DIONYSIUS

TRIAL

“”Does Increasing Oxygen Nurture Your Symptomatic Ischemic Ulcer Sufficiently?”

Clinical Study Protocol

Version 1.1

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)
AE	Adverse Event
AR	Adverse Reaction
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CV	Curriculum Vitae
DFU	Diabetic Foot Ulcer
DSMB	Data Safety Monitoring Board
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)
HBOT	Hyperbaric Oxygen Therapy
IB	Investigator's Brochure
IC	Informed Consent
METC	Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)
MAMS	Multi-arm, multi-stage study design
PAOD	Peripheral arterial occlusive disease
(S)AE	(Serious) Adverse Event
SmPC	Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst
Sponsor	The sponsor is the party that commissions the organisation or performance of the research.
SUSAR	Suspected Unexpected Serious Adverse Reaction
UAVG	Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG
WMO	Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale: Diabetes is a major healthcare problem with a high incidence and morbidity. Diabetic foot ulcers (DFUs) are a major complication of diabetes, often associated with peripheral arterial occlusive disease. Currently available evidence shows HyperBaric Oxygen Therapy (HBOT) can reduce major amputation rate, but clinicians remain sceptical about the (cost-) effectiveness and feasibility of HBOT for ischemic DFUs in clinical practice. Therefore, international vascular surgeons and HBOT-physicians feel a strong need for a sufficiently powered clinical trial to determine whether and how many HBOT-sessions may be a (cost-) effective adjunctive treatment to ischemic DFUs.

Objective: The primary objective is to assess the (cost-) effectiveness of HBOT in addition to standard wound care and vascular surgical treatment for patients with a DFU and leg ischemia.

Study design: An international, multi-arm multi-stage (MAMS) design is chosen to conduct an efficient randomised clinical trial. At a planned interim analysis the best performing study arm(s) will be chosen to continue.

Study population: We need up to 544 patients with a Meggitt-Wagner stage 3 or 4 DFU and proven peripheral ischaemia.

Intervention: Patients will be randomised to receive standard care (wound treatment and surgical interventions following international guidelines) with either 0, 20, 30 or 40 sessions of HBOT. These sessions will compromise 90-120 minutes of HBOT at a pressure of 2.2-2.5 ATA according to international standards.

Main study parameters/endpoints: Primary endpoints are amputation rates and amputation-free survival after 12 months. Secondary objectives are wound healing, health-related quality of life and cost-effectiveness of the interventions.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: All patients will receive standard wound care and (if possible) endovascular or surgical revascularization, irrespective of their allocation. HBOT is a generally accepted and reimbursed indication for the treatment of DFUs. The risks of HBOT are regarded as low. Patients in the HBOT groups will undergo 20, 30 or 40 HBOT sessions. Patients will be asked to fill in questionnaires at four time points, and to score their pain weekly during the first 8 weeks. Three visits to their outpatient surgical clinic will be scheduled, which do not deviate from usual care.

1. INTRODUCTION AND RATIONALE

Diabetes is a major healthcare problem with an estimated incidence of 422 million people worldwide (WHO 2019) and 1.2 million in the Netherlands (Diabetesfonds 2019). Besides blindness, kidney failure, heart attacks and strokes, a major burden of diabetes is the occurrence of diabetic foot ulcers (DFUs). Diabetes is also often associated with peripheral arterial occlusive disease (PAOD). Despite optimal treatment, DFUs are the main cause of lower extremity amputations, especially in the presence of leg ischemia. Two out of three amputations are diabetes-related, with a yearly amputation rate of 2.5% for diabetic patients (Lombardo et al., 2014).

The treatment of diabetic foot ulcers (DFU) is an approved indication for hyperbaric oxygen therapy (HBOT), as acknowledged by the European, American and South-Pacific hyperbaric societies (EUBS, UHMS and SPUMS; Weaver 2014). Therefore, HBOT is reimbursed for this indication in many countries, including the Netherlands. The evidence for this effectiveness has been based on systematic reviews of (relatively few and small) clinical trials that included both ischemic and non-ischemic DFUs (O'Reilly 2013, Stoekenbroek 2014, Kranke 2015). Later systematic reviews, including more recent evidence, showed that HBOT appears effective for ischemic DFUs (Brouwer 2020), rather than for non-ischemic DFUs (Lalieu 2019). Hence, ischemia should be discerned when considering HBOT for DFUs (Brouwer 2020).

