Effect of Hyperbaric Oxygen on Persistent Post-Concussive Symptoms after Mild Traumatic Brain Injury (HOINPCS)

Lead Investigator: Olayinka David Ajayi, MD, MPH

Funded by: State of North Dakota, Dakota Medical Foundation

July 7, 2025

Version 1.9

NCT# 05173818

PROTOCOL DEVELOPMENT TEAM

Lead Investigators (LI):

Olayinka David Ajayi, MD, MPH
Principal Investigator, Essentia Health

Study Team:

Marilyn Klug, PhD
Co-Investigator, Statistician, University of North Dakota

Richard Ferraro, PhD
Co-Investigator, Psychologist, University of North Dakota

Paulina Kunecka, MD
Co-Investigator, Neurologist, Essentia Health

Rebecca Quinn, MSW, LMSW
Co-investigator, Licensed Clinical Social Worker, Center for Rural Health, University of North Dakota

Effect of Hyperbaric Oxygen on Persistent Post-Concussive Symptoms after Mild Traumatic Brain Injury

A. Introduction
   - Study abstract
   - Primary hypothesis
   - Purpose of the study protocol

B. Background
   - Prior literature and studies
   - Rationale for this study

C. Study Objectives
   - Primary aim
   - Secondary aims
   - Rationale for the selection of outcome measures
   - Study limitations

D. Study Design
   - Overview or design summary
   - Subject selection and withdrawal
     - Inclusion criteria
     - Exclusion criteria
     - Ethical considerations
     - Subject recruitment plans and consent process
     - Randomization method and blinding
     - Risks and Benefits
     - Early withdrawal of subjects
     - When and how to withdraw subjects
     - Data collection and follow-up for withdrawn subjects
   - Study Drug (Intervention)
     - Description
     - Treatment Regimen
     - Method for assigning subjects to treatment groups
     - Administration of study intervention
     - Subject compliance monitoring
     - Blinding of study intervention

E. Study Procedures
   - Screening for eligibility
   - Schedule of measurements
   - Study outcome measurements and ascertainment
   - Safety and adverse events
     - Safety and Compliance Monitoring
     - Medical Monitoring (Investigator only, Institutional Data and Safety Monitoring Board)

- Definitions of Adverse Events
- Classification of Events (severity and expectedness)
- Data collection procedures for adverse events

F. Statistical Plan
- Sample size determination and power
- Hypothesis
- Interim monitoring and early stopping
- Planned interim analysis and stopping rule
- Analysis plan and statistical methods
- Missing outcome data
- Unblinding procedures

G. Data Handling and Record Keeping
- Confidentiality and security
- Training
- Records retention
- Participant unblinding

H. Study Administration
- Organization
- Funding Source and Conflicts of Interest
- Subject stipends or payments
- Study timetable

I. Publication Plan

References

Attachments – Study Documents

- Informed Consent documents
- Questionnaires, logs, or surveys

A

INTRODUCTION

Study abstract

The objectives of this study are to determine whether hyperbaric oxygen improves neuropsychological status and ameliorates symptoms in adults with persistent post-concussive symptoms (PPCS) following mild traumatic brain injury. Persistent post-concussive symptoms (PPCS) are a source of significant burden among a subset of patients with concussion with prevalence rates previously reported between 11 – 82% based on timing of assessment, diagnostic criteria, or population under study.

Current literature has largely been from military population with its result mostly equivocal. One recent study suggests patients with concurrent post-traumatic stress disorder (PTSD) as likely to benefit from hyperbaric oxygen.[1] The same study also reported a loss in initial clinical benefits reported among patients treated with hyperbaric oxygen compared to sham/control after 13 weeks. Most studies to date have been unable to conclusively eliminate potential placebo effect experienced among patients in the sham arm. As such, additional studies such as this proposed study are needed to include non-military population and remove the effect of a "therapeutic sham" while maintaining the positives from previous studies.

In addition to the proposed randomized, controlled trial; we plan to recruit subjects from the general population as opposed to strictly military population and use a sham regimen that excludes any pressure or oxygen therapeutic effect. We will describe changes from baseline values at specific time interval from the intervention. This information will be useful in refuting or advocating a therapeutic role of hyperbaric oxygen treatments in the management of post-concussive syndrome among civilian populations.

Hypotheses

The main hypothesis of the proposed research is "hyperbaric oxygen will be an effective and tolerable treatment in improving neuropsychological status among adult patients with PPCS". The secondary hypotheses are hyperbaric oxygen relieves post-concussive related symptoms and reduces serum levels of a novel concussion biomarker, neurofilament light chain.

Although the main hypothesis has been previously studied, this study is distinct from its predecessors because it recruits participants from the civilian & military populations, uses RBANS as its primary outcome measurement, a validated screening tool used in dementia and other forms of brain injury not used hitherto, in previous randomized control trial that investigated the effectiveness of hyperbaric oxygen in the management of PPCS.[9-10] Furthermore, this study includes an objective secondary endpoint - endpoint, the neurofilament light chain; this study also attempts to eliminate a therapeutic sham arm.

Purpose of the study protocol

The purpose of this study protocol is to describe how the clinical trial will be conducted while ensuring the safety of study participants and integrity of the data collected.

B

BACKGROUND

Prior literature and studies

Hyperbaric oxygen therapy (HBO_2) as defined by the Hyperbaric Oxygen Committee of the Undersea and Hyperbaric Medical Society (UHMS) entails breathing 100% oxygen while inside a chamber pressurized to 1.4 absolute atmosphere (1.4 ATA) or more. The FDA and UHMS recognize and, have endorsed HBO_2 as either primary or adjunctive treatment for several conditions such as gas gangrene, decompression sickness, necrotizing soft tissue infection, carbon monoxide poisoning, crush injuries, intracranial abscess and selected non-healing diabetic foot ulcers. It has also been used as off-label for treating patients with acute, sub-acute and chronic traumatic brain injury with varying degree of success and is not considered part of the standard of care for these patients.

A concussion or mild traumatic brain injury is caused by disruption in the brain's delicate homeostatic balance following a blow or jolt to the head with accepted varying severity of experienced clinical sequelae including loss of consciousness for 0-30 minutes, alteration in consciousness for up to 24 hours and amnesia lasting up to 24 hours. Higher-resolution neuroimaging may show abnormalities, but this is not true in some cases.

Concussion among non-military population is usually associated with motor vehicle collision, sport-related injuries and falls among the elderly. Approximately 2.87 million TBI-related emergency department visits, hospitalizations and deaths were reported in the United States in 2014.[2] Mild traumatic brain injury (concussion) accounts for at least 75% of all traumatic brain injuries.[3] Estimates of the incidence of developing post-concussive symptoms after a mild traumatic brain injury is within a range of 10% to 25% with the actual range depending on timing of assessment, diagnostic criteria and population under consideration.[4-6] Most cases resolve within the next 7-10 days. Other patients experience re-emergence of symptoms after weeks of relief.[7]

Patients with symptoms after three months are considered as persistent post-concussive symptoms (PPCS) and they are the focus of this research. These constellations of symptoms continue to adversely affect their quality of life. Examples of persistent post-concussion symptoms include balance problems, headaches, fatigue, poor concentration, forgetfulness, anxiety, irritability, and sleep disturbance. Few proposed therapies have proved successful in the management of persistent post-concussion symptoms following mild TBI.

Table 1. Classification of Mild, Moderate and Severe TBI

Mild TBI	Moderate TBI	Severe TBI
Normal structural imaging	Normal or abnormal structural imaging	
Loss of consciousness 0 - 30 minutes	Loss of consciousness > 30 minutes and < 24 hours	Loss of consciousness > 24 hours
Alteration of consciousness for a moment up to 24 hours	Alteration of consciousness for > 24 hours. Severity based on other criteria	
Post-traumatic amnesia 0 - 1 day	Post-traumatic amnesia > 24 hours & < 7 days	Post-traumatic amnesia > 7 days
Glasgow Coma Scale 13 - 15	Glasgow Coma Scale 9 - 12	Glasgow Coma Scale 3 - 8

Current treatment approach for post-concussive syndrome includes neurocognitive rehabilitation therapy: education and support, reassurance, graded increase in activity and cognitive restructuring. Other forms of treatment are driven by the patient's primary complaints and include antidepressants, vestibular rehabilitation, visual training, treatment of cervical spine dysfunction and acetylcholinesterase inhibitors for patients with memory impairment. Overall, these treatment options have resulted in limited success. The Department of Veterans Affairs and Department of Defense issued a clinical practice guideline for management of concussion, including post-concussive syndrome (persistent post-concussive symptoms) following mild traumatic brain injury, yet there is no treatment standard of care.

The role of hyperbaric oxygen in the management of traumatic brain injury is an area of active interest and research. There is an ongoing adaptive phase II multi-centered RCT (HOBIT trial) investigating the efficacy of hyperbaric oxygen in the management of severe TBI. Table 2 shows previous clinical studies that have explored the role of HBO_2 among patients with mild traumatic brain injury with persistent post-concussive symptoms with the vast majority among military population and, the results suggesting no effect of HBO_2 on persistent post-concussive symptoms following mild TBI.

