressing provided antimicrobial coverage to reduce biofilm burden. The entire wound site was sealed with an occlusive dressing film, Tegaderm™ (3 M Health Care, St. Paul, MN). The wound dressing was changed twice a week.

The ulcer size was captured following wound debridement each week using a standardized photographic protocol and the photos were encrypted and anonymized for confidentiality and data protection. All the images were assessed independently to significantly reduce the risk of any bias within the trial.

Safety Assessments

- The wound was inspected as per the above protocol. These types of wounds are prone to infection and may deteriorate clinically while in the trial, and as such were always kept under close clinical review. Infection and wound deterioration were dealt with as felt appropriate by the primary provider, documented as an adverse event and oral or intravenous antibiotics were prescribed as deemed fit and patients allowed to remain in the study unless an alternative wound therapy was deemed more appropriate by the PI.
- It was possible that the fine bore tube delivering the oxygen to the wound could cause pressure trauma if it were not appropriately padded. Clinical teams were alerted to this fact so that precautions could be taken. Any such event was logged as an adverse event.

Statistical Analysis

Baseline variables were summarised with the use of descriptive statistics. Continuous variables were reported as the mean and standard deviation, or median and range, as appropriate, and categorical variables as absolute number and %. The primary endpoint of the change in ulcer size from baseline to 12 weeks was analysed using a linear mixed model. For the secondary endpoints, continuous data were analysed using linear mixed models. Categorical variables were analysed using a Chi-squared test. A two-sided p-value of $< .05$ was considered significant. Data were analysed on an intention to treat basis. All analyses were performed in R version 3.5.1.[19]

Results

The trial screened 33 patients for inclusion between August 2018 and April 2021. This resulted in 20 patients going forward to TOT at Visit 4. Baseline demographics are shown in Table 1. There were 13 (65%) males and mean age was 65.7 ± 4.3 years. Majority of patients were either Chinese/Malay in origin (16/20; 80%). There were only 4/20 (20%) active smokers. The mean HbA1c at enrolment

Table 1. Patient Demographics.

	Number (%), n = 20
Mean Age, years (±sd)	65.7 ± 11.6
Mean BMI, kg/m^2 (±sd)	24.6 ± 4.3
Male	13 (65.0)
Ethnic Group	
Chinese	11 (55.0)
Malay	5 (25.0)
Indian	3 (15.0)
Sikh	1 (5.0)
Smoking status	
Non-smoker	14 (70.0)
Smoker	4 (20.0)
Ex-smoker	2 (10.0)
HbA1c (±sd) (mmol mol^{-1})	6.9 ± 1.3
Co-Morbidities (%)	
Diabetes Mellitus	20 (100.0)
Hypercholesterolemia	18 (90.0)
Hypertension	18 (90.0)
Ischemic Cardiomyopathy	15 (75.0)
End Stage Renal Failure (ESRF)	12 (60.0)
Wound Type	
Chronic Diabetic Foot Ulcers	8 (40.0)
Surgical wounds (post-amputation)	12 (60.0)
Concomitant Angioplasties for CLTI	18 (90.0)
Mean Transcutaneous Oxygen Measurement (±sd), mm Hg	34.1 ± 19.6
Mean Toe Pressure (±sd), mm Hg	50.8 ± 24.1
Mean Wound Duration prior to Natrox™ application, days	114.4 ± 79.1
Average wound area at baseline, cm^2 (Min–Max)	12.5 (.6 to 44.0)
Number of wounds with >75% closure	14 (70.0)
Mean time taken for 100% closure, days (±sd)	77.6 ± 32.5

was 6.9 ± 1.3 mmol mol^{-1}. The majority had some degree of ischaemic heart disease (15/20; 75%) and 12/20 (60%) had ESRF established on dialysis.