The largest and most recent DAMOCLES trial on HBOT for ischemic DFUs (Santema 2018a) showed some promising results for HBOT as an adjunctive treatment for patients with ischemic DFUs. However, the treatment effect was not convincing, due to the limited number of patients available for inclusion in a single country, as well as the inclusion of relatively superficial, uninfected (Meggitt-Wagner class 2) DFUs (Santema 2018b). They found 10% fewer major amputations after 12 months in patients treated with HBOT in their intention-to-treat analysis, but this was not statistically significant. In the per-protocol analysis a significant reduction of major amputations (17%) observed. Amputation-free survival (AFS) after HBOT was 13% higher in the intention-to-treat analysis, which was not statistically significant. In the per-protocol analysis the AFS was significantly (26%) higher as opposed to standard treatment. No significant effect was found on complete ulcer healing in neither the intention-to-treat nor the per-protocol analysis.

The recent meta-analysis (Brouwer 2020) of currently available evidence shows that HBOT may lead to a 15% reduction of major amputation rate, but shows no effect on minor amputation, CUH, or mortality. To date, substantial scepticism still exists among clinicians due to the limited and conflicting evidence for its (cost-)effectiveness to promote wound healing and prevention amputations or amputation-free survival. Hence, a strong need is felt internationally among vascular surgeons and HT-physicians for a sufficiently powered clinical trial that is able to determine whether HBOT is a (cost-)effective treatment for ischemic DFUs.

2. OBJECTIVES

The aim of the study is to confirm or refute the hypothesis that HBOT is (cost-) effective as an adjunctive treatment to standard wound care for patients with an ischemic DFU to prevent major amputations and to establish the optimal number of sessions to obtain this purported effect.

Primary Objective:
Amputation rate and amputation-free survival

Secondary Objective(s):
- Cost-effectiveness and budget impact
- Complete wound healing
- Health-related quality of life
- Pain scores
- Need for additional (vascular) interventions
- $TcpO_2$ before, during and after HBOT

3. STUDY DESIGN

Multinational randomized clinical trial with an adaptive (multi-arm, multi-stage; MAMS) design, with a follow-up of 12 months, following the (extended) CONSORT-statement. We will have four study arms and two stages (one interim and one final analysis; see figure 1 below). Patients will be randomized to receive standard treatment with 0, 20, 30 or 40 HBOT treatments.

The more efficient MAMS design (Sydes 2018, Sydes 2012, Wason 2012) for clinical trials includes a planned interim analysis, based on which only the best performing study arms will be chosen to continue. This will reduce the total number of patients required and the number of patients receiving an ineffective treatment, because after the interim analysis no patients will be included in less effective treatment arms.