Table 2. Hyperbaric Oxygen Therapy and Persistent Post-concussive Symptoms Trials

Study	Design	Intervention	Criteria	Result
Brain Injury and Mechanism of Action of HBO_2 (BIMA) 2012 - 2016	Randomized, double-blinded, sham-controlled, multicenter Military N – 71 (36:35)	Intervention: HBO_2 @ 1.5 ATA, 100%; Sham @ 1.2 ATA, 21% 40 treatments, 60 minutes each	>3 months - < 5 years Loss/decreased level of consciousness < 30 min Loss of memory / alteration in mental status < 24 hours 3 or more post-concussive symptoms Stable on concomitant medications > 30 days	Significant differences between HBO_2 & sham at week 13 for RPQ-3, PCL-C, CVLT-II Long delay cued recall at week 13

Study	Design	Intervention	Criteria	Result
Hyperbaric Oxygen for Persistent Post-concussive symptoms after mTBI (HOPPS) 2011 - 2012	Randomized, double-blinded, sham-controlled, multicenter Military N – 72 (24:25:23)	Intervention: HBO_2 @ 1.5 ATA, 100%; Sham @ 1.2 ATA, TBI standard care no HBO_2 40 treatments, 60 minutes each	>3 months - < 5 years Loss/decreased level of consciousness < 30 min Loss of memory / alteration in mental status < 24 hours 3 or more post-concussive symptoms Stable on concomitant medications > 30 days	No difference between HBO_2 & sham for RPQ but both improved, PCL-C more improved in sham, No improvement in local care group
Hyperbaric Oxygen therapy for post-concussive symptoms after mTBI (DARPA/VCU) 2011 - 2013	Randomized, double-blinded, sham-controlled Single center Military N – 61 (19:21:21)	Intervention: HBO_2 @ 2.0 ATA, 100% O_2; HBO_2 @ 2.0 ATA, 75% O_2; sham @ 2.0 ATA, 10.5% O_2 40 treatments, 60 minutes each	At least one mild TBI < 3 years prior to screening, PCS for at least 3 months Mild TBI definition per DoD	No significant difference for RPQ and PCL-M or any neuropsychological or self-report measures
Cognitive Function in a traumatic Brain Injury HBO_2 (USAFSAM) 2009 - 2011	Randomized, double-blinded, sham-controlled Single center Military N – 50 (25:25)	Intervention: HBO_2 @ 2.4 ATA, 100% O_2; Sham @ 1.3 ATA, 21% O_2 30 treatments, 90 minutes each	Neurologist-confirmed TBI diagnosis based on DoD definition of TBI Psychiatric status stable for 2 or more months, stable psychotropic medication history 1 or more months Injury more recent than 10/2001	Improvement with HBO_2 vs. sham for PCL-M in PTSD subgroup, no significant difference between both groups.
HBO_2 can improve post-concussion syndrome years after mild TBI – Randomized Prospective Trial	Prospective, randomized, controlled, 2-group crossover trial. Practically, this was a delayed	Intervention: HBO_2 @ 1.5 ATA, 100% vs. No HBO_2 in cross-over group 40 treatments, 60 minutes each	Less than 30 minutes loss of consciousness Mild TBI occurred 1-5 years prior to inclusion	Significant improvements were demonstrated in cognitive function & quality of life in both groups, but no

Study	Design	Intervention	Criteria	Result
2008 – 2012 Boussi-Gross & Efrati	intervention / incomplete crossover trial Non-military N – 56 (24:32)	2nd period Crossover group receives HBO_2 @ 1.5 ATA, 100% 40 treatments, 60 minutes each	Persistent PCS and impaired cognition > 1 year No brain damage from neuroimaging No changes to cognitive function one month prior to the beginning of the study	significant improvement was observed following the control period Elevated brain activities in agreement with the cognitive improvements on SPECT
A Phase I Study of low-pressure HBO_2 for blast-induced PCS and PTSD Harch PG et al.	Proof-of-concept pilot study No control group Case series Military N - 16	Intervention: HBO_2 @ 1.5 ATA, 100% Treated twice a day 40 treatments, 60 minutes each	Mild-to-moderate TBI characterized by loss of consciousness Blast injury	Improvement in neurological exam, PCS symptoms, WMS IV delayed memory, WMS-IV working memory, stroop test, TOVA impulsivity & variability, Grooved Peg-board, RPQ, PTSD symptoms & depression

Rationale for this study

Critiques of previous studies include underpowered for efficacy, limited randomized controlled trials among civilian population and noise around a potential therapeutic sham arm.

Previous studies have been underpowered for efficacy but included small sample size to calculate variances, identified the best parametric fit of response distributions and calculated correlations and associated confidence intervals between responses and changes in responses on neuropsychological, neurological & neuroimaging measures.[8]

Traditionally, patients in the sham group have been exposed to 1.2 - 1.3 ATA (25% equivalent of oxygen) that provides more oxygen than inspired while breathing air at sea level (20.9% equivalent of oxygen). Additionally, there is a possible therapeutic pressure effect from exposing patients to pressure greater than 1.0 ATA. It is difficult to exclude some "crossover / blunting" effect when patients in the sham group are treated at higher oxygen equivalent and ambient pressure than what is expected by breathing air at sea-level. This may contribute to the difficulty in separating participation effects (placebo effect) from true therapeutic effects.

It is also pertinent to note that > 80% of all studies investigating the role of HBO_2 on mild TBI recruited subjects from the military sub-population.

We propose a new study powered for efficacy with a non-therapeutic sham arm and recruitment of patients from the general population. The objectives of this study are to determine whether hyperbaric oxygen improves neuropsychological status and ameliorates symptoms in adults with persistent post-concussive symptoms (PPCS) following mild traumatic brain injury.

Study Limitation

Composite and surrogate measures present a universal drawback in post concussive research because the outcome in interest (post-concussive symptoms) may be numerous, vague and difficult to assess in each patient. Surrogate endpoints are indirect outcome measures that may not correlate with clinical response. We have included serum neurofilament light chain as an objective direct secondary endpoint to mitigate this limitation.

Composite outcome measures may lead to bias, make it difficult to interpret individual components with the composite score and/or lead researchers to select convenient components to report after the study. We have determined the components of each composite endpoint that will be used in our analysis in a theoretical fashion and will report results for the components of each composite outcome measure in addition to the composite score.

C

STUDY OBJECTIVES

Primary aim

The objectives of this study are to determine whether hyperbaric oxygen improves neuropsychological status in adults with persistent post-concussive symptoms (PPCS) following mild traumatic brain injury

Secondary aims

Secondary aims of this study:

- To describe the side effects of hyperbaric oxygen and determine discontinuation of HBO_2 due to side effects.
- To compare changes in persistent post-concussive symptoms as documented along sleep related, PTSD related, quality of life related and neurobehavioral symptoms among adults with PPCS treated with hyperbaric oxygen versus standard care.
- To compare changes in a novel serum concussive biomarker, neurofilament light chain, among patients treated with hyperbaric oxygen versus standard care.

Rationale for the selection of outcome measures

We have chosen our outcome measures based on results from previous studies. We chose 'Repeatable battery for the assessment of neuropsychological status' (RBANS) because it is a validated screening tool used in medical conditions with neuropsychological components such as mild traumatic brain injury and it measures several items that may be compromised in PPCS

such as memory and attention.[9-10] Other reasons for using RBANS include short duration of administration in a busy setting and alternate forms to track improvement while avoiding content practice effect (when a participant gets higher scores due to familiarity with the content of a questionnaire with subsequent tests).[9-10] We have included secondary outcome measures based on recommendations from the Brain Injury and Mechanisms of Action of Hyperbaric Oxygen (HBO_2) for Persistent Post-Concussive Symptoms after Mild Traumatic Brain Injury (BIMA study). These include Neurobehavioral Symptom Inventory (NSI), PTSD measure for civilian population, a sleep questionnaire, and a Quality-of-Life measure.[1]

Based on recent studies, we have also added a potential prognostic serum biomarker for post-concussive symptoms, neurofilament light chain. Serum neurofilament light chain is a novel potential biomarker in concussion that can detect concussion, estimate its severity and assist with predicting recovery.[11,12] Due to its objectiveness, measuring serum neurofilament light chain allows this study to circumvent issues around surrogate / composite outcome measures. We expect a decrease in the level of serum neurofilament light chain with hyperbaric oxygen treatments but are cautious to propose an expected change in its level because our assessment at this point is exploratory. The outcome measures of this study are expected to contribute to our understanding of the role of hyperbaric oxygen in post-concussive symptoms.