Post-digital amputation wounds made up 12/20 (60%) of the wounds and 18/20 (90%) patients had CLTI that required a pre-Natrox™ endovascular revascularization procedure, to optimize blood flow to the foot. There were 13/20 (65%) UTC Grade III wounds. The mean size of the foot ulcer at baseline was 11.3 ± 14.8 cm^2 and mean transcutaneous oxygen measurement value was 34.1 (±19.6) mmHg. The mean wound duration prior to TOT application was 114 ± 79.1 days.

Wound closure of >75% was observed in 14/20 (70.0%) patients (Figure 3). There was a 91.3% (±SD) wound area reduction by 3 months from baseline and mean time for 100% closure was 77.6 ± 32.5days. There was 1/2 (50%), 5/5 (100%) and 8/13 (62%) complete healing of UTC grade I, II and III ulcers respectively with the device. Mean pain scores reduced from 2.4 (±1.8) at baseline to .5 (±1.0) at 3 months (P = .008). Figure 4 shows examples of wound progression using TOT of several trial patients. There were no deaths and 4 adverse events (all due to secondary infection) using the TOT device directly, but all patients were very satisfied using the ambulatory device.

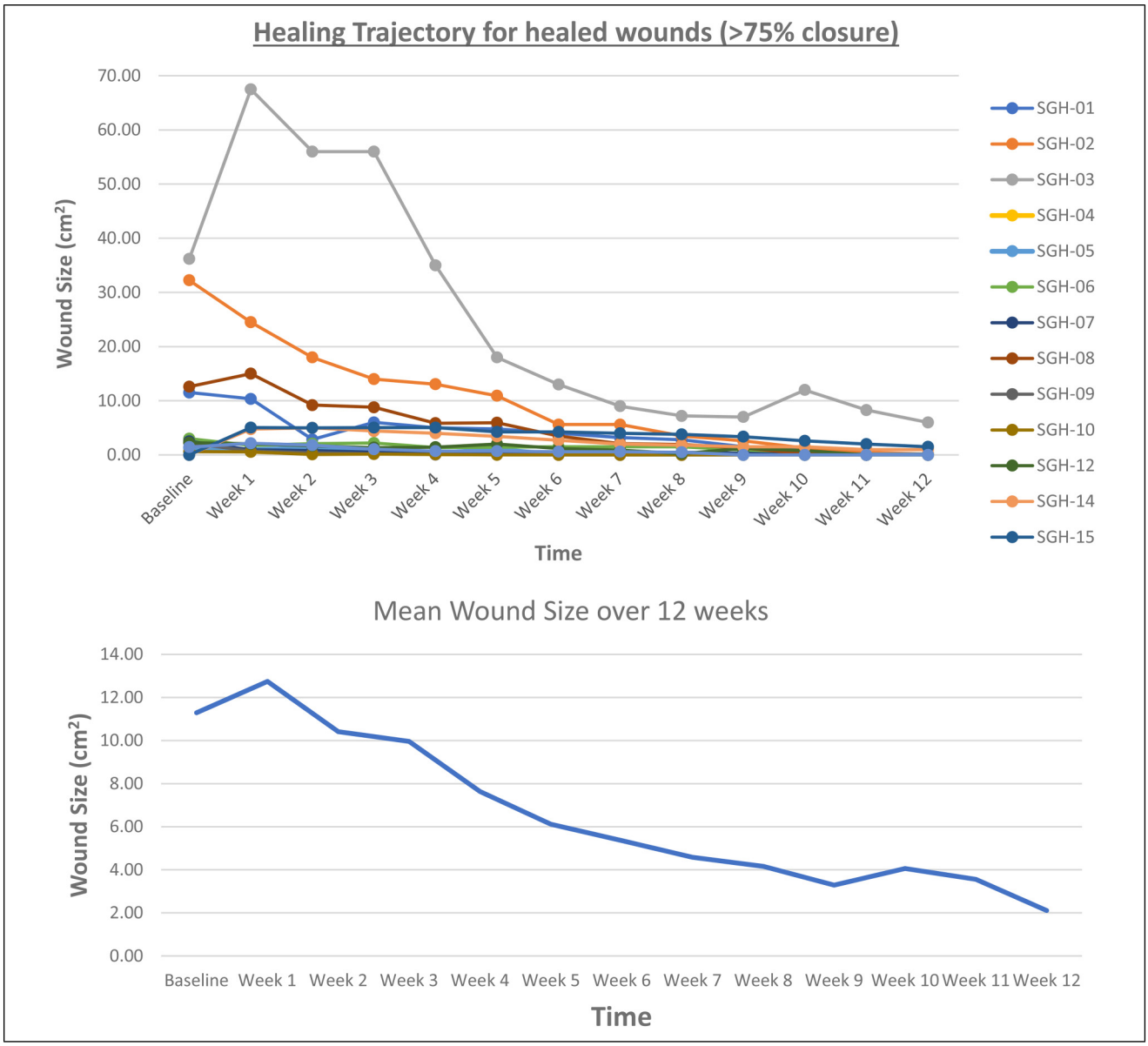

Figure 3. Healing trajectory and mean wound size.

