BMJ Open

How biomarkers reflect the prognosis and treatment of necrotising 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

Peter Polzik,[1] Pär I Johansson,[2] Ole Hyldegaard[1]

To cite: Polzik P, Johansson PI, Hyldegaard O. How biomarkers reflect the prognosis and treatment of necrotising 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. *BMJ Open* 2017;**7**:e017805. doi:10.1136/bmjopen-2017-017805

► Prepublication history for this paper is available online. To view these files please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2017-017805).

Received 18 May 2017
Revised 1 August 2017
Accepted 16 August 2017

[1]Department of Anesthesiology, Center of Head and Orthopedics, Copenhagen University Hospital (Rigshospitalet), Copenhagen Ø, Denmark
[2]Department of Clinical Immunology, Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark

Correspondence to
Dr Peter Polzik;
pyotrpolzik@gmail.com

ABSTRACT

Introduction Not enough is known regarding the prognosis and treatment of necrotising soft tissue infections (NSTIs). Mortality has been shown to be 25%–35%, with survivors coping with amputations and prolonged rehabilitation. This study will evaluate soluble urokinase-type plasminogen activator receptor (suPAR) as a possible prognostic marker of NSTI severity and mortality, as well as whether hyperbaric oxygen therapy (HBOT) can modulate markers of endothelial damage during NSTI. We hypothesise that in patients with NSTI, suPAR can provide prognostic risk assessment on hospital admission and that HBOT can reduce the endothelial damage that these patients are exposed to.

Methods and analysis This is a prospective observational study. Biomarkers will be measured in 150 patients who have been diagnosed with NSTI. On admission, baseline blood samples will be obtained. Following surgery and HBOT, daily blood samples will be obtained in order to measure endothelial and prognostic biomarkers (soluble thrombomodulin, syndecan-1, sE-selectin, vascular endothelial (VE)-cadherin, protein C and suPAR levels). Clinical data will be acquired during the first 7 days of stay in the intensive care unit. The primary outcomes in studies I and II will be endothelial biomarker levels after HBOT, and in study III suPAR levels as a marker of disease prognosis and severity.

Ethics and dissemination The study has been approved by the Regional Scientific Ethical Committee of Copenhagen (H-16021845) and the Danish Data Protection Agency (RH-2016-199). Results will be presented at national and international conferences and published in peer-reviewed scientific journals.

Trial registration number ClinicalTrials.gov Identifier NCT03147352. (Pre-results)

Strengths and limitations of this study

- It is the largest, single-centre prospective cohort study of biomarkers during necrotising soft tissue infections (NSTIs).
- The study will measure biomarkers never previously examined in patients with NSTI.
- The study's outcomes may provide valuable evidence for future studies of optimisation of NSTI prognosis and treatment.
- Due to the non-randomised design, we may be subject to biases due to differences in hyperbaric oxygen therapy allocation and result interpretation.

INTRODUCTION

Necrotising soft tissue infections (NSTIs) are serious and deadly. They are characterised by rapidly progressing soft-tissue inflammation with necrosis and can quickly cause multiple organ failure and death. They have a wide range of presentations. Patients can become mortally infected in hours. Mortality has been shown to be 25%–35%, with survivors coping with amputations and prolonged rehabilitation.[1] Septic shock accompanies death due to NSTI.

Currently, we lack the proper tools to evaluate the severity and prognosis of NSTI in individual patients. This results in necessary, yet sometimes overzealous surgical debridement, culminating in prolonged patient rehabilitation and amputations. Hyperbaric oxygen therapy (HBOT) may be added as adjunctive therapy of NSTI.[2–4] Large database surveys indicate that HBOT improves survival of patients with NSTI in hospitals capable of providing HBOT—the effect being most prominent for severely ill patients, with septic shock.[2–4] Large randomised controlled trials (RCTs) are lacking, in large part due

to ethical concerns. However, in the present prospective cohort, HBOT is already being used as part of the standard NSTI treatment in a multidisciplinary setting in a tertiary hospital, with centralised treatment expertise and an in-hospital HBOT unit. We wish to use this unique opportunity to examine the effects of HBOT during NSTI by means of biomarkers, in order to obtain pathophysiological knowledge about the effects of HBOT. The data will also contribute to improved decision-making with respect to the proper design and ethical justification of future RCT studies on the effects of HBOT.