Table 3. ENDPOINTS

Objectives	**Endpoints**	**Justification for endpoints**
Primary		
To investigate the effect of hyperbaric oxygen in patients from the general population with persistent post-concussive symptoms due to mild trauma brain injury.	Repeatable battery for the assessment of neuropsychological status (RBANS). This is a validated screening tool initially used to assess dementia but has been extended to other neuropsychological conditions, including mild traumatic brain injury. Advantages includes short administration time, alternate forms, co-normed index scores and inclusion of a summary score.	RBANS can assess a broad range of independent neuropsychological domains, a characteristic of mild traumatic brain injury (affects different domains such as sleep, cognition). Alternate forms of RBANS allow for repeated evaluation and sensitivity to milder brain impairment. It is easy to administer. It has also been found to be more sensitive to TBI-related deficits that other cognitive screening tools such as MMSE and Neurocognitive Status.[9,10]
Secondary		
To describe any effect of hyperbaric oxygen as an adjunct therapeutic option across specific domains affected by mild Traumatic Brain Injury		
Sleep	Pittsburg Sleep Quality Index. It is a validated self-rated	Persistent post-concussive symptoms following mild TBI

Objectives	Endpoints	Justification for endpoints
	subjective questionnaire comprising 19 individual questions that generate seven "component" scores {sleep quality, latency, duration, efficiency, disturbances, use of sleep medication and daytime dysfunction}. <5 indicate good overall sleep & >5 indicate poor sleep with a diagnostic sensitivity & specificity of 89.6% & 86.5% respectively.	usually affects several domains and patients could present with sleep disturbances, cognitive deficits, headache, dizziness, and behavioral changes. Any interventional study on mild TBI should consider each domain to proof efficacy (or otherwise). As a result, we have included a validated sleep outcome measure (PSQI). Significant changes in PSQI scores will support HBO_2 role in managing mild TBI.
Neurobehavioral Symptoms	Neurobehavioral Symptom Inventory (NSI). The NSI is a 22-item validated self-report measure of post-concussion symptoms, grouped into 4 cluster (somatosensory, cognitive, affective and vestibular). A total score and 4 cluster scores can be obtained.	Based on BIMA results, the NSI is a reasonable simple primary outcome for future studies.[1] It has been recommended as a common data elements outcome measure for TBI. Minimal clinical changes in the total and/or cluster scores supports effectiveness of HBO_2 in the management of post-concussive symptoms.
Quality of Life (QoL) Questionnaire	World Health Organization Quality of Life -BREF (WHOQOL-BREF). This is a practical abridged version of WHOQOL-100 that includes 26-item grouped into four domains (physical health, psychological, social relationships, environment). It has been shown to have good psychometric properties. Its brevity makes it suitable for brief assessments of QoL where there are other questionnaires & measures each participant needs to complete.	As aforementioned in sleep outcome measure section, persistent post-concussive symptoms affect multiple domains and frequently affects quality of life. Improvement in QoL could be the single most practical change to some patients and it supports effectiveness of HBO_2 as a therapeutic option in persistent post-concussive symptoms. It was also suggested as an outcome measure for future studies following the exhaustive BIMA.[1]
Post-traumatic Stress Disorder (PTSD) Questionnaire	PTSD-Civilian version (PCL-C). This is a 17-item validated self-report measure that assesses PTSD symptoms. It is further subcategorized into three clusters (reexperiencing, avoidance, hyperarousal). A	Persistent post-concussive symptoms are often non-specific and overlap with those of PTSD. Some patients with mild TBI may also have concurrent PTSD. The BIMA study reported more beneficial effect

Objectives	Endpoints	Justification for endpoints
	total score and cluster scores can be obtained based on DSM-IV-TR criteria	of HBO_2 among patients with mild TBI and concurrent PTSD.[1] This requires further study and external validation, hence its inclusion in our study.
To describe any changes to prognostic biomarkers for post-concussive symptoms	Neurofilament Light chain (NfL). This is a protein marker of neuro-axonal damage that can be measured in serum and cerebrospinal fluid, which allows for repeated measurements.	Recent studies have suggested that serum NfL has better diagnostic and prognostic utility than other neuro-axonal damage biomarkers such as GFAP, tau, UCH-LI in patients with TBI. There is Class III evidence that it distinguishes patients with mild TBI from healthy controls.[11,12] To date, no hyperbaric oxygen-related interventional study in patients with mild TBI has included NfL as an outcome measure. We have included this because changes in its levels is an objective measure of efficacy and it could serve as the focal point for future interventional studies in this field.

D

STUDY DESIGN

Overview or design summary

In this randomized controlled study, we will enroll 100 patients with persistent post-concussive symptoms as defined by persistence of concussion-related symptoms beyond the normal course of recovery (set as 3 months) following an initial mild traumatic brain injury. Each participant must have documented persistent post-concussive symptoms as diagnosed by a neuropsychologist or neurologist. Participants will be randomized to standard treatment plus sham-control or standard treatment plus hyperbaric oxygen treatments. All RCT participants will have baseline neurobehavioral, sleep, quality of life, PTSD evaluations and neurofilament light chain evaluations and subsequent evaluations as outlined by the study's timeline. The timeline stipulates repeating the evaluations at 13 weeks, 6 months, 12 months and 18 months post-intervention.

In the sham control arm of the study, we will enroll 50 participants with persistent post-concussive symptoms as defined by persistence of concussion-related symptoms beyond the

normal course of recovery (set as 3 months) following an initial mild traumatic brain injury. Each participant must have documented persistent post-concussive symptoms as diagnosed by a neuropsychologist or neurologist. These potential participants must meet all the same inclusion and exclusion criteria as the treatment arm except for not receiving the study's therapeutic agent - hyperbaric oxygen. Participants in this arm will have baseline neurobehavioral, sleep, quality of life, PTSD evaluations and neurofilament light chain evaluations and subsequent evaluations as outlined by the study's timeline.

Subject selection and withdrawal

Inclusion Criteria

For the standard treatment plus hyperbaric oxygen treatment arm of the study, the inclusion criteria are as follows:

1. Volunteers must have a history of mild traumatic brain injury, specifically, persistent post-concussive symptoms after examination by a neuropsychologist or neurologist.
2. Volunteers time from concussive incident (Mild traumatic brain injury) must be between 3 months – 5 years following head trauma incident
3. TBI was caused by non-penetrating trauma
4. Volunteers must have current complaints of TBI symptoms such as headache, dizziness, or cognitive or affective problems
5. Volunteers must be 18-year-old or more
6. Willing and able to provide informed consent
7. Able to speak and read English, as primary language
8. Agrees to provide blood samples for clinical lab tests
9. Willing and committed to comply with research protocol and complete all outcome measures
10. Able to equalize middle ear pressures or willing to undergo needle myringotomies if needed
11. Based on participant's reproductive status at time of enrollment, the following are required:
    1) **A combination of two contraceptive methods will** be required for non-pregnant women that are of reproductive potential and hetero-sexually active during the period of exposure to hyperbaric oxygen. These methods include:
        - Barrier method of contraception such as condoms with or without spermicidal agents (male partners or female), diaphragm or cervical cap with spermicide
        - Intra-uterine device (hormonal or non-hormonal)
        - Hormone-based contraceptive
    2) **A combination of two contraceptive methods will** be required for non-pregnant women who are of reproductive potential but not hetero-sexually active during the period of exposure to hyperbaric oxygen. These methods include:
        - Barrier method of contraception such as condoms with or without spermicidal agents (male partners or female), diaphragm or cervical cap with spermicide
        - Intra-uterine device (hormonal or non-hormonal)
        - Hormone-based contraceptive

3) No contraceptive requirement for participants (men and women) with very low or no reproductive potential

Pregnancy Testing will be done at the following period/conditions:

1) For all women of reproductive potential at baseline prior to randomization
2) End of the 4th week of hyperbaric oxygen therapy
3) End of study intervention (systemic exposure to hyperbaric oxygen therapy)
4) Additional test for delayed menstrual period (over one month between menstruations)

Contraception should be maintained during study intervention period (8 weeks of hyperbaric oxygen treatments). Lactating women are not excluded from this study.

Inclusion criteria are the same for both arms.

Exclusion Criteria

For the standard treatment plus hyperbaric oxygen treatment arm of the study, the exclusion criteria are as follows:

1. Prior treatment with hyperbaric oxygen treatment
2. Hyperbaric chamber inside attendant, professional, SCUBA diver (technical, commercial, operational/military or recreational)
3. We exclude pregnant women from this study because potential fetal adverse reactions triggered by high oxygen partial pressures such as retrolenticular fibroplasia though careful review of human data are generally reassuring such as the use of hyperbaric oxygen to treat acute carbon monoxide poison in the pregnant patient.[13]
4. Prisoners
5. Minors
6. Unable to protect airway or requires frequent suctioning
7. Known or suspected peri-lymphatic fistula
8. Presence of tracheostomy (due to limitations in auto-inflating the middle ear)
9. Unable to participate fully in outcome assessments
10. Unable to cooperate with hyperbaric oxygen treatments
11. Unable to speak and read English, as primary language
12. Not cooperating with instructions during hyperbaric oxygen treatments such as bringing contraband items into the chamber (such as fire lighter)
13. Not competent to sign their own consent. They will not have the capacity to participate in a full range of neurocognitive testing
14. Pre-existing diagnosis of a psychotic disorder(s) that is not stable: schizophrenia, bipolar, dissociative disorder. To be stable, patient must have been on medication(s) and/or therapy for the psychotic disorder for more than 30 days and his/her treating provider agrees the patient is medically stable
15. History of moderate or severe traumatic brain injury
16. History of brain injury not of traumatic etiology such as stroke or drug-induced coma
17. Verifiable diagnosis of learning disability. Other pre-existing cognitive issues may make it difficult for potential participants to fill out certain outcome assessments such as

neurobehavioral symptomatic inventory. This could lead to measurement bias or confound our result.
18. Claustrophobia and unwilling to enter hyperbaric chamber
19. Untreated pneumothorax
20. Presence of implanted device with un-verifiable test to pressure by manufacturer
21. Concurrent enrollment in an alternate interventional trial for post concussive symptoms

Exclusion criteria for standard treatment plus sham control arm is the same with the standard treatment group plus hyperbaric oxygen treatment.