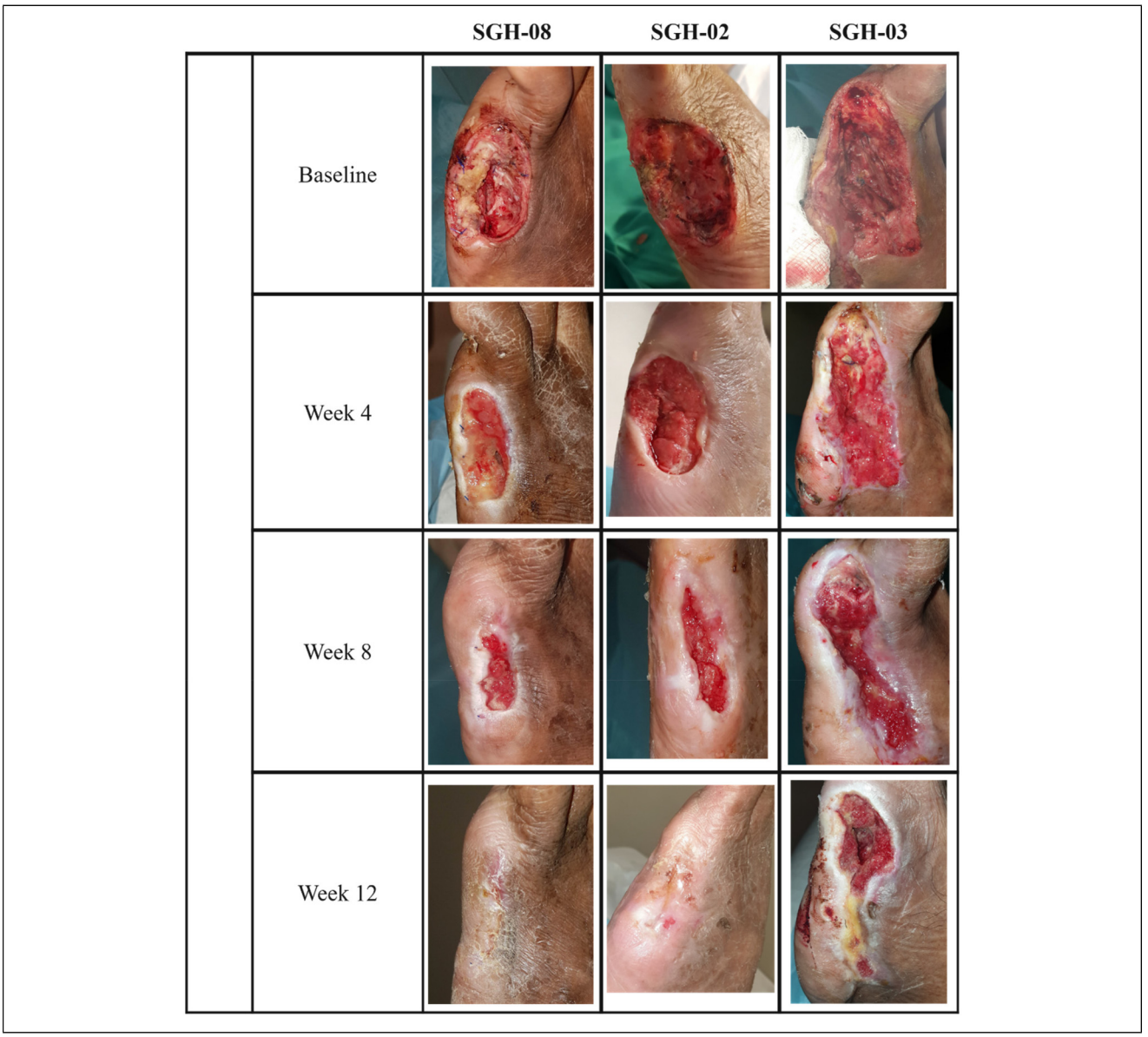

Figure 4. Example of wound healing course.

The mean follow-up after finishing the 3 months of TOT was 70.9 ± 33.0 days with no ulcer recurrence reported.

There were five withdrawals from the study ranging from 21 to 63 days after starting TOT. The reasons for withdrawal are stated in Table 2. 3/5 (60%) cases were due to physician choice and switching to vacuum assisted closure therapy based on wound healing progression and the rest were because of secondary infection, requiring further debridement and washout. Decision to not restart Natrox™ therapy post-surgery was also made by the physician in charge.

There were significant improvements between baseline and 3 months of the "leisure" and whether "bothered by the ulcer care" components of the DFS survey. Negative emotions improved between baseline and 2 months of treatment (Figure 5). Interestingly there was a significant difference in the "worried about ulcer" domain between those patients whose wounds had healed and those that didn't, across all time points.

Qualitative Observations From OTONAL

Wounds treated with TOT were qualitatively different from those with SOC dressings. The quality of granulation tissue was superior and less liable to contact bleeding. We also noted that the amount of exudate from the DFU increased

Table 2. AE Reporting and Withdrawals.