Accordingly, our first and second study will analyse markers of endothelial function in order to examine the effect of HBOT on patients with NSTI. Our third study will look at the prognostic value of soluble urokinase-type plasminogen activator receptor (suPAR) in NSTI. SuPAR is a biomarker reflecting immune system activity.[5]

Studies I and II: endothelial function during NSTI and the effects of HBOT

Endothelial dysfunction during sepsis is the result of damage to the endothelial glycocalyx, which leads to platelet aggregation, leucocyte adhesion and an increase in endothelial permeability. The result is capillary leakage and tissue oedema. At the same time, the patients' blood is anticoagulated endogenously. This capillary leakage and anticoagulation ultimately lead to intravascular volume depletion.[6–9] Tissue dysfunction is due to inflammation, reduced tissue blood flow and ischaemia, which can lead to multiorgan failure and death.[6–9] Recently, we have demonstrated in more than 4400 patients with acute critical illness (sepsis,[10–12] trauma,[13] myocardial infarction[14] and resuscitated cardiac arrest[15]) that endothelial breakdown as evaluated by the biomarkers soluble thrombomodulin (sTM) and syndecan-1 is independently associated with development of multiorgan failure and death. We are interested in examining whether this also is the case with NSTIs, since most of these patients are also septic. sTM and syndecan-1 have been shown as markers of endothelial and glycocalyx damage, respectively.[16 17] sTM is released from endothelial cells on damage, while damage to the glycocalyx releases syndecan-1. Increases in these markers therefore correspond to increased levels of endothelial damage.[18]

In septic rats, HBOT has been shown to attenuate levels of proinflammatory cytokines and prevent coagulation disorders.[19–22] Furthermore, HBOT may improve microcirculation by inducing the formation of reactive oxygen species[23 24] and decreasing the adherence of polymorphonuclear neutrophils to the endothelial cell wall,[25–28] possibly by downregulation of intracellular adhesion molecule-1.[29 30] sE-Selectin and vascular endothelial (VE)-cadherin are markers of leucocyte adhesion and endothelial barrier function, respectively.[31 32] sE-Selectin is responsible for interactions between leucocytes and the endothelium, and increased expression is due to endothelial activation.[33] Lower concentrations of VE-cadherin result in loss of vascular integrity.[34]

We believe it is plausible to consider the potential beneficial effects of HBOT on patients with NSTI in septic shock due to HBOT mediating an endothelial/glycocalyx protective effect, which enhances the endothelial integrity with its effects on coagulation and platelet reactivity and functionality.[26–30] Also, HBOT has been shown to induce a cytoprotective and angiogenic response in human endothelial cells.[35] A deeper understanding of endothelial dysfunction during NSTI, and the possible countering effect of HBOT, could contribute to a better understanding of this disease.

Therefore, the purpose of studies I and II will be to investigate the effect of HBOT on possible endothelial dysfunction in patients with NSTI. We will do this by measuring sTM and syndecan-1 in study I, as well as sE-Selectin, VE-cadherin and protein C in study II.

Study III: suPAR as a prognostic biomarker for NSTI

suPAR receptor has been shown to predict the risk of developing a wide range of chronic conditions, as well as predicting mortality during acute infectious conditions. The risk of developing cardiovascular disease, diabetes mellitus, cancer,[36 37] acute exacerbation of chronic obstructive lung disease,[38] mortality during bacteraemia,[39–41] mortality during bacterial meningitis,[42] mortality from systemic inflammatory response syndrome[43] as well as negative prognosis during sepsis[44] are all correlated with higher than normal levels of suPAR. Likewise, since NSTIs are also infectious diseases, we are interested in examining suPARs potential during NSTIs.

In our third study, we will assess suPARs possible value as a prognostic biomarker for mortality and morbidity as well as clinical condition during NSTI.