Ethical considerations

Exclusion of Vulnerable population

We will not include children younger than 18-year-old in our study. We will also exclude prisoners or pregnant women in this study.

Subject recruitment plans and consent process

Patients receiving care within Essentia Health (West Market) with persistent post-concussive symptoms diagnosis will be referred to neurology clinic at Essentia Health Fargo for a clinic visit with any neurologist (preferably Dr. Kunecka) to assess diagnostic qualification for possible enrolment in the study. Referral may be made from any of Essentia Health's outpatient clinic, hospitals across Fargo-Moorhead area. Essentia Health Fargo neurology clinic will contact Hyperbaric research nurses with the referred patient's details to complete an eligibility check prior to visiting Dr. Kunecka (or any other Essentia Health Fargo neurologist). Dr. Kunecka will inform the patient if he/she meets diagnostic criterion and complete the screening process consent with the patient. This is the 1st consent for the study. Dr. Kunecka will use the screening consenting process for patients she deemed qualified to be in the study based on diagnostic and eligibility criteria. Research nurses may also complete an eligibility check for any patient that was referred directly to the hyperbaric department before sending qualifying patients to Dr. Kunecka for diagnostic screening purposes. Hyperbaric research nurses will let the potential participants know that their next visit with Dr. Kunecka will assist with confirming or refuting they have persistent post-concussive symptoms and inform the patient it is a randomized trial with 8-week period of intervention and 18-month total study time.

Post Screening Consent: Dr. Kunecka and/or a delegate from her team will inform the hyperbaric medicine research staff through a direct phone call to the research phone (701.364.8505) or an email directed to either hyperbaric research nurses on any participants that agrees to and provides consent to the screening consent. Hyperbaric research nurse(s) will contact any patient that has agreed and signed the screening consent with the intention of scheduling the patient for other study activities, namely: evaluation by hyperbaric medicine physician and/or principal investigator, completing baseline assessments (primary and secondary endpoints), blood draw (neurofilament light chain and pregnancy test, if applicable), hyperbaric

nursing visit, main informed consenting and chest x-ray. Participants (patients) that provide consent to the main informed consent form will be randomized via RedCap afterwards.

Patients that meet screening criteria and sign the screening consent will be referred by Dr. Kunecka or anyone she delegates from the neurology clinic, to the hyperbaric department for continuation of the enrolment process with the hyperbaric medicine physician (Principal Investigator). Hyperbaric physician will assess the patient for medical fitness in the hyperbaric environment. Hyperbaric research nurses will immediately follow the hyperbaric physician's visit with nursing related assessments. Hyperbaric medicine physician and/or hyperbaric research nurses will complete a more detailed research consent (combined biomedical and HIPAA consent). Patients that consent (sign and date) the 2nd consent form will be sent to graduate studentto undergo baseline assessments; the lab for neurofilament light chain blood draw and radiology department to get a chest X-ray unless the PI decides not to get a chest x-ray.

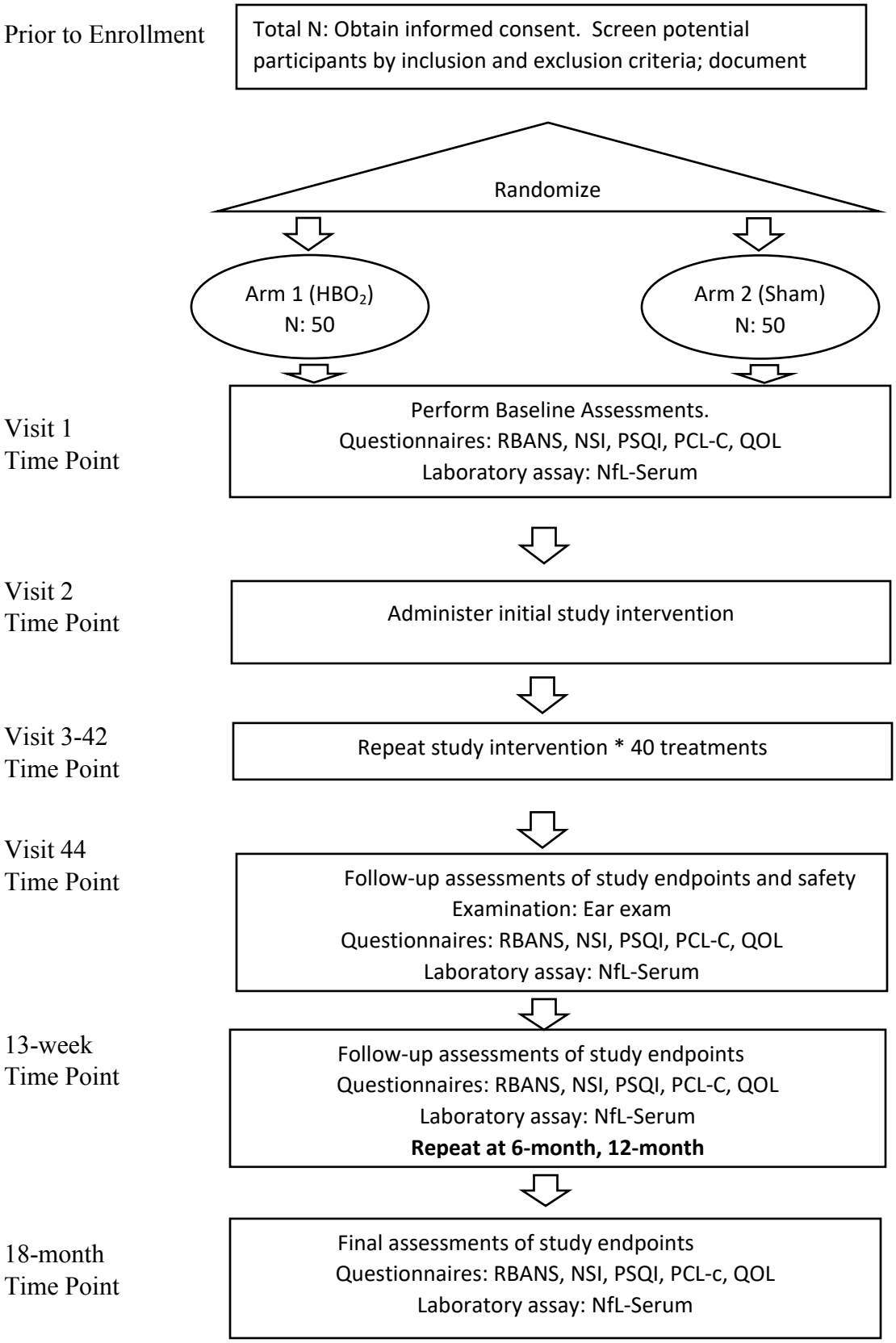
Patients can also be referred from external health facilities in the state of North Dakota or surrounding communities but will be evaluated by a member of the research team (neuropsychologist or neurologist) to confirm diagnosis and referred to study coordinator to ascertain appropriateness for inclusion into the study.

North Dakota Brain Injury Network may also serve as a source of external referral for the study. Potential patients should be referred to Essentia Health Fargo neurology clinic with a sentence stating the reason for referral is "Persistent Post concussive symptoms and possible enrollment in the Hyperbaric Oxygen Therapy study". Mild traumatic brain injury that occurred less than 3 months to presentation will not be included in the study. Patients meeting study criteria will be approached by a member of our research team for consent at the conclusion of their clinical visit. Patients who meet study criteria except being symptomatic (PCS) for less than 3 months will be followed in subsequent weeks and re-considered if their post concussive symptoms persist beyond 3 months, at which point, they will be deemed approachable for study consent. Eligible patients interested in the study will have the study explained to them, consent explained, reviewed and questions answered in a confidential manner in a private consulting room.

Randomization method and blinding

Participants will be randomized after their consult visit with the hyperbaric medicine physician, hyperbaric medicine nurse(s) (HBO Nurse) and have provided consent for the study. Randomization will be done via REDCap and will be based on gender and age. HBO Nurse will select/click the randomization section in REDCap to initiate and complete the randomization process. Post randomization, patients will be identified on the chamber console with either HOINPCS 1 (sham group) or HOINPCS 2 (treatment group) protocols.

Figure 1. Flow diagram



Risks and benefits

Hyperbaric oxygen treatments are administered at 2.0 – 3.0 atmosphere absolute ATA for durations of 90 minutes or longer for FDA-approved disease conditions. Exposure at these levels is usually well tolerated even for critically ill patients.[14] Potential adverse events of HBO_2 are usually related to pressure effects such as middle ear and sinus barotraumas, air embolism and pneumothorax; other adverse events related to oxygen toxicity includes ocular changes, neurologic and cardiovascular effects. Claustrophobia is also a recognized adverse event. At the proposed pressure and duration (1.5 ATA for 60 minutes), one would expect the incidence of oxygen toxicity to be lower than in patients undergoing clinical hyperbaric oxygen treatments for currently approved medical conditions.