Subject ID	Duration of Natrox™ treatment (days)	% Wound Size Reduction	Withdrawal	Adverse Event	Event Description/Reason for withdrawal
SGH03/ TCK	90	83	No	Yes – Wound Infection	Maggots noted at index wound during follow-up visit. Wound was debrided, no overt signs of infection, nil pus expressed, nil surrounding erythema and nil overt warmth. Continued treatment but subsequent week noted sudden discolouration of toe. Further amputation required but went back on Natrox post-op.
SGH13/ CKC	35	65	Yes	Yes – Wound Infection	Admitted through ED for foot pain post 5 weeks of treatment. Noted to have a wound infection that subsequently required a forefoot amputation.
SGH16/ MNBA	21	0	Yes	No	No improvement in wound after three weeks of treatment. Physician decided to change dressing to VAC. Patient undergo major amputation 1 month later and subsequently died of sepsis.
SGH17/ RS	63	64	Yes	Yes – Wound Infection	Index wound was doing well, but developed a wound infection 9 weeks post treatment. Patient was switched to VAC therapy post-wound debridement.
SGH18/ LKK	63	30	Yes	No	There was no significant improvement in wound size despite 2 months of treatment. Physician's decision to switch to VAC therapy.
SGH19/ TKC	30	43	Yes	Yes – Wound Infection	Wound has shown good healing progress throughout the month. However, extensive infection was noted at follow-up. Patient subsequently undergo a major amputation.