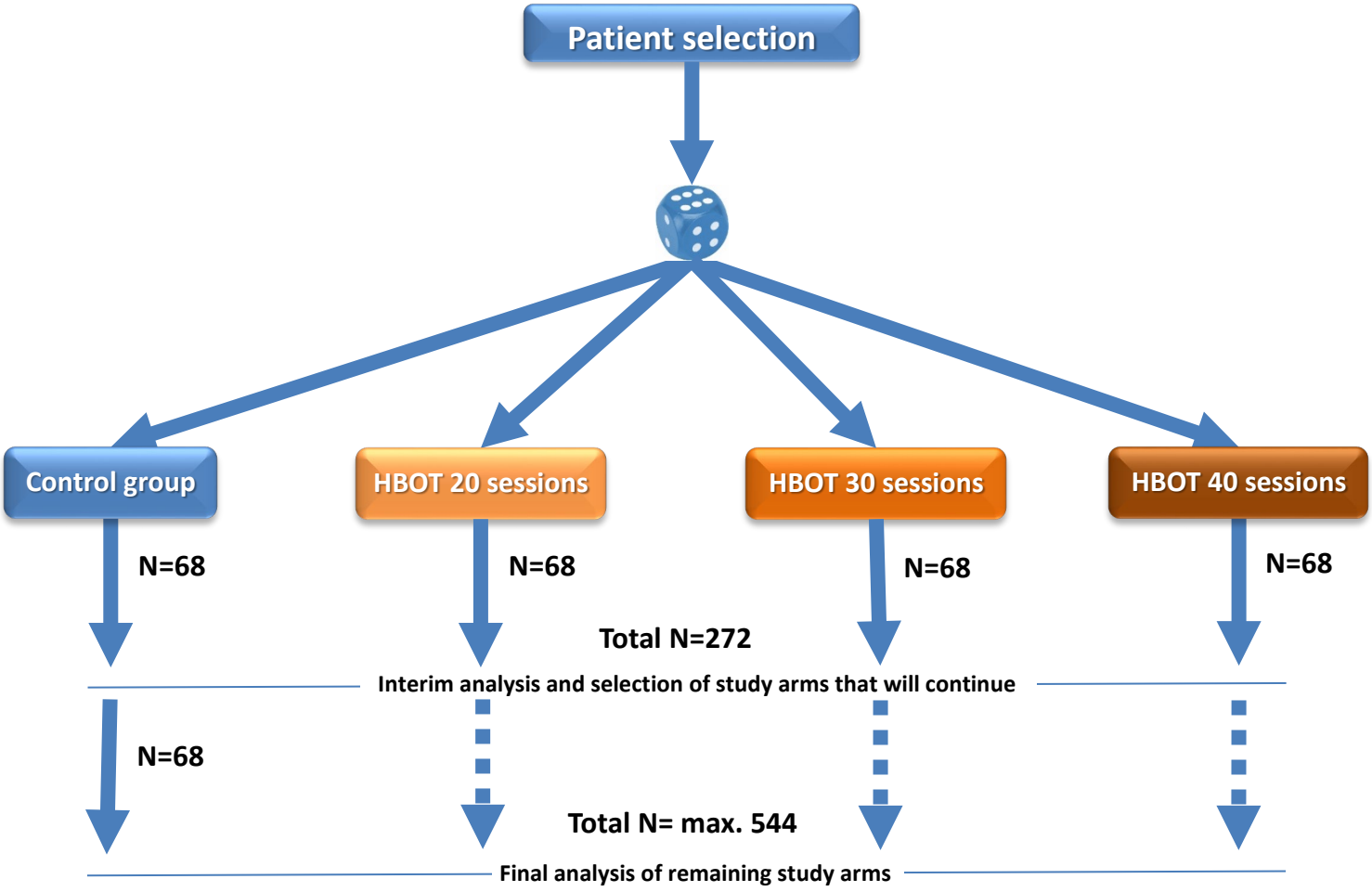

Figure 1 Flow of patient inclusion and analysis

4. STUDY POPULATION

4.1 Population (base)

Consecutive patients with type I or II diabetes, a DFU and leg ischemia, presenting at the vascular surgery departments of the participating hospital. Also, HBOT centers and general practitioners may refer patients to the vascular surgery departments for inclusion in the study. Both patients suitable and unsuitable for vascular repair are eligible for inclusion.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:
1. Type I or II diabetes
2. Meggitt-Wagner 3 or 4 lower extremity ulcer(s), present for at least 4 weeks or after a minor amputation because of a previously existing ischemic DFU on a toe or forefoot. In case more than one ulcer is present, the largest will be observed as target ulcer
3. Leg ischemia, characterized by a highest ankle systolic blood pressure < 70 mmHg, **or** a toe systolic pressure < 50 mmHg **or** a TcpO2 < 40 mmHg
4. Complete assessment of peripheral arterial lesions from the aorta to the pedal arteries with duplex ultrasonography, magnetic resonance angiography, computed tomography angiography and/or intraarterial digital subtraction angiography of the ipsilateral leg
5. Adults
6. Written informed consent

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:
1. Chronic Obstructive Pulmonary Disease (COPD) GOLD IV
2. Treatment with chemotherapy, immunosuppressive drugs or systemic corticosteroids within last 3 months, as this interferes with normal wound healing
3. End-stage renal disease requiring dialysis
4. Metastasized malignancy
5. Left ventricular failure with ejection fraction (EF) <20% or external pacemaker
6. Pregnancy
7. Insufficient proficiency of local language/English, or inability to complete the questionnaires

4.4 Sample size calculation

The primary outcome is the probability of amputations after 12 months. The meta-analysis showed a 15% difference (11% vs. 26%; Brouwer 2020) of amputations between patients treated with HBOT and patients treated conservatively. The sample size calculation is based on a one-sided type I error of 5% and a power of 90% to control for a type 2 error. A

generalization of the power requirements for testing multiple active treatments to one control group was used as proposed by Dunnett (Dunnet 1984).
The critical values for continuation of a treatment arm are odds ratios of 0.76 and 0.352, which are in accordance with a drop in amputations from 26% to 21% and to 11%. If the difference is <5% the HBOT treatment arm is deemed futile, while a difference of 15% or higher is deemed superior. Based on these critical values the required number of patients in each group in each stage is 68, leading to a required maximum number of 544 patients. The sample size calculation is based on a generalization of Whitehead and Jagi (Whitehead 2009, Jaki 2019). Taking into account a possible loss to follow-up of 5%, we will need a total of (up to) 573 patients.
This leads to the following stopping rules and possible scenarios after the interim analysis (using the chi-square test for differences in percentages between the groups) (see figure 2 below):