A potential benefit is decreased symptoms (frequency or intensity) among participants randomized to the hyperbaric oxygen arm. There is a global benefit of increased knowledge surrounding HBO_2 role in the management of post concussive symptoms. Currently, there is no gold standard or standard comparators for HBO in TBI.

Early withdrawal of subjects

Participants will be informed that their participation in this study is voluntary at the time of consent. They will also be informed at time of consent that they may discontinue participation without penalty at any time.

When and how to withdraw subjects

Withdrawal due to subject's decision:

- Participants may decide to withdraw from the research at any time by notifying the principal investigator (PI) and/or another member of the research team by call the office at 701-364-8505 and letting the office staff know the participant needs to speak with the PI and/or member of the research team. The investigator will clarify whether the participant wishes to withdraw from all components of the trial or only a specific part of the trial.

Withdrawal due to Principal Investigator's decision:

- If participant were to have a serious adverse reaction to hyperbaric oxygen, the investigator would recommend discontinuation.
- To protect a participant from excessive risk or to maintain integrity of the data (an example includes a participant not following study procedures or intentionally providing false information), investigator may recommend discontinuation
- If the investigator terminates a participant's enrolment in the trial, the investigator should explain to the subject the reason(s) for this action and provide other treatment options as appropriate

Data collection and follow-up for withdrawal subjects

Data collected prior to subject withdrawal may be retained and used in a manner that is consistent with the study protocol and supplemental documents, unless the subject notifies the investigator that all participant information must be removed from the study.

Study drug

Description

Oxygen is a drug by FDA and given at atmospheric absolute (ATA) pressure greater than 1.4, is considered the active ingredient in hyperbaric oxygen therapy. It entails breathing >99.9% oxygen intermittently or continuously while pressurized inside a hyperbaric chamber at a pressure of at least 1.4 ATA.[15] Theoretical premise for using hyperbaric oxygen in mild traumatic brain injury include addressing chronic hypoxia & ischemia and augmenting neuroplasticity through mobilization of stem cells that may alter the biochemical messengers within the secondary penumbra region of the primary injury. A single pre-clinical study demonstrated benefits of hyperbaric oxygen (HBO_2) in rats with chronic unilateral cortical contusion. Rats that received 1.5 ATA for 90 minutes, twice a day for a total of 80 sessions had improvement in spatial learning and increased vascular density in the hippocampus.[16]

Treatment Regimen

HBO_2 is currently used off label with a physician's prescription for patients with brain injury. A typical course is hyperbaric oxygen 100% oxygen at 1.5 ATA for 60 minutes, given once or twice a day with a total of 40 or more sessions. Previous studies have used this regimen with two exceptions (2.0 ATA, DARPA/VCU study; 2.4 ATA, USAFSAM). There are concerns that > 2.0 ATA may be toxic to healing brain because the brain tissue has a lower threshold for oxygen toxicity. Two arms of this study will use the following regimen:

A. Standard Care* and sham for 60 minutes, once a day with a total of 40 sessions. Sham treatment includes pressurizing the chamber to 1.2 ATA with air for 5 minutes with noise of circulating air, return the chamber to 1.0 ATA after 5-7 minutes and maintain it at that pressure for until the end of the treatment, with noise of circulating air. Internal control knobs will be concealed. Participants will be instructed to undergo intermittent ear clearing technique during the initial 5 minutes of "descent"
B. Standard Care* and hyperbaric oxygen therapy at 1.5 ATA for 60 minutes, once a day with a total of 40 sessions. Participants will be instructed to undergo intermittent ear clearing technique during the initial 5 minutes of "descent"

* Standard Care: Patients will not be forbidden to use other therapies during the trial period. Information about these therapies will be documented by completing the periodic "concomitant medication log" and "alternative therapy questionnaire" built into the protocol. We define *standard care* as current appropriate, acceptable, and

widely used treatment plan for patients with post concussive symptoms; this usually entails symptomatic management of the most worrisome complaints.

There will be no treatments (study intervention) on Federal Public Holidays.

Method for Assigning Subjects to Treatment Groups

Participants will be randomized after their consult visit with the hyperbaric medicine physician, hyperbaric medicine nurse(s) (HBO Nurse) and provide consent for the study. Randomization will be done via REDCap and will be based on gender and age. HBO Nurse will select/click the randomization section in REDCap to initiate and complete the randomization process. Post randomization, patients will be identified on the chamber console with either HOINPCS 1 (sham group) or HOINPCS 2 (treatment group) protocols.

Preparation and Administration of Study Drug

The choice of sham-control and blinding we propose is to prevent therapeutic pressure effect, increased oxygen effect while keeping participants and non-HBO_2 technician research staff blinded. The sham-control arm will be exposed to 21% oxygen with 1.2 ATA pressure in the first 5 minutes of the session with noise of circulating air. The participant will be exposed to 21% of oxygen at 1.0 ATA during the next 50 minutes of intervention. Noisy circulating air will be introduced from the manifold during the final 5 minutes of the treatment. This sham-protocol replicates all experiential aspects of HBO_2 therapy, including a pressure sensation and noise of air circulation. All participants will be instructed to undergo intermittent ear clearing technique during the initial 5 minutes of "descent". All participants will have otoscopic examination after each treatment.

Subject Compliance Monitoring

Protocol deviations

A member of the research team will be expected to complete a protocol deviation log for each participant at baseline assessment, randomization, during their hyperbaric oxygen treatments, at 13-week post intervention, 6-month post intervention, 12-month post intervention and 18-month post intervention. Participants will also be contacted by a member of the research team (by phone) at 4/5-month post intervention and 8/10-month post intervention to answer questions related to protocol deviation. Each participant's response will be recorded into the study's password protected, electronic de-identified database.

Concomitant medications

Participants concomitant medications will be reviewed periodically as outlined here. All participants will be expected to complete a concomitant medication use form as part of their baseline assessment, during their hyperbaric oxygen treatments, at 13-week post intervention, 6-month post intervention, 12-month post intervention and 18-month post intervention. They will also be contacted by a member of the research team (by phone) at 4/5-month post intervention and 8/10-month post intervention to complete the same concomitant medication form. Each

participant's response will be recorded into the study's password protected, electronic de-identified database.

Alternative therapy

A member of the research team will be expected to complete an alternative therapy survey for each participant as part of their baseline assessment, during their hyperbaric oxygen treatments, at 13-week post intervention, 6-month post intervention, 12-month post intervention and 18-month post intervention. This entails periodically asking participants if they are undergoing/receiving other therapies that could confound the results. Participants will also be contacted by a member of the research team (by phone) at 4/5-month post intervention and 8/10-month post intervention to complete the same alternative therapy survey. Each participant's response will be recorded into the study's password protected, electronic de-identified database.

Blinding of Study Drug

Hyperbaric medicine physician will be unblinded for safety reasons. Chamber operators will not be blinded. Outcome assessor(s) and participants will be blinded. Dive console will be hidden so that the pressure gauge is not visible to non-chamber operating staff. Interior pressure gauge will be covered. Participants will be scheduled as such to reduce the possibility of the sham group meeting and comparing chamber experiences with the intervention arms.

E

STUDY PROCEDURES

Screening for eligibility

New patients with a chief complaint related to persistent post-concussive symptoms will be prescreened by the neurologist or neuropsychologist, a member of the research team. Established patients receiving care for persistent post-concussive symptoms within Essentia Health network will also be prescreened by the research neuropsychologist / neurologist or a designated member of the research team. Items from the patient's clinical records that may be used to identify potential participants for recruitment include but not limited to name, date of birth, primary diagnosis addressed at the visit, other historical diagnoses, medication history, previous therapies related to post concussive symptoms and primary language spoken.

Primary care providers within Essentia Health network that identify any existing patient with a new diagnosis of post-concussive symptom will notify the members of the research team performing screening that the existing patient will be coming to the office for a new diagnosis of post concussive symptoms. Prior to the patient's appointment, he/she will be prescreened by a member of the research team. If the patient appears eligible, any missing information will be obtained during routine clinical care during the patient's appointment to complete the screening process. If a patient is an existing patient within the neurology division with concussion but not

persistent post-concussive symptoms, then such patient may be screened if they continue to have persistent symptoms for 3 months or more.

If a patient is noted to be eligible during an office visit (new diagnosis of persistent post-concussive symptoms) and therefore the patient was not prescreened prior to the appointment, then a member of the research team will be notified as soon as possible to complete the screening process to see if the patient is eligible for study participation.

North Dakota Brain Injury Network will serve as a source of external recruitment for the study. Patients can also be referred from external health facilities in the state of North Dakota or surrounding communities but will be evaluated by a member of the research team (neuropsychologist or neurologist) to confirm diagnosis and referred to study coordinator to ascertain appropriateness for inclusion into the study.

