PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	How biomarkers reflect the prognosis and treatment of necrotizing soft tissue infections and the effects of hyperbaric oxygen therapy: The protocol of the prospective cohort PROTREAT study conducted at a tertiary hospital in Copenhagen, Denmark
AUTHORS	Polzik, Peter; Johansson, Pär I; Hyldegaard, Ole

VERSION 1 – REVIEW

REVIEWER	Darin James Saltzman Olive View-UCLA Medical Center, USA
REVIEW RETURNED	01-Jul-2017

GENERAL COMMENTS	The authors present a prospective cohort study design title, " ProTreat-Prognosis and treatment of necrotizing soft tissue infections: A prospective cohort study. The authors correctly point out that necrotizing soft tissue infections (NTSI) continue to carry a high mortality with significant morbidity and as such deserves ongoing investigation with regard to prognosis and treatment of the disease process. The study design, however, is more related to how hypobaric oxygen therapy (HBOT) influences the clinical outcome of NTSI and how the prognosis is related to elevated biomarkers. In general, the authors should be applauded in their quest; however, there are significant concerns with regard to their experimental design. Major Comments: The study design presented lacks detail and comes across as a "rough draft" and rushed. There many times the reader may wonder "why and how". Hyperbaric Oxygen Therapy is intensely debated and it would have been nice to add significant detail why the authors believed this treatment to be beneficial and give some arguments to studies that have shown HBOT not to be therapeutic. Similarly, the use of biomarkers is a new and exciting field. However, the this manuscript would have a greater impact if the authors could go into more detail why and how these specific markers will improve our understanding of the disease. Minor Comments: Abstract: Page 3 Line 11: Please spell out "suPAR" before using the abbreviation.

	Introduction: Page 4 Line 18: This is not strength of the study, but an outcome. If the data analysis is inconclusive some may question the value of this evidence. Page 4 Line 24: …be subject to various biases. Please be more specific. Where is the bias, in patient selection, in result interpretation, etc. Page 4 Line 32: …are among the most…known. May think to rephrase as ….is a serious and deadly infection. Otherwise please state "known to who" (medical community, in rural community, etc. Page 4 Line 44: ……patient rehabilitation and "invalidity". Unclear, please rephrase. Page 4 Line 45: …(HBOT) may be added as adjunctive therapy of NSTI. Please site reference. Page 5 Line 4: ….a biomarker reflecting the immune system activity. Please reference. Page 5 Line 20: ….contributes to intravascular depletion. Please reference Page 5 Line 33: …by inducing formation of reactive oxygen species (ROS). Is this true? Some may consider ROS detrimental to the microvasculature. Please reference this statement. Page 5 Line 48: Is it possible to give a little more description/background why and how these markers correlate with endothelial integrity? For example, are increases in these markers associated with less or more endothelial membrane damage? 4.3 Data Collection: Page 7 Line 43: Please spell out EDTA Page 7 Paragraph 1: can drop the use of "once". 4.5 Hypotheses; primary and secondary outcomes Page 10 Line 4: Please define sepsis, severe sepsis, and septic shock. Page 10 Line 20: Please describe how HBOT will be delivered, i.e. is it directed to the wound or whole body. What objective data will be used to determine that an elevated oxygen level has been achieved. Page 11 Line 16: Please spell out SAPS II and SOFA Page 11 Line 45: "Middle"… is this equivalent to "mean" arterial pressure? 4.6 Sample Size Page 13 Line 11: …since NSTI patients are also septic. Is this true? Are all patients with NSTI septic or are the authors implying that some may be septic?

REVIEWER	Massimo Sartelli Macerata hospital Italy
REVIEW RETURNED	01-Jul-2017

GENERAL COMMENTS	Interesting study We look forward to reading the results

VERSION 1 – AUTHOR RESPONSE

Dear Editor and Reviewers!

See below for our response.

Major Comments:
The study design presented lacks detail and comes across as a “rough draft” and rushed. There many times the reader may wonder “why and how”. Hyperbaric Oxygen Therapy is intensely debated and it would have been nice to add significant detail why the authors believed this treatment to be beneficial and give some arguments to studies that have shown HBOT not to be therapeutic.
Similarly, the use of biomarkers is a new and exciting field. However, the this manuscript would have a greater impact if the authors could go into more detail why and how these specific markers will improve our understanding of the disease.