METHODS AND ANALYSIS

Study design

The PROTREAT study is a prospective observational substudy of the INFECT project (ClinicalTrials.gov Identifier: NCT01790698). The INFECT project involves five centres (Copenhagen University Hospital, Karolinska Institute, Blekinge Hospital, Sahlgrenska Hospital, University Hospital of Bergen) with the objective of improving the outcome in patients with NSTI.

The PROTREAT study will be conducted at Copenhagen University Hospital and Hvidovre Hospital. The study will use data gathered in Denmark on patients diagnosed with NSTI, who are admitted to Copenhagen University Hospital. The patients are enrolled immediately on diagnosis using predefined criteria for NSTI, as specified below. The first patient was enrolled on 26 February 2013 and inclusion is ongoing. Due to the low incidence of NSTI, the enrolment period of this study will extend over 4.5 years, with expected closure by the end of August 2017.

For study III, only the patient with NSTI cohort will be used. For studies I and II, we will also use data gathered from a group of 65 elective orthopaedic surgery patients,

Table 1 Overview of blood sampling procedures

Day 0 (time of admission)	Day 1	Day 2	Day 3
Patients with NSTI	Patients with NSTI	Patients with NSTI	Patients with NSTI
EDTA blood (two collection tubes)	EDTA blood (two collection tubes)	EDTA blood (two collection tubes)	EDTA blood (two collection tubes)

NSTI, necrotising soft tissue infection.

functioning as controls for our patients with NSTI. Furthermore, data on endothelial function from the Scandinavian Starch for Severe Sepsis/Septic Shock trial (ClinicalTrials.gov: NCT00962156) of patients with sepsis will be used to illustrate and compare with the modulation of endothelial function in patients with sepsis who do not receive HBOT.

Inclusion and exclusion criteria

Studies I, II and III: patients with NSTI

Patient inclusion criteria are (all of which must be met):

1. Diagnosed with NSTI based on surgical findings (necrosis of any soft tissue compartment; dermis, hypodermis, fascia or muscle)
2. Age ≥18 years
3. Admitted to the intensive care unit (ICU) and/or operated for NSTI at Copenhagen University Hospital.

Patient exclusion criteria are:

1. They are categorised as non-NSTI in the operating theatre.

Studies I and II: orthopaedic control patients

Control patient inclusion criteria are (all of which must be met):

1. Undergoing elective orthopaedic surgery (non-pathological fractures, joint replacement surgery or spine surgery) at Copenhagen University Hospital
2. Age ≥18 years.

Patient exclusion criteria are:

1. Ongoing infection or inflammatory condition.

Data collection

A blood sample taken from an arterial line from each patient with NSTI is collected into tubes containing EDTA at four time points: on admission and each of the following 3 days, always between 08:00 and 14:00 (see

Table 2 Baseline characteristics and clinical data

Data	Description
Baseline characteristics	► Sex and age – ComorbiditiesDiabetes mellitus, cirrhosis of the liver, renal disease, heart disease, vascular disease, hepatitis, intravenous drug abuse, history of cancer, COPD, immunosuppression ► Body mass index ► Primary infection site – Origin of infection Chronic wound, injection, boil/furuncle, animal bite, idiopathic, trauma, postoperative infection, perianal abscess, other – Symptoms registered at the primary hospital Oedema, erythema, tachycardia, fever, bullae ► Responsible micro-organism ► Time between admission to primary hospital and first debridement ► Time between admission to primary hospital and admission to ICU ► Steroid treatment (injection/oral) prior to development of NSTI (Time frame: up to 7 days prior to surgical diagnosis at primary hospital) ► Other medication
Clinical data from the ICU	► MAP (mm Hg) ► Heart rate (bpm) ► Arterial blood gas values: pO_2, pCO_2, HCO_{3-}, base excess, pH ► K^+, Na^+, Ca^{2+}, glucose, creatinine, haemoglobin, haematocrit ► Norepinephrine infusion ► Ventilator treatment ► Vasopressor treatment ► Renal replacement treatment ► LRINEC score

bpm, beats per minute; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; LRINEC, Laboratory Risk Indicator for Necrotising Fasciitis; MAP, mean arterial blood pressure; NSTI, necrotising soft tissue infection; pO_2, partial pressure of oxygen; pCO_2, partial pressure of carbon dioxide.

table 1). During the first 7 days in the ICU, clinical data will be gathered (see table 2). For the orthopaedic control group, the blood samples have been drawn at three time points: once at baseline (preoperatively), once 2–6 hours postoperatively and once on the day after surgery between 08:00 and 12:00. For both patient groups, the anticoagulated blood is put on ice until centrifugation (within 40 min of collection, at 3500 rpm for 10 min). The supernatant (serum) is stored in 1 mL vials at −80°C until analysis.