Eligible patients interested in the study will have the study explained to them and any questions answered or concern addressed in a confidential manner in a private consulting room.

Schedule of testing/ measurements

Table 4. Schedule of Activities

Study visit	**Pre-screen**	**Base-line Assess ments**	**Ran-dom-ization**	**Cham-ber sessions 1-40**	**13-week (post-chamber assess-ments)**	**Phon e call mo. 4-5**	**6-month assess-ments**	**Phone call mo. 8-10**	**12-month assess-ments**	**18-month assess-ment**
Visit location	Phone/ local	local	local	local	local	phone	local	phone	local	local
Initial contact	X									
Informed consent		X								
Inclusion/e xclusion eval		X	X							
Demograph ics		X								
Medical history		X								
Physical exam		X		X (40th chamber session)			X		X	X
Vital signs BP, PR, RR, SpO2, Temp, PL		X		X						
Chest Xray		X								
Pregnancy Test		X		‘X’ at chamber # 20, ‘X’ at chamber # 40						

Study visit	Pre-screen	Base-line Assess ments	Ran-dom-ization	Cham-ber sessions 1-40	13-week (post-chamber assess-ments)	Phon e call mo. 4-5	6-month assess-ments	Phone call mo. 8-10	12-month assess-ments	18-month assess-ment
TEED Scale -ear barotrauma		X		X						
Test of Pressure & tolerance		X								
Con-comitant medication s		X		X	X	X	X	X	X	X
Alternate Therapy		X		X	X	X	X	X	X	X
Adverse Event Monitoring		X	X	X	X	X	X	X	X	X
Protocol deviations		X	X	X	X	X	X	X	X	X
NSI		X			X		X		X	X
PSQI		X			X		X		X	X
OSU TBI-ID		X			X		X		X	X
PCL-C		X			X		X		X	X
RBANS		X			X		X		X	X
WHOQOL -BREF		X			X		X		X	X
NfL- serum		X			X		X		X	X
X										
NSI: Neurobehavioral Symptom Inventory, PSQI: Pittsburgh Sleep Quality Index, RBANS: Repeatable Battery for the Assessment of Neuropsychological Status, WHOQOL-BREF: World Health Organization Quality of Life, PCL-C: Post-Traumatic Stress Disorder Checklist-Civilian Version, OSU TBI-ID: Ohio State University Traumatic Brain Injury Identification, NfL serum: Neurofilament Light Chain serum, TEED -Ear barotrauma classification (modified) **Vital signs to be included at local visit: Blood Pressure(BP), Heart Rate (PR), Respiratory Rate (RR), Temperature (Temp), Oxygen Saturation at room air (SpO2) and Pain level (PL)**										

Study outcome measurements and ascertainment

Study outcomes will be measured as shown on **Table 4 (Schedule of Activities).** As a control check within the study protocol, participant(s) who miss the 13-week outcome measurement mark will be contacted by a follow up phone call to ascertain the reason & accommodation will be made to re-schedule the appointment (phone or direct contact) provided it's within 1-10 days from the initial date. Participants who miss 6-month, 12-month or 18-month outcome will be contacted by a follow up phone call or text message to ascertain the reason & accommodation will be made to re-schedule their appointment provided it's within 1- 14 days (more flexibility in the winter months – up to 21 days).

Medical History

The principal investigator, research nurse(s) or other designee will collect a baseline medical history and symptom review relevant to persistent post-concussive symptom(s) that started prior to randomization. This activity will occur before adverse event reporting period and will be recorded on the "other medical history" CRF. Each entry will include date of onset (or date of diagnosis), severity of the condition or symptom and status of the disease condition or symptom. The "other medical history" report will be reviewed prior to determining if any future complaint is a new diagnosis/symptom or worsening of a baseline diagnosis/symptom.

Safety

Safety and Compliance Monitoring

All participants will be asked to complete several forms/questionnaires at specific points during the study. Only members of the study team will have access to this information for compliance. Participants will be assigned a unique study ID that will be used capture their information for database reporting. These forms are further explained below:

Protocol deviations: A member of the research team will be expected to complete a protocol deviation log for each participant at baseline assessment, randomization, during their hyperbaric oxygen treatments, at 13-week post intervention, 6-month post intervention, 12-month post intervention and 18-month post intervention. Participants will also be contacted by a member of the research team (by phone) at 4/5-month post intervention and 8/10-month post intervention to answer questions related to protocol deviation. Each participant's response will be recorded into the study's password protected, electronic de-identified database.

Alternative therapy: Participants will be expected to complete an alternative therapy survey as part of their baseline assessment, during their hyperbaric oxygen treatments, at 13-week post intervention, 6-month post intervention, 12-month post intervention and 18-month post intervention. They will also be contacted by a member of the research team (by phone) at 4/5-month post intervention and 8/10-month post intervention to complete the same alternative therapy survey. Each participant's response will be recorded into the study's password protected, electronic de-identified database.

Concomitant medications: Participants concomitant medications will be reviewed periodically as outlined here. All participants will be expected to complete a concomitant medication use form as part of their baseline assessment, during their hyperbaric oxygen treatments, at 13-week post intervention, 6-month post intervention, 12-month post intervention and 18-month post intervention. They will also be contacted by a member of the research team (by phone) at 4/5-month post intervention and 8/10-month post intervention to complete the same concomitant medication form. Each participant's response will be recorded into the study's password protected, electronic de-identified database.

Medical Monitoring

i Investigator only

Data will be collected via direct contact or phone during their 40 hyperbaric oxygen treatments, at 13-week post intervention, 6-month post intervention, 12-month post intervention and 18-month post intervention. They will also be contacted by phone/text at 4/5-month post intervention and 8/10-month post intervention regard to any side effects experienced during the study period.

ii Institutional Data and Safety Monitoring Board

After the two ten participants have completed the study, review of adverse events experienced by the first two participants will be compiled and assessed. The data and compiled information will be made available to an institutional data and safety monitoring board (DSMB) for independent analysis. A similar sequence of events will occur after half of the participants in the study have completed the study and again at completion of all participants in the study. The DSMB will include an individual with expertise and experience with conducting randomized controlled trials in the field of hyperbaric medicine, an individual with expertise in neurology or neuropsychology, and a statistician. Additional ad hoc meetings of the DSMB may be scheduled if requested by either the PI, IRB, FDA or the DSMB.

Definitions of Adverse Events

Adverse events will include hyperbaric-related side effects including middle ear barotrauma, claustrophobia, progressive myopia, pulmonary oxygen toxicity, seizures from central nervous system (CNS) oxygen toxicity, cataract development and sinus squeeze.

Classification of Events

i Severity

Pulmonary oxygen toxicity, severe middle ear barotrauma (TEEDs classification 3 or more), progressive myopia and cataract development are considered of moderate severity while the other expected adverse events are of mild severity.

For this study, the following standard adverse event (AE) definitions are used:

Definitions (21 CFR 312.32(a))

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. Reasonable possibility means there is evidence to suggest a causal relationship between the drug and the adverse event.

An adverse event or suspected adverse reaction is considered unexpected if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect.

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Classification of An Adverse Event

For adverse events (AEs) in this study protocol, the following guidelines will be used to describe severity.

Mild – An event that is transient and requires minimal or no treatment and does not interfere with usual daily activities.

Moderate – An event that results in a low level of inconvenience, alleviated with simple therapeutic treatment and may interfere with daily activities.

Severe – An event that interrupts a participant usual daily activity and requires therapeutic intervention. Severe events are usually incapacitating or life-threatening. Severe does not mean serious but if hospitalization is involved, it becomes serious adverse event.

Relationship to Study Intervention

The principal investigator (PI) or a PI-designated clinician will assess all adverse event to determine their temporal relationship to study intervention based on his/her clinical judgment. The degree of certainty to causality will be graded as shown below:

Not Related – There is not a reasonable possibility that the study intervention caused the event in question, the temporal relationship is inconsistent with causality or an alternate etiology has been established.

Related – The adverse event is known to occur with the study intervention, there is a **reasonable possibility** that the study intervention caused the adverse event or there is a plausible temporal relationship between the intervention and adverse event. Reasonable possibility means there is evidence to suggest a causal relationship between the drug and the adverse event.

- Definitely Related: There is clear evidence to suggest a causal relationship and other contributing factors can be ruled out.
- Probably Related: There is evidence to suggest a causal relationship and influence of other factors in unlikely.
- Potentially Related: There is some evidence to suggest a causal relationship, however, other factors may have contributed to the event.

- Unlikely to be related: The temporal relationship between the event and administration of the study intervention makes it improbable and other medications or interventions may provide plausible explanations
- Not Related: The adverse event is independent of study intervention administration and/or evidence exists that the adverse event is related to another etiology.

Expectedness[18 - 20]

Common: Middle ear barotrauma and sinus barotrauma. A need for myringotomy following otic barotrauma or history of Eustachian Tube Dysfunction is within routine clinical care in hyperbaric medicine.