Authors’ response to the major comments:

Thank you for your time and valid points!

We believe that the presented revisions have made the study protocol better and more precise, and hope that you agree. Please see the yellow marked text at the bottom of protocol page 4 for our arguments regarding HBOT. In general, although randomized HBOT trials are lacking, numerous studies have continued to demonstrate the beneficial effects of HBOT in the management of NSTI. Recent, larger database studies provide evidence that HBOT may improve the survival of patients with NSTI (see references 2, 3, and 4, included in the protocol) to warrant our present study’s focus. It is especially important considering the present lack of large RCTs, due to the ethical concerns. Although some authors claim HBOT remains controversial due to the lack of RCTs, others have concluded that the improvements in morbidity and mortality when compared to historical data would make it unethical to perform an RCT, as it would deny a well-substantiated adjunctive treatment for a disease with a high rate of morbidity and mortality, with generally few risks of complications from the treatment (UHMS Indications, 2014). However, the current study protocol will add important and additional information in describing possible pathophysiological effects of HBOT in NSTI, thereby providing an excellent chance to produce data that may lead to properly designed RCTs in the future. Lastly, there are in fact no prospective, randomized or controlled trials of any kind whatsoever, regarding surgery or surgery and antibiotics for NSTI, nor any studies evaluating different surgical techniques of for example skin sparing, yet these interventions are used without any question of efficacy and based on similar retrospective and historical data.

Regarding the biomarkers, the text highlighted in yellow on protocol pages 5 and 6 addresses the concerns regarding why and how specific biomarkers improve our understanding of the disease.

We have added multiple new references and gone into more detail regarding the why and how, as suggested.

Minor Comments:

1. Abstract:
Page 3 Line 11: Please spell out “suPAR” before using the abbreviation.

Revised as suggested.

2. Introduction:
Page 4 Line 18: This is not strength of the study, but an outcome. If the data analysis is inconclusive

some may question the value of this evidence.

Revised as suggested.

3. Page 4 Line 24: …be subject to various biases. Please be more specific. Where is the bias, in patient selection, in result interpretation, etc.

Revised as suggested.

4. Page 4 Line 32: …are among the most…known. May think to rephrase as ….is a serious and deadly infection. Otherwise please state “known to who” (medical community, in rural community, etc.

Revised as suggested.

5. Page 4 Line 44: ……patient rehabilitation and “invalidity”. Unclear, please rephrase.

Revised as suggested.

6. Page 4 Line 45: …(HBOT) may be added as adjunctive therapy of NSTI. Please site reference.

Reference added.

7. Page 5 Line 4: ….a biomarker reflecting the immune system activity. Please reference.

Reference added.

8. Page 5 Line 20: ….contributes to intravascular depletion. Please reference

Reference added.

9. Page 5 Line 33: …by inducing formation of reactive oxygen species (ROS). Is this true? Some may consider ROS detrimental to the microvasculature. Please reference this statement.

Reference added.

Page 5 Line 48: Is it possible to give a little more description/background why and how these markers correlate with endothelial integrity? For example, are increases in these markers associated with less or more endothelial membrane damage?

We have added more background and references, as suggested.

10. 4.3 Data Collection:
Page 7 Line 43: Please spell out EDTA

Revised as suggested.

11. Page 7 Paragraph 1: can drop the use of “once”.
Revised as suggested.

12. 4.5 Hypotheses; primary and secondary outcomes
Page 10 Line 4: Please define sepsis, severe sepsis, and septic shock.

Revised as suggested.

13. Page 10 Line 20: Please describe how HBOT will be delivered, i.e. is it directed to the wound or whole body. What objective data will be used to determine that an elevated oxygen level has been achieved.

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14. Page 11 Line 16: Please spell out SAPS II and SOFA
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15. Page 11 Line 45: “Middle”… is this equivalent to “mean” arterial pressure?

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16. 4.6 Sample Size
Page 13 Line 11: …since NSTI patients are also septic. Is this true? Are all patients with NSTI septic or are the authors implying that some may be septic?

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