1) If one of the HBOT treatment groups leads to a significantly less than 5% improvement in amputation rate as compared to controls (based on the expected 26% vs 21% difference, i.e. an OR of 0.76), then this treatment group will be discontinued as it is futile.
2) IF a HBOT treatment group proves to decrease amputation rate significantly by >15% (26% vs 11%; OR: 0.352) as compared to controls, then superiority of a treatment arm is proven and the trial will be discontinued as it has reached its aim. If more than one HBOT-treatment arm shows this superiority, the group with the lowest number of HBOT treatments is deemed superior.
3) If the HBOT group(s) show(s) minor improvements in amputation rate (between 5% and 15%) over control, then these groups will continue in the second stage of the trial.

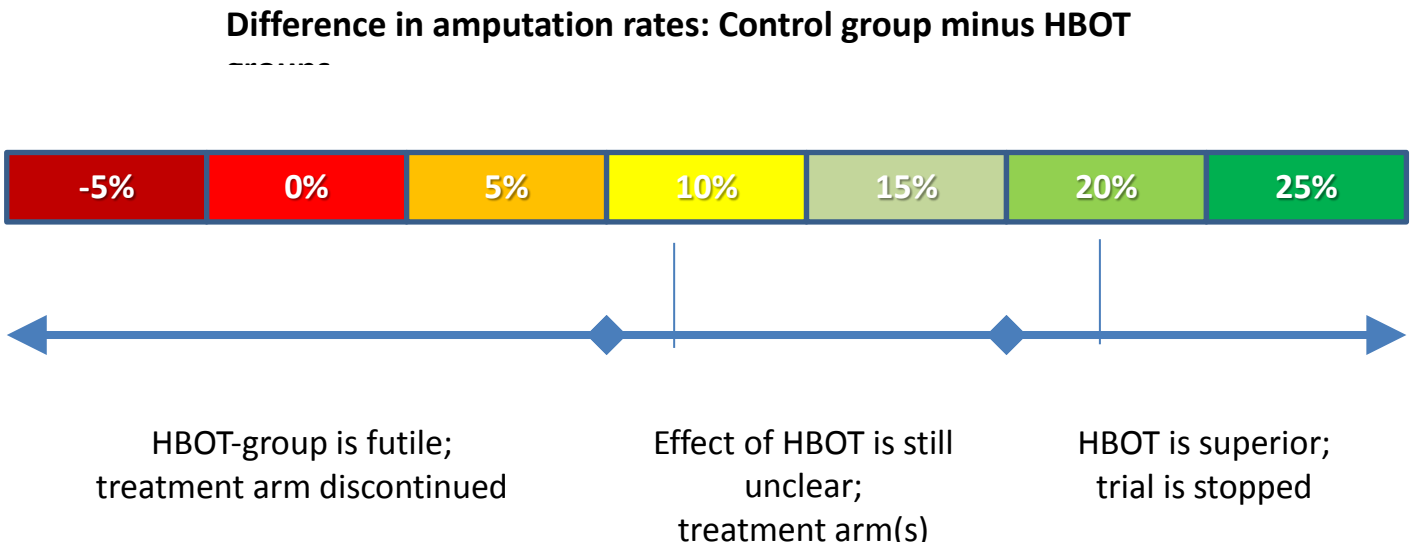

Figure 2 Possible scenarios at the interim analysis

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

Patients allocated to the intervention group will receive 20, 30 or 40 sessions of HBOT adjunctive to standard care, as determined by the randomisation. A HBOT session will take 90 to 120 minutes at 2.2-2.5 ATA. Besides the 90-120 minutes of treatment, approximately 20 minutes are required for compression and decompression. During the treatment session the patients will breathe 100% FiO_2 except for 3 blocks of 5 minutes during which atmospheric air will be administered to prevent oxygen intoxication. This HBOT-regimen has been approved by the hyperbaric medicine specialists from all contributing HBOT-centers as the optimum and safest HBOT procedure for these patients. Patients will either lay in bed or sit in a chair during the session. At least for the first time, cabin personnel will accompany patients in the cabin and instruct them about the use of the oxygen masks or hoods. The cabin will be pressurized gradually in about 10 minutes. Subsequently, the supervising staff member will supply patients with masks through which the oxygen will be administered. The staff member will then leave the cabin, but will remain in constant contact with the patients through cameras and microphones. In case of adverse events requiring termination of the session, patients will be able to leave the cabin after a short decompression period. After the treatment, the cabin will be decompressed in about 10 minutes.