Less common: claustrophobia, seizures due to central nervous system oxygen toxicity

Rare: progressive myopia

Very rare: pulmonary oxygen toxicity

PLAN FOR COLLECTING AEs, SAEs and TEARs

Time Period and Frequency for Event Assessment and Follow-up

The study coordinator or a PI designated study member will inquire about the occurrence of adverse events or serious adverse event since the last phone or in-person study visit. The occurrence of an adverse event or serious adverse event may be initiated by participants through secured text to dedicated study phone or noted at study visits (in person or via phone calls as outlined in the schedule of activities.

Adverse events not meeting the serious adverse events criteria will be captured with the adverse event worksheet. All adverse events will be followed to adequate resolution while on the study and must be documented regardless of relationship. Information to be collected includes the following:

- Adverse event description
- Start date and time
- Date study team was made aware
- Severity
- Relationship to study intervention
- Action taken by study team (such as withdraw from study or treatment given)
- Stop date and time

Medical condition(s) present at screening will not be reported as an adverse event but any deterioration will be considered as an adverse event. Changes in adverse event severity will be documented to allow an assessment at each level of severity.
The study coordinator will record all reportable events with start dates occurring any time after informed consent is obtained until:

- 7 days for non-serious adverse events after last day of study participation

- 30 days for serious adverse events after last day of study participation.

Adverse Event Reporting

The Principal Investigator (PI) will immediately report to the sponsor any serious adverse event, whether or not considered study intervention-related and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event.

Serious adverse events (SAE) will be followed until adequately resolved or the PI deems it chronic/ the participant is stable. Study endpoints that are SAEs will be reported according to the protocol unless a causal relationship between the study intervention and the event is made. In this case, the PI-study sponsor reports the event immediately to the Food and Drug Administration (FDA). The Study sponsor is responsible for notifying the FDA of any unexpected fatal or life-threatening adverse reaction as soon as possible, but no later than 7 calendar days after the sponsor's initial receipt of the information. The sponsor will also notify FDA of potential serious risks as soon as possible but no later than 15 calendar days after the sponsor determines that the information qualifies for reporting.

Participants will be informed about adverse events and serious adverse events and study related results on an individual or aggregate level without HIPAA violation. Incidental findings will be monitored by the PI and reported appropriately as required.

Participants that become pregnant during the study will be immediately withdrawn from the study (taper to hyperbaric oxygen is not required) after education and counseling from the Principal Investigator.

Unanticipated Problem (UP) Reporting

The principal investigator will report unanticipated problems to the reviewing IRB (Essentia Institute of Rural Health IRB) and to the Data and Safety Monitoring Board (DSMB). Reported information will include study ID, protocol identification, description of the event, basis for determining the event is an "unanticipated problem" and any changes to the protocol or corrective actions that have been taken or proposed.

- UPs that are SAEs will be reported within 25 hours of the PI becoming aware
- Other UPs will be reported within one week of the PI becoming aware.
- All UPs should be reported to appropriate institutional officials and Office of Human Research Protections within 7 business days of the IRB's receipt of the problem from the PI.

Unanticipated Problem is any incident, experience or outcome that meets **all** the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)- approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied

- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

F

STATISTICAL PLAN

Sampling size determination and power

A sample size determination was calculated based on our primary endpoint- Repeatable battery for the assessment of neuropsychological status (RBANS). Mean and standard deviation of RBAN scores in non-TBI population is estimated at 100 and 10-15 respectively. Very few studies have reported the mean, standard deviation, and minimal clinical change in RBAN total scores in patients with mild TBI. One retrospective study among active military population reported a mean RBANS composite score of 79.14 among soldiers that were unable to return to duty following blast related mild TBI while those who were able to return to duty had a mean RBANS composite score of 88.45, a 9.3 difference that was significant.[17] This suggests a score change of 5-20 (SD of change 4-10) may be used as the minimal RBANS total score change to determine our sample size.

We derive the sample size from longitudinal calculations for our primary endpoint while simultaneously looking at our secondary endpoints and ensuring the number needed are within the same range (except for QOL measures). Statistical tests used may include one-sided paired t-tests of change or paired Wilcoxen signed rank tests measured between two means. Wilcoxen can be used when assumptions for the paired t-test (e.g., underlying normal distribution) are not met. A type I error of .05 and a type II error of .2 to .1 were used. Other assumptions made about the data included a correlation between measured values of 0.2 to 0.8. Adjustments to the estimated sample sizes were made of 20% as maximum dropout rate.

Estimated sample sizes varied primarily by type of test and assumptions used. A middle value for sample size would be 16, or 22 assuming a 20% dropout rate. This will provide good power (.8 or higher) for the primary outcome RBANS to detect a mean change in scores of 5 to 20.

Table 5. Mean, standard deviation and estimated sample size for primary and secondary endpoints.

	Population/Target		TBI		Change		
	MN	SD	MN	SD	Mean	SD	Paired t
RBANS	100	10-15	70-90	10-15	+ 5-20	4 – 10	4-38 (5-48)
PQSI	4.4	.5	13.6	3.5	- 3 - 9	3 – 5	4-28 (5-35)
NSI	39.8	15.0	53.9	15.7	- 8 - 14	4 – 7	4-10 (5-13)

CPCL	57.21	5.08	62.29	11.56	- 5 - 20	4 – 10	4-38 (5-48)
Reexperience	16.2	5.0	17.5	4.5	- 1.1 – 1.5	0.4 – 0.9	4-9 (5-12)
Avoidance	16.2	5.0	17.5	4.2	- 1.1 – 1.5	0.4 – 0.9	4-9 (5-12)
Hypervigilance	17.7	4.8	19.2	3.8	- 1.3 – 1.7	0.4 – 0.9	7-7 (5-9)
QOL							
Physical	73.5	18.1	45.5	16.5	+ 1 - 10	2.5 – 5.5	4-274 (5-343)
Psychological	70.6	14.0	54.1	19.7	+ 1 - 10	2.5 – 5.5	4-274 (5-343)
Social	71.5	18.2	60.0	21.8	+ 1 - 10	2.5 – 5.5	4-274 (5-343)
Environmental	75.1	13.0	67.3	15.9	+ 1 - 10	2.5 – 5.5	4-274 (5-343)
NfL-Serum	20-45	5-15	12.8	12-16	+ 5 - 20	4 – 10	3-44 (4-55)

Hypothesis

Primary endpoint: Using superiority hypothesis, participants with persistent post-concussive symptoms treated with hyperbaric oxygen therapy at 1.5 atmosphere absolute (ATA) for 40 sessions will have better RBANS scores (higher scores) compared to those in the sham-controlled arm (did not receive hyperbaric oxygen therapy during the duration of the study). H_0: $\mu_t-\mu_c <= \delta$ and H_1: $\mu_t-\mu_c>\delta$ where δ is clinically meaningful difference.

Secondary endpoints: All superiority hypotheses.

- Participants with persistent post-concussive symptoms treated with hyperbaric oxygen therapy at 1.5 atmosphere absolute (ATA) for 40 sessions will have lower PSQI scores compared to those in the sham-controlled non HBO_2 arm
- Participants with persistent post-concussive symptoms treated with hyperbaric oxygen therapy at 1.5 atmosphere absolute (ATA) for 40 sessions will have lower NSI scores compared to those in the sham-controlled non HBO_2 arm
- Participants with persistent post-concussive symptoms treated with hyperbaric oxygen therapy at 1.5 atmosphere absolute (ATA) for 40 sessions will have higher WHOQOL-BREF scores compared to those in the sham-controlled non HBO_2 arm
- Participants with persistent post-concussive symptoms treated with hyperbaric oxygen therapy at 1.5 atmosphere absolute (ATA) for 40 sessions will have lower PCL-C score compared to those in the sham-controlled non HBO_2 arm
- Participants with persistent post-concussive symptoms treated with hyperbaric oxygen therapy at 1.5 atmosphere absolute (ATA) for 40 sessions will have higher serum NfL values compared to those in the sham-controlled non HBO_2 arm

Statistical Analysis Plan

Our study statistician will conduct analyses following CONSORT standards. Intention-to-treat analysis will be applied to all data obtained from study participants. Subsequent analyses will be as outlined by the protocol. Baseline tests and change from baseline differences to test values at later dates will be compared between both groups and within subgroups. Continuous variables will be modeled with response beginning from the 13-week point. Four more measures of outcomes will be taken. RMD comparing between cases and controls will be the primary statistical method to determine changes in outcomes longitudinally (including trend determination) and between cases and controls. Paired t-tests and multiple comparison tests may be used for specific pairs of means. Other demographics (e.g., gender age) may be entered into the model as covariates.

Interim Monitoring and Early Stopping

Study data will be assessed on a weekly basis to determine the need for early stopping.

Criteria being used for weekly interim monitoring and early stopping.

A) Evidence of harm (weekly check)

- Increased frequency of expected side effects of hyperbaric oxygen treatments. Accepted range: as > 15% of moderate-to-severe middle ear barotrauma (modified TEEDs 3-5), need for myringotomy more than 2.2% - 48%, Seizure resulting from CNS oxygen toxicity < 0.024%, pulmonary oxygen toxicity < 0.01%, pulmonary barotrauma in patients with lung pathology (rare), progressive myopia (<0.001%)
- Any case of pulmonary barotrauma requiring chest tube placement
- Poor compliance with study treatment regimen
- Death related to study intervention

B) Evidence of benefit: This study will not be stopped before its planned duration if interim analysis shows significant beneficial effect of hyperbaric oxygen treatment.