Data analysis

Studies I, II and III: routine blood analysis

These tests will be run at the Department of Clinical Biochemistry, Copenhagen University Hospital. Among others: platelets, pH, base excess, fibrinogen, International Normalized Ratio (INR), D-dimer, C-Reactive Protein (CRP), procalcitonin, lactate, bilirubin, potassium, sodium, calcium, glucose, creatinine, haemoglobin, leucocytes.

Studies I and II: sTM, syndecan-1, sE-Selectin, VE-cadherin and protein C levels

These tests will be conducted at the Department of Clinical Immunology, Copenhagen University Hospital. All the biomarkers will be measured using ELISA methods from various companies (Nordic Biosite for sTM, syndecan-1 and sE-Selectin; R&D systems for VE-cadherin; Orion Diagnostica for protein C).

Study III: suPAR levels

These tests will be conducted at the Clinical Research Department, Hvidovre Hospital. suPAR levels will be measured using ELISA from ViroGates. Using a double monoclonal antibody sandwich ELISA assay, samples and peroxidase-conjugated anti-suPAR are mixed together. Incubation is done in anti-suPAR precoated micro wells. Calibration of the recombinant suPAR standards is done against healthy human blood donor samples. suPAR levels are reported in nanograms per millilitre of plasma.

Hypotheses; primary and secondary outcomes

Study I

Study I hypotheses

1. In patients with NSTI stratified into no sepsis, sepsis and septic shock groups as defined by standardised criteria,[45] HBOT[i] lowers sTM more than 1.75 ng/mL per day.
2. The aforementioned reduction in sTM in patients with NSTI after HBOT is statistically significantly larger than any reduction in sTM seen in both an elective orthopaedic surgery control group and sepsis control group.

[i]HBOT is applied by placing the patient with NSTI inside a HBOT chamber, where the patient is continuously breathing 100% O_2 through a ventilator and endotracheal intubation or if awake through a transparent hood and where the entire chamber is pressurised to 2.8 atmospheres absolute (ATA) for 90 min.

Study I primary outcome

Changes in plasma sTM and syndecan-1 concentrations, measured on admission and once daily the first 3 days in the ICU.

Study I secondary endpoint

A subanalysis of the differences in the aforementioned endothelial biomarkers between patients with NSTI who do not receive HBOT within the first 24 hours of ICU admission (because they are deemed too unstable for HBOT) versus those who receive HBOT within the first 12 and 24 hours of ICU admission.

Study II

Study II hypothesis

1. Inpatients with NSTI stratified into no sepsis, sepsis and septic shock groups as defined by standardised criteria,[45] HBOT[i] lowers sE-selectin more than 1.1 ng/mL per day.
2. The aforementioned reduction in sE-selectin in patients with NSTI after HBOT is statistically significantly larger than any reduction in sE-selectin seen in both an elective orthopaedic surgery control group and sepsis control group.

Study II primary endpoint

Changes in plasma sE-selectin, VE-cadherin and protein C concentrations, measured on admission and once daily the first 3 days in the ICU.

Study II secondary endpoint

A subanalysis of the differences in the aforementioned biomarkers between patients with NSTI who do not receive HBOT within the first 24 hours of ICU admission (because they are deemed too unstable for HBOT) versus those who receive HBOT within the first 12 and 24 hours of ICU admission.

Study III

Study III hypotheses

Inpatients with NSTI stratified into no sepsis, sepsis and septic shock groups as defined by standardised criteria,[45] suPAR levels are a predictor for mortality.