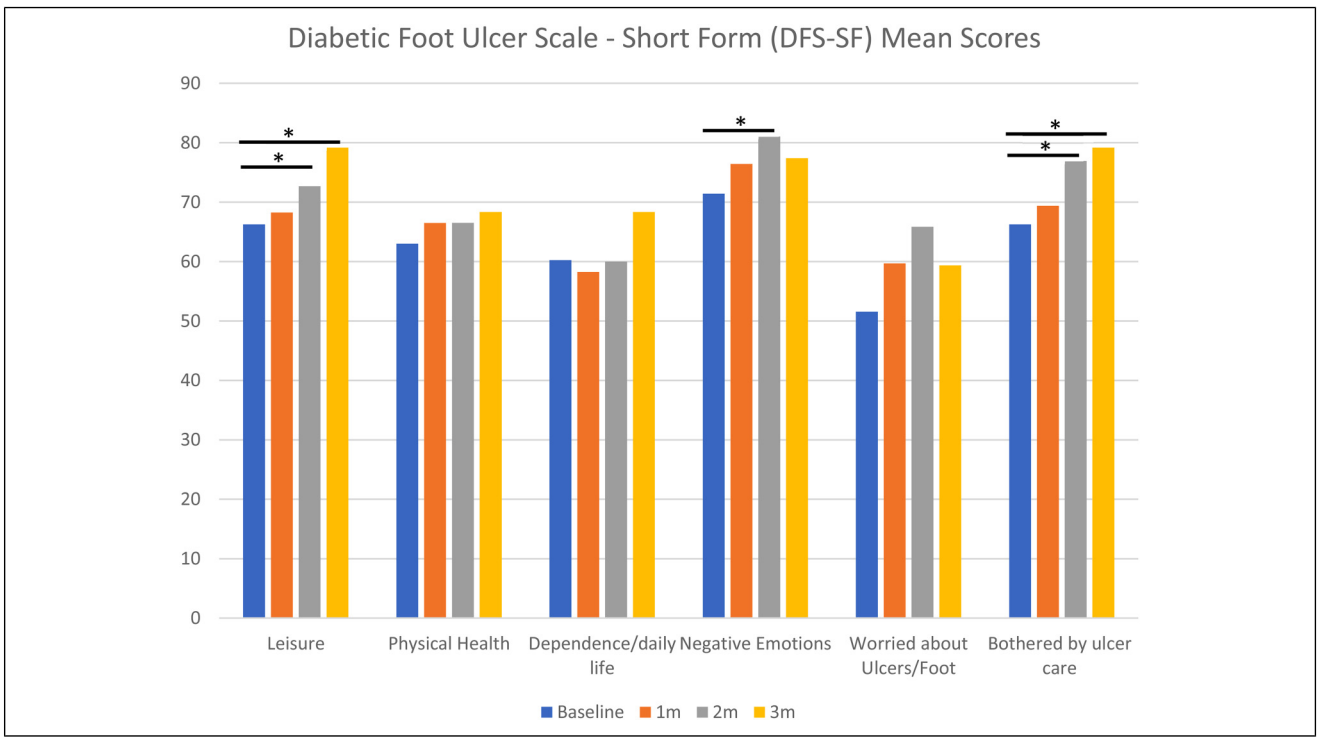

Figure 5. Diabetic foot ulcer scale-short form scores.

significantly in the first 2 weeks of treatment with TOT (yellow exudate), which typically preceded the granulation and contraction phases of the wound. To the inexperienced eye, this could look like worsening infection and could pre-empt stopping topical oxygen. To counter the increase in exudate, extra foam dressing was added to allow for added absorption. It is important to get some granulation tissue to appear before use of TOT and negative pressure therapy was a useful adjunct to help this process. Those wounds that did not heal had very little granulation before onset of TOT.