C) Evidence of futility: Worsening persistent post-concussive symptoms in the treatment arm (arm receiving hyperbaric oxygen) at planned interim analysis. Planned interim analysis is recommended at end of 18 months from study start date.

Planned Interim Data Analysis and Stopping rules

Planned interim analysis is recommended at the end of eighteen (18) months as we expect approximately 50% of the participants to have completed their intervention and 30% of participants followed for at least six months. The sample size is constructed to maintain the overall study power of at least.80. If the longitudinal and case control comparisons exceed the level of significance (all p-values < .05), then the study could be considered for early termination due to emerging differences. The study could also be terminated if the rate of adverse events exceeds previous studies or the rates in daily clinical practice such as > 15% of moderate-to-severe middle ear barotrauma (TEEDs 3-5).

Missing Outcome Data

Missing data and trends will be assessed monthly by the study data manager. Remedial measures, including retraining of research staff, will be used as needed to minimize missing data. Data will be analyzed using intent-to-treat approach. To accomplish this, attrite participants will remain in the study arm they were randomized to and, will be considered in the primary analysis of the trial.

Unblinding Procedures

Hyperbaric medicine physician will be unblinded for safety reasons. Chamber operators will not be blinded. Outcome assessor(s) and participants will be blinded. If unblinding is required, the PI and/or any delegated research staff will be contacted at 701-364-8505 for further evaluation and consideration. Reason for unblinding, date and time of unblinding will be included in the participant's REDCap information.

G

DATA HANDLING AND RECORD KEEPING

Confidentiality and security

Informed consent will be obtained in a private consultation room. Every precaution will be taken to ensure data confidentiality. Only research team members will have access to the paper and electronic data. Participants will be assigned a unique ID that will be used on all case report forms and database reporting. The database that will be used is Research Electronic Data Capture (REDCap), a HIPAA compliant, password protected and encrypted database. Electronic data will be accessible only on password protected computers. Any hard copies will be maintained in a locked cabinet in a locked office by a member of the study team. SMS service that does not collect PHI will be used for text messaging should the need arise. There will be strict adherence to good data management protocols.

Training

Every member of the research team will be educated on the study protocol and take CITI program courses as required by the institution's IRB. During study protocol training meetings, members of the research team will be able to ask questions and after such education sessions with respect to all aspects of the study protocol. All members of the research team that will access REDCap.

Records retention

Every participant will be assigned a unique study identifier. Any hard copies will be maintained in a locked cabinet in the locked office of the Principal Investigator (PI). The PI will keep a log in his locked drawer, in the locked office of subject ID's that links up their names and information. The PI's office number is **3000 32nd Avenue S, Room 5C319, Fargo, ND 58103.** The Principal Investigator and clinical research coordinator (or research nurse) are the only research staff that will have access to identifiable data.

Electronic data will be stored in REDCap which is HIPAA compliant, encrypted and password protected. Research work will only be done on password protected computers with only research members having study information access. Other members of the research team such as Co-Principal Investigators, Statistician, Psychologist, Neuropsychologist, Neurologist will get participants' information after it has been deidentified.

Participant Unblinding

Participants will remain blinded until the primary outcome analysis is finalized. Unblinding will occur after the study closure. The study team will contact participants via letter or phone call. Unblinding disclosures will be recorded in the participant's study file. No further study-related interventions will be required after unblinding.

H

STUDY ADMINISTRATION

Organization

The location of this study will be at the hyperbaric chamber center Essentia Health -Fargo at 3000 32nd Avenue South, Fargo ND and hyperbaric chamber center at 45th Street, Fargo ND.

Funding sources and conflicts of interest

Funding for this project will be supplied by a grant provided by the state of North Dakota.

Subject stipends or payments

Participants will not be reimbursed for their participation in this research study. Participants in the treatment arm will receive hyperbaric oxygen as part of the study.

Figure 2. Study timetable

Study Measures (mo = months)	0-3 mo	3-6 mo	7-20 mo	20 – 24 mo	18 – 24 mo	36 mo
Patient enrollment						
Intervention: hyperbaric oxygen vs sham control						
Data Analysis Interim (brown) Final (green)						
Manuscript preparation and submission						

I

PUBLICATION PLAN

We intend to present result(s) of this study as a manuscript for publication in a reputable peer-review medical journal in a timely fashion. Journal choice will likely be related to the field of neurology, hyperbaric medicine, sport medicine and may be dependent on study findings.

REFERENCE

1. Weaver LK, Wilson SH, Lindblad AS, et al. Hyperbaric oxygen for post-concussive symptoms in United States military service members: a randomized control trial. UHM Journal 2018; 45(2):129-156.
2. Centers for Disease Control and Prevention (2019). Surveillance Report of Traumatic Brain Injury-related Emergency Department Visits, Hospitalizations, and Deaths—United States, 2014. Centers for Disease Control and Prevention, U.S. Department of Health and Human Services.
3. Centers for Disease Control and Prevention (CDC), National Center for Injury Prevention and Control. Report to Congress on mild traumatic brain injury in the United States: steps to prevent a serious public health problem. Atlanta (GA): Centers for Disease Control and Prevention; 2003.
4. Ruff RM. Mild traumatic brain injury and neural recovery: rethinking the debate. NeuroRehabilitation (2011) 28:167–80. doi: 10.3233/NRE-2011-0646.
5. Cassidy JD, Cancelliere C, Carroll LJ, Côté P, Hincapié CA, Holm LW, et al. Systematic review of self-reported prognosis in adults after mild traumatic brain injury: results of the international collaboration on mild traumatic brain injury prognosis. Arch Phys Med Rehabil. (2014) 95(3 Suppl.):S132–51. doi: 10.1016/j.apmr.2013.08.299.
6. Hiploylee C, Dufort PA, Davis HS, et al. Longitudinal study of postconcussion syndrome: not everyone recovers. J Neurotrauma (2016) 34:1511-1523 doi: 10.1089/neu.2016.4677.
7. Vanderploeg RD, Curtiss G, Luis CA, et al. Long-term morbidities following self-reported mild traumatic brain injury. J Clin Exp Neuropsychol (2007) 29(6):585-598.
8. Weaver LK, Chhoeu A, Lindblad AS, et al. Hyperbaric oxygen for mild traumatic brain injury: Design and baseline summary. Undersea and Hyperbaric Medicine Journal (2016) 43(5):491-509.
9. Moser RS, Schatz P. Enduring effects of concussion in youth athletes. Archives of Clinical Neuropsychology. 2002; 17:91-100.
10. Carone et al. A comparison of three cognitive screening tests in a traumatic brain injury sample. Poster session presented at the 32nd meeting of the international neuropsychological society. Baltimore MD. Feb 2004.
11. Shahim P, Politis A, Van der Merwe A, et al. Neurofilament light as a biomarker in traumatic brain injury. *Neurology*. 2020 Aug 11;95(6):e610-e622.
12. Shahim P, Politis A, Van der Merwe A, et al. Time course and diagnostic utility of NfL, tau, GFAP and UCH-L_1 in subacute and chronic TBI. Neurology. 2020 Aug 11;95(6):e623-e636.
13. Van Hoesen KB, Camporesi EM, Moon RE, Hage ML, Piantadosi CA. Should hyperbaric oxygen be used to treat the pregnant patient for acute carbon monoxide poisoning? A case report and literature review [published correction appears in JAMA

1990 May 23-30;263(20):2750]. *JAMA*. 1989;261(7):1039-1043. doi:10.1001/jama.261.7.1039
14. Weaver LK. Hyperbaric oxygen in the critically ill. Critical Care Medicine 39(7):1784-1791
15. Gesell L, Ed. (2008). Hyperbaric Oxygen Therapy Indications. Durham, NC, Undersea and Hyperbaric Medical Society.
16. Harch PG, Kriedt C, Van Meter, et al. Hyperbaric oxygen therapy improves spatial learning and memory in a rat model of chronic traumatic brin injury. Brain Res 2007; 1174:120-129.
17. Cook PA, Johnson TM, Martin SG, Gehrman PR, Bhatnagar S, Gee JC. A retrospective study of predictors of Return to Duty versus Medical Retirement in an Active Duty Military Population with Blast-Related Mild Traumatic Brain Injury. Journal of Neurotrauma Apr 2018; 35:991-1002
18. Paganini M, Camporesi EM. Chapter 16: Side Effects of Hyperbaric Oxygen Therapy. 335 – 342. Moon RE. "Undersea and Hyperbaric Medical Society's Hyperbaric Oxygen Therapy INDICATIONS. BPC. 14th Edition". 2019
19. Shupak A, Clark JM, Butler FK, et al. Section V; Side Effects and Complications: Chapters 22 – 26. Neuman TS, Thom SR. Physiology and Medicine of Hyperbaric Oxygen Therapy. Saunders Elsevier. 2008
20. McLelland CA: Incidence of complications from use of tympanostomy tube. Arch Otolaryngol 106:97 – 99, 1980