Inpatients with NSTI stratified into no sepsis, sepsis and septic shock groups as defined by standardised criteria,[45] suPAR levels reflect patients with NSTI' clinical condition as assessed by Simplified Acute Physiology Score II (SAPS II) and Sequential Organ Failure Assessment (SOFA) scores.

Study III primary endpoint

Association between plasma suPAR levels, measured on admission and once daily during the first 3 days in the ICU, and NSTI mortality, SAPS II and SOFA scores.

Joint secondary endpoints for studies I, II and III

In studies I, II and III, the following outcomes will be analysed for the NSTI group only:

- Mortality in the ICU and at 30, 90 and 180 days

- Amputations.

The following characteristics are registered in the INFECT database, which we will also be using for our studies:

- Age and sex
- Comorbidities: diabetes mellitus, liver cirrhosis, kidney disease, cardiovascular disease, HIV/AIDS, hepatitis, intravenous drug use, malignancy
- Body mass index
- Mean arterial pressure
- Heart rate
- Arterial blood gas: partial pressure of oxygen, partial pressure of carbon dioxide, HCO_3, base excess, pH
- Standard biochemistry: K^+, Na^+, Ca^{2+}, glucose, creatinine, haemoglobin etc
- Norepinephrine use
- Mechanical ventilation
- ICU scores: SAPS II, SOFA without Glasgow Coma Scale (GCS), Laboratory Risk Indicator for Necrotising Fasciitis
- Primary infectious focus
- Primary symptoms: pain, erythema, tachycardia, fever
- Pathogen type
- Time between admittance at primary hospital to the first surgery
- Definitive treatment at Copenhagen University Hospital: antibiotics, immunoglobulin and HBOT
- Treatment at primary hospital: antibiotics, immunoglobulin, surgical treatment
- Immunocompromising drugs prior to admission.

Sample size

Study I

The test kits we will be using to measure our primary outcome sTM (Human sCD141 ELISA kit, Nordic Biosite) have an interassay standard variation of 0.58 ng/mL. In order to be certain that measured changes in sTM concentration are not a result of interassay SD, we have set our minimum relevant difference in sTM to three times the interassay standard variation, thus 1.75 ng/mL.

We prepared a power calculation using a Wilcoxon rank-sum test. Assuming an estimated SD of 4.6 ng/mL and a mean of 9.9 ng/mL,[12] we will need to include a maximum of 150 patients with NSTI and 50 elective surgery patients to reach a statistical power of at the very least 60% (a very conservative estimate) and presumably closer to 85% (more realistic estimate) at a 5% significance level. The estimates depend on data distribution.

Study II

The test kits we will be using to measure our primary outcome sE-selectin (Human CD62E ELISA kit, Diaclone) have an interassay standard variation of 0.37 ng/mL. In order to be certain that measured changes in sE-selectin concentration are not a result of interassay standard variation, we have set our minimum relevant difference in sE-selectin to three times the interassay standard variation, thus 1.1 ng/mL.

Assuming an estimated SD of 209 ng/mL (septic shock) vs 23 ng/mL (severe sepsis and sepsis) and means of 295 vs 181 ng/mL, respectively,[46] we will need to include at least 132 patients with NSTI and 50 elective surgery patients to reach a statistical power of 90% at a 5% significance level.

Study III

suPAR levels during NSTI have never previously been examined. In order to estimate sample size and since most patients with NSTI are also septic, we are basing our sample size calculation on a previous study concerning the correlation between suPAR and sepsis.[29] This study found statistically significant correlation between suPAR levels and mortality in 141 patients. This is also our goal. Further studies have also found significant correlations between suPAR, sepsis and mortality in 132 patients.[30] We will include at least 150 patients with NSTI during this study.

Statistical considerations

Studies I and II

To check whether the HBOT treatment has an effect on the range of biomarkers, we will analyse the means and variances of the biomarkers in the NSTI group and the two control groups, the orthopaedic patients and the patients with sepsis. Non-parametric data will be log-transformed and will be presented as median values with IQR. Wilcoxon rank-sum tests will be used for group comparisons. Fisher's exact test will be used for categorical data. Correlation analysis will be performed using Spearman rank correlation or Pearson correlation.