Discussion

DFU can be difficult to heal despite advances in wound therapy treatment options. Less than half of all new DFUs are closed by 3 months and even in specialised centres, only 22 to 30% of DFUs are healed by 5 months.[20,21] This can be explained by microvascular dysfunction and ischaemic neuropathy present in the diabetic foot, as well as mechanical changes in the foot architecture and an increased incidence of infection in this population.[22] The presence of microvascular disease (MVD) independently increases the risk of major lower extremity amputation (LEA) and synergistically increases the risk of limb loss in peripheral arterial disease patients with CLTI.[23] Microvascular dysfunction is a systemic phenomenon, and both retinopathy and nephropathy are associated with impaired skin microvascular function, poor wound healing and increased risk of major LEA.[24,25] Angioplasty surgery re-establishes the macrocirculation to the ankle but when patients have co-existing MVD, the microcirculation of the foot is not addressed. Currently, there is no surgical treatment to improve the micro-circulation of the foot among patients with MVD. Therefore, non-surgical treatments should be considered and trialed early once subtypes of CLTI involving MVD are established including TOT,[26] the rationale being that if oxygen delivery cannot be achieved from the vasculature, then it can be given directly onto the wound. The addition of TOT to SOC dressings has increased the DFU proportion healed at 3 months in several recently reported randomized controlled trials and *OTONAL* gives further credence to the efficacious role of TOT to close hard-to-heal chronic DFUs.[15,27,28]

The International Working Group on the Diabetic Foot (IWGDF) has recently published evidence-based guidelines on the use of oxygen to aid wound healing.[29] They suggest "consider(ing) the use of systemic hyperbaric oxygen therapy as an adjunctive treatment in non-healing ischaemic diabetic foot ulcers despite best standard of care (weak; moderate)" and suggest "not using topical oxygen therapy as a primary or adjunctive intervention in diabetic foot ulcers including those that are difficult to heal. (weak; low)". With recent new evidence in the form of RCTs and our data from Asian CLTI diabetic foot wounds,[14,15] this statement should potentially be revised moving forward.

We have shown that using a run-in period of 4 weeks to help enrich a population of wounds that are chronic and already difficult to heal, there was wound closure of >75% observed in 70.0% patients and over an 80% reduction in wound area after 12 weeks of TOT. Furthermore, average time for complete wound healing was just over 2 months, which is relatively short when there was minimal ulcer healing for either 4 to 12 weeks prior to enrolment. *OTONAL*'s trial design was similar to Serena *et al*'s multicentre RCT from the US, which also used Natrox™ device as their TOT. They showed that significantly more wounds in the patients who had SOC + TOT (44.4%) healed completely compared to the SOC group (28.1%) and that the wound area reduction was also better in the SOC + TOT arm (70% vs. 40%; P = .005). However, there was no significant differences in the changes of pain levels between the two groups. Limitations of the study included lack of blinding and a relatively high withdrawal rate of 18.6% like in our trial reflecting the complex nature of these patients and potential for multiple procedures and reinterventions for wound deterioration and co-morbidities. However, there are caveats to our study making our results all the more compelling. Firstly, the mean baseline wound size was much larger in our trial (11.3 vs. 2.9 cm^2) and we included more complex wounds with the majority (65%) being UTC Grade III in nature, which were more likely to heal completely than not – Serena's RCT only included Grade I and II wounds, majority being Grade I in nature. This shows that TOT can heal even larger and more complex DFUs than previously demonstrated in the same time frame of 3 months. Yu *et al* showed a 50% DFU healing rate with TOT in their Grade III ulcers although the duration of treatment was shorter (8 weeks) in their RCT.[14] Also, the majority of our patients (90%) had underlying CLTI and required pre-Natrox™ angioplasty to optimise blood flow to the foot. These patients with underlying peripheral artery disease are usually the most challenging because they are frail and are prone to cardiovascular adverse events and blood supply is already suboptimal to start with. A significant number (60%) also had ESRF and were on dialysis, which is an independent co-morbidity associated with poor wound healing and major LEA.[30] Kaufman *et al* recently suggested that using TOT prior to revascularization may be a helpful adjunctive treatment to reduce wound size prior to surgery and that longer treatment times achieved better wound healing outcomes.[13] A larger study of >4000 wounds treated with TOT also found a link between longer treatment times with TOT and improved wound healing.[31] The fact that Natrox™ is designed as a portable device and can be worn on an outpatient basis is therefore particularly advantageous for this reason. Also, given the clear increase in the