HBOT will be administered in a hyperbaric chamber. HBOT is preferably given 5 times per week, but at least 3 times per week, until the total number of sessions has been reached. To minimize interruption of HBOT and masking of the effect by additional vascular interventions, any (additional) (endo)vascular procedure will preferably be performed after randomisation but before the start of HBOT treatment. In case a vascular intervention is required (due to progression of the disease) while the patient is undergoing the HBOT sessions, these are interrupted for the period of the vascular intervention and continued directly afterwards until completion of the sessions.

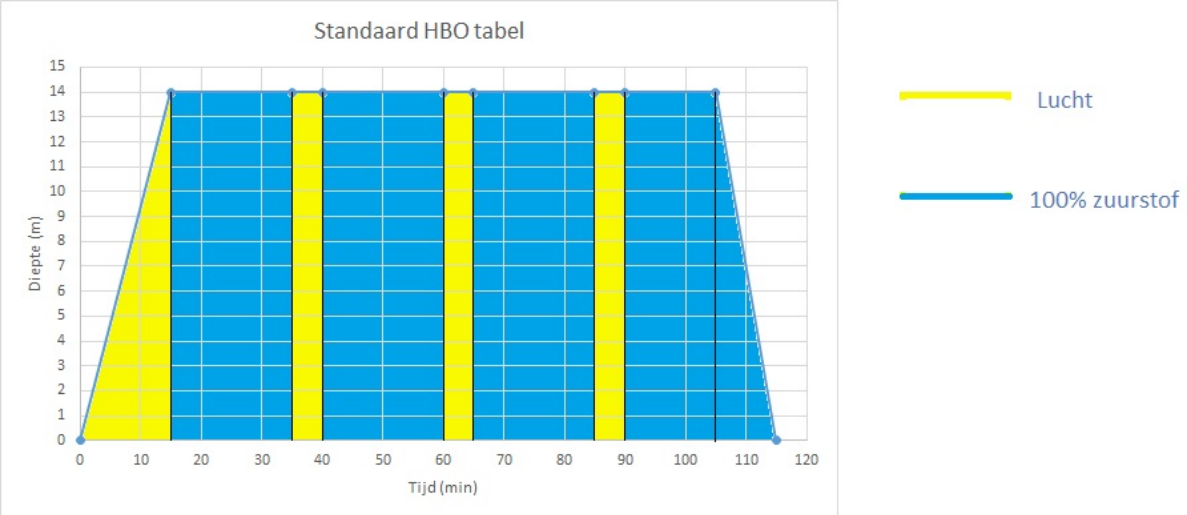

Figure 3 Example of HBOT tabel

5.2 Use of co-intervention (if applicable)

HBOT will be given in addition to standard care according to local best practice in expertise centers and will not intervene with the usual care. Although (vascular) interventions are preferably performed before the start of HBOT, participation in the study will not influence any additional interventions that might be needed during follow-up ((endo)vascular interventions, amputations, wound debridement, medication, etc).

5.3 Escape medication (if applicable)

Not applicable.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

In this trial, oxygen will be used under hyperbaric conditions. In addition, 100% oxygen will be administered. Both can be either cryogenic preserved oxygen or compressed oxygen.

6.2 Summary of findings from non-clinical studies

For the scope of this study the finding in non-clinical studies are not relevant, since HBOT is already widely implemented in humans and clinical studies are available.

6.3 Summary of findings from clinical studies

HBOT has a variety of mechanisms of action: it improves tissue oxygenation; inhibits the pro-inflammatory reaction by reducing cytokines; improves neo-vascularization; has a bacteriostatic effect on anaerobic bacteria and stimulates stem cells and growth factors (Camporesi 2014). There are 14 indications of HBOT approved by the UHMS, including the treatment of air or gas embolism, arterial insufficiencies, carbon monoxide poisoning and gas gangrene (Weaver 2014). Also see the attachments D1, page 1.