Study III

To assess the quality of suPAR as a predictor of health outcomes, a model selection exercise will be conducted with various types of regression models. The type of regression will vary with the type of health-outcome, with suPAR as the predictor in all cases. Non-parametric data will be log-transformed and will be presented as median values with IQR. Fisher's exact test will be used for categorical data. Receiver operating characteristic curve analysis will be applied to determine suPARs accuracy as a marker of severity and mortality in patients with NSTI. We will construct Kaplan-Meier curves for survival data. Statistically significant results are when $p<0.05$. Corrections for multiple comparisons will be done using Wilcoxon rank-sum tests.

ETHICS AND DISSEMINATION

The study will be conducted in accordance with the principles of the Declaration of Helsinki. The Regional Health Research Ethics Committee and the Danish Data Protection Agency (responsible for the correct processing of confidential patient data) have approved the study (RHREC document number: H-16021845; DDPA j. no.: RH-2016–199). The investigator will inform the Research Ethics Committee

and the Danish Data Protection Agency of any significant changes to the protocol.

Written informed consent will be acquired from either the patients themselves or their next of kin as well as from their primary healthcare physician, as required by Danish law. This study itself poses no additional risk to the patients, as patients will receive standard NSTI treatment at Copenhagen University Hospital, in no way different from the usual treatment. To maintain confidentiality, each patient is assigned a pseudonymous research code. Access to patient data analysis is restricted to the investigators.

The study has been registered at the international database of clinical trials (www.clinicaltrials.gov; NCT03147352).

Results will be disseminated at national and international conferences and then published in international peer-reviewed scientific journals. Positive, negative and any inconclusive results will be published. The advanced knowledge of NSTIs generated by the above studies will be used to create evidence-based guidelines for classification and management. Through the INFECT project, we have access to the UK NSTI patient organisation together with the NSTI clinical consortium. This provides excellent means for efficient dissemination of guidelines and other advances made in the project to relevant end-users, including medical staff, patients and their relatives, small and medium enterprises and researchers.

Contributors PP and OH designed and wrote the research protocol. PP is responsible for the sample size calculations and statistical methods. PP is responsible for data acquisition. PIJ contributed to drafting the protocol. PIJ is responsible for the laboratory work in studies I and II.

Funding The INFECT project is supported by The European Seventh Framework Programme (grant number 305340).

Competing interests None declared.