device's efficacy using a longer treatment regimen, it is important to show patience and not switch to another wound product because of lack of ulcer improvement at the start. 50% of our withdrawals were because physician decided to switch to VAC therapy and perhaps if they had waited longer, the wound would have begun to respond to TOT. As learnt from the study, it is important for the ulcer base to have some evidence of granulation tissue prior to TOT and VAC can be a helpful adjunct to accelerate this process. Finally, although offloading was achieved using dressings alone, there was no contact casting performed as this is not well tolerated by our patients in Singapore in a temperate climate. Another unique point of *OTONAL* is the QoL outcome measures reported, showing improvement between baseline and 3 months of several components of the DFS. The "leisure" aspect was markedly improved and this may be because of how mobile and flexible this allows the patient to be with the TOT device on.

Limitations

Although the rate of decrease in wound area was significant with TOT, this is a small, single centre study, with a relatively high withdrawal rate albeit with a tight prospective follow-up protocol in place. There was no placebo control group of SOC dressing alone to compare with. Furthermore, SGH is an experienced centre for diabetic foot salvage in CLTI patients, with established multidisciplinary protocols for endovascular revascularization and wound care, which may limit the reproducibility elsewhere. However, the patients included in this study are representative of daily clinical practice, where we deal with Asian CLTI patients who have chronic wounds that can be resistant to heal. This study has taken some time to recruit and complete and has been surpassed with several larger RCTs published to date. However, the population is unique and frail in that patients were type II diabetic with majority having ESRF and underlying CLTI with ulcers, which were relatively large and complex at baseline. The very significant costs of treatment of diabetic foot ulcers and the impact of major amputations on quality of life and life expectancy, suggest that Natrox™ treatment is likely to be cost effective, a conclusion that was also evident from the brief independent health economics study linked to a previous pilot study from Cambridge, UK.[17] However, input efficacy data for definitive economic modelling requires a larger, more appropriately-designed study. Unanswered questions regarding the use of Natrox™ therapy include:

1. what is the optimal oxygen flow through the tube to induce wound healing – should it be higher or lower than 15 ml/hr?
2. How many "ODS" are required for bigger wounds and what is the radial spread of oxygen under a wound dressing that may require multiple ODS use?
3. Which DFUs are best for TOT and at what stage should the wound be in before application of the device?
4. Can there be concomitant use of hyperbaric oxygen and TOT and under what instances? Can Natrox™ be used synergistically with other wound therapies such as topical haemoglobin to increase oxygen delivery to the chronic DFU?[32]

Conclusions

Use of TOT in chronic diabetic foot wounds stimulate a healing state, underpinning the concept that oxygen plays a central role in wound healing. Our results are more compelling if you consider that the cohort had a larger mean baseline wound size and complexity compared to other TOT studies and majority of patients were frail with underlying peripheral artery disease. This study adds to the growing body of evidence confirming TOT is effective in hard to heal chronic DFUs.

Author Contributions

TYT was primarily involved in study design, protocol development, and analysis of the data. MYQM, CJQY, JECB, SXYS, IABI, RWLL, XJS, WXG were involved in patient data collection. TYT drafted the manuscript, and all edited the final version. SLC aided with the data analysis and statistical prowess. All authors have read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: TYT has received travel and speaking honoraria from *Inotec AMD Ltd*.

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Ethical Approval

Not applicable, because this article does not contain any studies with human or animal subjects.

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