6.4 Summary of known and potential risks and benefits

Risks: HBOT might cause middle ear and sinus barotrauma, myopia and epileptic seizures; see the structured risk analysis (see chapter 12).
Benefits: A recent meta-analysis (Brouwer 2020) shows evidence that HBOT can prevent major amputation in patients with ischemic DFUs.

6.5 Description and justification of route of administration and dosage

Patients will undergo sessions of 90 to 120 minutes under 2.2-2.5 ATA according to international treatment standards (Weaver 2014). Usually every 20 minutes an air break will be implemented, where patients only breathe room air. This reduces the risks of oxygen toxicity. This falls within the limits of standard use of 1.4-3.0 ATA for 45 to 300 minutes (attachments D1, page 2).

6.6 Dosages, dosage modifications and method of administration

Depending on local protocols, patients will breathe 90 to 120 minutes of HBOT under 2.2-2.5 ATA. The total number of sessions will vary between 20 and 40, depending on the randomisation.

6.7 Preparation and labelling of Investigational Medicinal Product

The oxygen has to be prepared and labelled according to the standard operation procedures of the AMC (attachment K6 SOP). All centers have to record the quality certificates of the oxygen used for patients included in the trial.

6.8 Drug accountability

The shipment, receipt, disposition, return and destruction of oxygen will not be affected by this study. All HBOT-centers already have to record the quality certificates of the oxygen used for patients included in the trial.

7. METHODS

7.1 Study parameters/endpoints

The effectiveness of HBOT will be calculated from three different perspectives: Wound-related, patient-related and society-related.

- Wound-related outcome measures include limb salvage (major amputation rate), minor amputation rate, amputation-free survival and (time to) complete ulcer healing.
- Patient-related outcomes measures will be measured by a common disease-specific questionnaire (Vascuqol), and a generic questionnaire (SF-12), and a pain score (VAS)
- From a societal perspective, outcomes relevant for the calculation of the cost-effectiveness, cost-utility and budget impact will be measured. (volumes and treatment costs, EQ-5D-5L questionnaire)

7.1.1 Main study parameter/endpoint

- Major amputation rate (above ankle) and (major) amputation-free survival after 12 months of follow-up

7.1.2 Secondary study parameters/endpoints (if applicable)

- Complete wound healing
- Time to reach complete wound healing
- Freedom from minor amputation (below ankle)
- Pain score (VAS)
- Health-related quality of life scores
  - EQ-5D-5L
  - SF-12
  - Vascuqol-6
- Costs related to HBOT and conventional therapy during follow-up period
- Patients' out of pocket expenses related to disease or treatment (travel expenses etc.)
- Mortality
- Patients' perception of improvement (through one anchor question)
- Forefoot $TcpO_2$ before, during and after (HBOT) treatment (if equipment is available)

7.1.3 Other study parameters (if applicable)

- Complications directly related to HBOT treatment (e.g. middle ear and sinus barotrauma, epileptic seizure, myopia)

7.2 Randomisation, blinding and treatment allocation

Randomisation will be performed through Castor Electronic Data Capture (Amsterdam, The Netherlands), which also ensures concealed treatment allocation. Stratification will be performed for center and whether or not a patient is included with a minor amputation.

7.3 Study procedures

Patients from each (remaining) study arm will be monitored up to 36 months after randomisation. At the start of the trial, patients' baseline characteristics will be obtained. Pain scores will be collected through first phase of the trial. Questionnaires will be completed at baseline and will be sent to the patients' home after 3 months and 1 year.

Amputation rate, ulcer recurrence, additional interventions, mortality, adverse events and costs will be monitored after 1 year and 3 years.

Assessment	Before randomisation	Week 1-8, once a week	After 3 months	After 1 year	After 3 years
Patient characteristics	*				
Meggitt-Wagner classification	*		*	*	
Wound measurements	*				
Ankle blood pressure	*		*	*	
Toe systolic pressure	*		*	*	
TcPO2	*		*	*	
VAS		*	*	*	
Freedom of amputation			*	*	*
Complete ulcer healing			*	*	*
Recurrent ulcer			*	*	*
Nr of additional vascular events		*	*	*	*
Adverse events		*	*	*	
Patient's out of pocket expences			*		
Questionnaires	*		*	*	*

Tabel 1 Assessments and follow-up

7.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The (local) investigator can decide to withdraw a subject from the study for urgent medical reasons.