Ethics approval The Regional Health Research Ethics Committee of Denmark.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1. Hakkarainen TW, Kopari NM, Pham TN, *et al*. Necrotizing soft tissue infections: review and current concepts in treatment, systems of care, and outcomes. *Curr Probl Surg* 2014;51:344–62.
2. Shaw JJ, Psoinos C, Emhoff TA, *et al*. Not just full of hot air: hyperbaric oxygen therapy increases survival in cases of necrotizing soft tissue infections. *Surg Infect* 2014;15:328–35.
3. Soh CR, Pietrobon R, Freiberger JJ, *et al*. Hyperbaric oxygen therapy in necrotising soft tissue infections: a study of patients in the United States Nationwide Inpatient Sample. *Intensive Care Med* 2012;38:1143–51.
4. Devaney B, Frawley G, Frawley L, *et al*. Necrotising soft tissue infections: the effect of hyperbaric oxygen on mortality. *Anaesth Intensive Care* 2015;43:685–92.
5. Thunø M, Macho B, Eugen-Olsen J. suPAR: the molecular crystal ball. *Dis Markers* 2009;27:157–72.
6. Reitsma S , *et al*. The endothelial glycocalyx. *Pflugers Arch* 2007;454:345–59.
7. Jallali N, Withey S, Butler PE, *et al*. Hyperbaric oxygen as adjuvant therapy in the management of necrotizing fasciitis. *Am J Surg* 2005;189:462–6.
8. van den Berg BM, Vink H, Spaan JA, *et al*. The endothelial glycocalyx protects against myocardial edema. *Circ Res* 2003;92:592–4.
9. De Backer D, Donadello K, Favory R, *et al*. Link between coagulation abnormalities and microcirculatory dysfunction in critically ill patients. *Curr Opin Anaesthesiol* 2009;22:150–4.
10. Ostrowski SR, Gaïni S, Pedersen C, *et al*. Sympathoadrenal activation and endothelial damage in patients with varying degrees of acute infectious disease: an observational study. *J Crit Care* 2015;30:90–6.
11. Johansen ME, Jensen JU, Bestle MH, *et al*. The potential of antimicrobials to induce thrombocytopenia in critically ill patients: data from a randomized controlled trial. *PLoS One* 2013;8:e81477.
12. Ostrowski SR, Berg RM, Windeløv NA, *et al*. Coagulopathy, catecholamines, and biomarkers of endothelial damage in experimental human endotoxemia and in patients with severe sepsis: a prospective study. *J Crit Care* 2013;28:586–96.
13. Johansson PI, Stensballe J, Rasmussen LS, *et al*. A high admission syndecan-1 level, a marker of endothelial glycocalyx degradation, is associated with inflammation, protein C depletion, fibrinolysis, and increased mortality in trauma patients. *Ann Surg* 2011;254:194–200.
14. Ostrowski SR, Pedersen SH, Jensen JS, *et al*. Acute myocardial infarction is associated with endothelial glycocalyx and cell damage and a parallel increase in circulating catecholamines. *Crit Care* 2013;17:R32.
15. Johansson PI, Bro-Jeppesen J, Kjaergaard J, *et al*. Sympathoadrenal activation and endothelial damage are inter correlated and predict increased mortality in patients resuscitated after out-of-hospital cardiac arrest. a post Hoc sub-study of patients from the TTM-trial. *PLoS One* 2015;10:e0120914.
16. Rehm M, Bruegger D, Christ F, *et al*. Shedding of the endothelial glycocalyx in patients undergoing major vascular surgery with global and regional ischemia. *Circulation* 2007;116:1896–906.
17. Blann A, Seigneur M. Soluble markers of endothelial cell function. *Clin Hemorheol Microcirc* 1997;17:3–11.
18. Johansson PI, Sørensen AM, Perner A, *et al*. High sCD40L levels early after trauma are associated with enhanced shock, sympathoadrenal activation, tissue and endothelial damage, coagulopathy and mortality. *J Thromb Haemost* 2012;10:207–16.
19. Lin HC, Wan FJ, Wu CC, *et al*. Hyperbaric oxygen protects against lipopolysaccharide-stimulated oxidative stress and mortality in rats. *Eur J Pharmacol* 2005;508:249–54.
20. Imperatore F, Cuzzocrea S, De Lucia D, *et al*. Hyperbaric oxygen therapy prevents coagulation disorders in an experimental model of multiple organ failure syndrome. *Intensive Care Med* 2006;32:1881–8.
21. Cuzzocrea S, Imperatore F, Costantino G, *et al*. Role of hyperbaric oxygen exposure in reduction of lipid peroxidation and in multiple organ failure induced by zymosan administration in the rat. *Shock* 2000;13:197–203.
22. Pedoto A, Nandi J, Yang ZJ, *et al*. Beneficial effect of hyperbaric oxygen pretreatment on lipopolysaccharide-induced shock in rats. *Clin Exp Pharmacol Physiol* 2003;30:482–8.
23. Thom SR. Hyperbaric oxygen: its mechanisms and efficacy. *Plast Reconstr Surg* 2011;127(Suppl 1):131S–41.
24. Thom SR. Oxidative stress is fundamental to hyperbaric oxygen therapy. *J Appl Physiol* 2009;106:988–95.
25. Mader JT, Brown GL, Guckian JC, *et al*. A mechanism for the amelioration by hyperbaric oxygen of experimental staphylococcal osteomyelitis in rabbits. *J Infect Dis* 1980;142:915–22.
26. Kalns J, Lane J, Delgado A, *et al*. Hyperbaric oxygen exposure temporarily reduces Mac-1 mediated functions of human neutrophils. *Immunol Lett* 2002;83:125–31.
27. Chen Q, *et al*. Functional inhibition of leukocyte B2 integrins by HBO. *J. Pharmacol. Exp. Ther* 1996;276:929–33.
28. Thom SR. Functional inhibition of leukocyte B2 integrins by hyperbaric oxygen in carbon monoxide-mediated brain injury in rats. *Toxicol Appl Pharmacol* 1993;123:248–56.
29. Buras JA, Stahl GL, Svoboda KK, *et al*. Hyperbaric oxygen downregulates ICAM-1 expression induced by hypoxia and hypoglycemia: the role of NOS. *Am J Physiol Cell Physiol* 2000;278:C292–302.
30. Zamboni WA, Roth AC, Russell RC, *et al*. Morphologic analysis of the microcirculation during reperfusion of ischemic skeletal muscle and the effect of hyperbaric oxygen. *Plast Reconstr Surg* 1993;91:1110–23.