7.5 Replacement of individual subjects after withdrawal

Subjects who withdraw from the study will not be replaced. We already adjust the sample size for an anticipated loss to follow-up of 10% in the sample-size calculation to keep sufficient power.

7.6 Follow-up of subjects withdrawn from treatment

Patients who withdraw from the study for any reason will not be followed conform ethical and legal protocols. They may continue their regular treatment (including HBOT if desired) follow-up moments.

7.7 Premature termination of the study

The study will be ended prematurely if the DSMB advises to stop the study for safety or superiority reasons (see figure 2).

8. SAFETY REPORTING

8.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

8.2 AEs, SAEs and SUSARs

All adverse events (AEs), serious adverse events (SAEs) and suspected unexpected serious adverse events (SUSARs) will be registered. The local investigator has to inform the coordinating investigator within 7 days when a SAE or SUSAR occurs.

8.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the experimental intervention. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

8.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for

SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

8.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
   - Summary of Product Characteristics (SPC) for an authorised medicinal product;
   - Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.
The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

8.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.
This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

8.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

8.5 Data Safety Monitoring Board (DSMB)

An independent DSMB, consisting of a statistician, an diabetologist and an independent vascular surgeon, is established prior to the start of the trial. The purpose of the DSMB is to advise the DIONYSIUS-trial investigators whether it is safe to continue the study. Interim-analyses will be performed after inclusion of 25%, 50% and 75% of the patients. The advice(s) of the DSMB will only be sent to the sponsor of the study. Should the sponsor decide not to fully implement the advice of the DSMB, the sponsor will send the advice to the reviewing METC, including a note to substantiate why (part of) the advice of the DSMB will not be followed. If a risk is detected that endangers patients, the study will be terminated prematurely.

9. STATISTICAL ANALYSIS

All analyses will be conducted according to the intention-to-treat principle. Baseline characteristics are summarized with descriptive statistics. Differences between dichotomous variables (like the amputation rate) will be expressed as a risk differences and Numbers Needed to Treat or Harm. Statistical precision will be expressed as 95% confidence intervals. Differences between continuous variable will be expressed as difference in means. If outcome parameters are unevenly distributed, they will be expressed as medians and inter-quartile ranges.

9.1 Primary study parameters

At the interim analysis, statistical analyses of the differences in amputation rates between the study arms will be conducted using the Chi-square statistic.
At the final analysis, major amputation rates and AFS will also be estimated using Kaplan-Meier survival analysis and differences between the groups will be analysed using the log-rank test.

9.2 Secondary and other study parameters

Differences in (semi-)continuous variables between the two treatment groups (such as the VAS, $TcpO_2$ and quality of life scores) will be analysed with the Student t-test in case of normally distributed data and otherwise the non-parametric Mann-Whitney U test, after correcting for baseline differences, if any.
Time to complete wound healing will be estimated using Kaplan-Meier survival analysis and differences between the groups will be analysed using the log-rank test. Changes over time of TcpO2 and quality of life scores will be analysed using the Wilcoxon test. Potential confounding factors will be adjusted using Cox proportional hazards regression modelling.
The economic evaluation of additional HBOT will be performed as a cost-effectiveness analysis from a societal perspective. Additionally, a cost-utility analysis will be performed with the costs per quality adjusted life-year (QALY) as outcome. As the time horizon is restricted to 12 months, no discounting (of costs and effects) will be performed.
The EQ-5D is used to generate health status scoring profiles over time, which will subsequently be translated in QALYs by applying time trade-off based health utility algorithms and assuming that interpolation between successive measurements best reflects a patient's health status during the year. Sensitivity analyses will be performed to account for sampling variability, for plausible ranges in unit costs, and for different health

utility algorithms. In addition, a budget impact analysis will be undertaken to assess the possible cost savingsof HBOT if applied to all eligible patients on a national scale.

9.3 Interim analysis

After inclusion of 68 patients in each group and three months of follow-up, an interim analysis will be conducted (see figures 1 and 2). From our previous trial (Santema 2018) we know that the vast majority of amputations occurs within three months of follow-up. Whether and how the trial will continue will depend on the outcome of the interim analysis, which is the main feature of the MAMS study design. This is described in chapter 4.4.

10. ETHICAL CONSIDERATIONS

10.1 Regulation statement

The DIONYSIUS trial will be conducted according to the principles of the Declaration of Helsinki (version of Seoul, 2008) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other guidelines, regulations and Acts.

10.2 Recruitment and consent

Patients will be recruited by their vascular surgeon in one of the participating hospitals and will be informed both verbally and through the patient information form (attachment E1). They will be asked for written informed consent (attachment E2) by a local investigator, or a co-worker in the contributing center who is not the vascular surgeon of the patient, in accordance with the guidelines of the medical ethics review board.

10.3 Objection by minors or incapacitated subjects (if applicable)

Not applicable

10.4 Benefits and risks assessment, group relatedness

All patients enrolled in this trial will receive the maximum vascular, endovascular or conservative (antibiotics, anticoagulants, glycaemic control) and local wound treatment according to best practice. The possible effect of adding HBOT tot the standard treatment may result in major benefits such as an improved limb salvage and higher wound healing rates. HBOT is regarded as a low-risk therapy.

10.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO. The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.
The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

10.6 Incentives (if applicable)

All Dutch recruiting centers receive 100 euro for each patient that completes the 12-month follow-up period (and data are recorded completely) as a compensation for their effort.

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Handling and storage of data and documents

All patient data will be stored in an eCRF in Castor, including a unique study number which will be allocated to each patient. This number identifies the patient and must be reported on all web-based case forms and questionnaires. The handling of personal data will be according the EU General Data Protection Regulation and the Dutch Act on Implementation of the General Data Protection Regulation (in Dutch: Uitvoeringswet AVG, UAVG). Patient data will only be available to the principal and coordinating investigators. The data will be stored for 15 years.

11.2 Monitoring and Quality Assurance

This study will be monitored following the current guidelines. Also, a DSMB is composed (see chapter 8.5). A full monitoring plan will be sent as soon as the funding for this study is obtained. A DSMB charter is added as attachment L5.

11.3 Amendments

A ‘substantial amendment’ is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

11.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

11.5 Temporary halt and (prematurely) end of study report

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

11.6 Public disclosure and publication policy

At least one full scientific article will be prepared by the coordinating and principal investigators. All local investigators will be acknowledged in the final manuscript when they include any number of patients. Co-authorship will be granted if they comply with the ICMJE guidelines.
The study results will be presented at national and international meetings by the coordinating and/or principal investigator. The subsets of data from each individual contributing center may be used for quality improvement purposes by the local investigator(s).

12. STRUCTURED RISK ANALYSIS

12.1 Potential issues of concern

a. Level of knowledge about mechanism of action

There is a high level of knowledge regarding the working mechanisms of HBOT. It has a variety of mechanisms of action: it improves tissue oxygenation; inhibits the pro-inflammatory reaction by reducing cytokines; improvers neo-vascularization; has a bacteriostatic effect on anaerobic bacteria and stimulates stem cells and growth factors (Camporesi 2014).

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

HBOT is widely used and implemented for 13 different indications (Weaver 2014)

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

The therapy does not target specific cells, but achieves its effect by an increased pO_2.

d. Selectivity of the mechanism to target tissue in animals and/or human beings

The therapy does not target specific cells, but achieves its effect by an increased pO_2.

e. Analysis of potential effect

See appendices D1. HBOT can cause sinus or middle ear barotrauma, myopia or an epileptic attack. Very high doses of HBOT over a prolonged period can cause central nervous system symptoms and pulmonary oxygen toxicity, which in very rare cases can be fatal (Camporesi 2014). The doses used in this study are much lower, in accordance withthe SmPC (appendix D1), and widely implemented.

f. Pharmacokinetic considerations

See attachments D1.

g. Study population

Patients with ischemic DFUs will be included. Patients with pregnancy will be excluded.

h. Interaction with other products

Also see appendices D1. Pulmonary toxicity of bleomycine, amiodarone, furadantine (and similar antibiotics) can be increased by high doses of oxygen. Patients using these drugs are excluded from participation.

i. Predictability of effect

There is no biomarker to predict the effect of HBOT. A forefoot Transcutaneous PO_2 measurement could be used to predict the changes of successful treatment (Fife 2002).

j. Can effects be managed?

There is no direct antidote or antagonist. When a patients shows signs of pulmonary toxicity or has an epileptic attack, the treatment will be immediately stopped. For barotrauma an ENT specialist will be consulted, who might place eardrum tubes if necessary.

12.2 Synthesis

HBOT is considered a cumbersome but low risk therapy. It has been implemented for a variety of indications and is routinely used, also for the treatment of (ischemic) DFUs. The risk of oxygen intoxication is minimised by using a well-known safe treatment window.

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