31. Giannotta M, Trani M, Dejana E. VE-cadherin and endothelial adherens junctions: active guardians of vascular integrity. *Dev Cell* 2013;26:441–54.
32. Hunt BJ, Jurd KM. A central pathophysiological process. *BMJ* 1998;316:1328.
33. Chong AY, Blann AD, Lip GY. Assessment of endothelial damage and dysfunction: observations in relation to heart failure. *QJM* 2003;96:253–67.
34. Gavard J. Endothelial permeability and VE-cadherin: a wacky comradeship. *Cell Adh Migr* 2014;8:158–64.
35. Godman CA, Chheda KP, Hightower LE, *et al*. Hyperbaric oxygen induces a cytoprotective and angiogenic response in human microvascular endothelial cells. *Cell Stress Chaperones* 2010;15:431–42.
36. Eugen-Olsen J, Andersen O, Linneberg A, *et al*. Circulating soluble urokinase plasminogen activator receptor predicts cancer, cardiovascular disease, diabetes and mortality in the general population. *J Intern Med* 2010;268:296–308.
37. Lyngbæk S, Marott JL, Sehestedt T, *et al*. Cardiovascular risk prediction in the general population with use of suPAR, CRP, and Framingham Risk Score. *Int J Cardiol* 2013;167:2904–11.
38. Gumus A, Altintas N, Cinarka H, *et al*. Soluble urokinase-type plasminogen activator receptor is a novel biomarker predicting acute exacerbation in COPD. *Int J Chron Obstruct Pulmon Dis* 2015;10:357–65.
39. Mölkänen T, Ruotsalainen E, Thorball CW, *et al*. Elevated soluble urokinase plasminogen activator receptor (suPAR) predicts mortality in Staphylococcus aureus bacteremia. *Eur J Clin Microbiol Infect Dis* 2011;30:1417–24.
40. Wittenhagen P, Kronborg G, Weis N, *et al*. The plasma level of soluble urokinase receptor is elevated in patients with Streptococcus pneumoniae bacteraemia and predicts mortality. *Clin Microbiol Infect* 2004;10:409–15.
41. Huttunen R, Syrjänen J, Vuento R, *et al*. Plasma level of soluble urokinase-type plasminogen activator receptor as a predictor of disease severity and case fatality in patients with bacteraemia: a prospective cohort study. *J Intern Med* 2011;270:32–40.
42. Ostergaard C, Benfield T, Lundgren JD, *et al*. Soluble urokinase receptor is elevated in cerebrospinal fluid from patients with purulent meningitis and is associated with fatal outcome. *Scand J Infect Dis* 2004;36:14–19.
43. Kofoed K, Eugen-Olsen J, Petersen J, *et al*. Predicting mortality in patients with systemic inflammatory response syndrome: an evaluation of two prognostic models, two soluble receptors, and a macrophage migration inhibitory factor. *Eur J Clin Microbiol Infect Dis* 2008;27:375–83.
44. Backes Y, van der Sluijs KF, Mackie DP, *et al*. Usefulness of suPAR as a biological marker in patients with systemic inflammation or infection: a systematic review. *Intensive Care Med* 2012;38:1418–28.
45. Singer M, Deutschman CS, Seymour CW, *et al*. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 2016;315:801–10.
46. Kayal S, Jaïs JP, Aguini N, *et al*. Elevated circulating E-selectin, intercellular adhesion molecule 1, and von willebrand factor in patients with severe infection. *Am J Respir Crit Care Med* 1998;157:776–84.