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CLINICAL INVESTIGATION

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Efficacy and safety of iloprost in trauma patients with haemorrhagic shock-induced endotheliopathy—Protocol for the multicentre randomized, placebo-controlled, blinded, investigator-initiated shine-trauma trial

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Abstract

Background: Traumatic injury accounts for 800 000 deaths in the European Union annually. The main causes of deaths in trauma patients are exsanguination and multiple organ failure (MOF). We have studied >1000 trauma patients and identified shock-induced endotheliopathy (SHINE), the pathophysiological mechanism responsible for MOF and high mortality. Pilot studies indicate that low-dose iloprost (1 ng/kg/min) improves endothelial functionality in critically ill patients suggesting this intervention may improve patient outcome in traumatic SHINE.

Material and Methods: This is a multicentre, randomized, blinded clinical investigator-initiated phase 2B trial in trauma patients with haemorrhagic shock-induced endotheliopathy. Patients are randomized 1:1 to 72 hours infusion of iloprost 1 ng/kg/min or Placebo (equal volume of saline). A total of 220 trauma patients will be included. The primary endpoint is the number of intensive care unit (ICU)-free days, within 28 days of admission. Secondary endpoints include 28- and 90-day all-cause mortality, hospital length of stay, vasopressor-free days in the intensive care unit (ICU) within 28 days, ventilator-free days in the ICU within 28 days, renal replacement-free days in the ICU within 28 days, number of serious adverse reactions and serious adverse events within the first 4 days of admission.

Discussion: This trial will test the safety and efficacy of administration of iloprost vs placebo for 72 hours in trauma patients with haemorrhagic shock-induced endotheliopathy. Trial endpoints focus on the potential effect of iloprost to reduce the need for ICU stay secondary to mitigation of organ failure.

Trial registration: SHINE-TRAUMA trial—EudraCT no. 2019-000936-24—Clinicaltrials.gov: NCT03903939 Ethics Committee no. H-19014482.

1 | INTRODUCTION

Traumatic injury is the fourth leading cause of death globally and the leading cause of death in patients <45 years and accounts for 800 000 deaths in the EU of which 500 are in Denmark annually.[1] The main causes of death in trauma patients are exsanguination and multiple organ failure (MOF) including traumatic brain injury (TBI).[2] The frequency of exsanguinations has decreased substantially since we introduced haemostatic resuscitation with early administration of plasma and platelets in a 1:1:1 ratio to red blood cells (RBC) and goal-directed haemostatic interventions based on whole blood thrombelastography (TEG). This transfusion strategy reduced haemorrhage-related mortality at Rigshospitalet, Denmark, from 50% in 2005 to 13% in 2013 and is recommended worldwide.[3]

To further reduce the risk of trauma patient exsanguination, the European Commission funded a FP7 Program for the International Trauma Research Network (INTRN) encompassing centres in Copenhagen (lead), London, Oxford, Cologne, Amsterdam and Oslo.[4] The aim of the Targeted Action for Curing Trauma Induced Coagulopathy (TACTIC) project is to develop evidence-based algorithms for haemostatic resuscitation in trauma patients and have these tested in a randomized clinical trial.[5] By studying 2300 trauma patients, two optimized treatment algorithms were developed and have currently been tested in 392 trauma patients with haemorrhagic shock [NTC02593877].

Apart from exsanguination also development of MOF remains a major problem with high mortality in trauma patients.[6] A potential explanation for the high non-bleeding mortality in trauma patients was provided by our group who in 2011 reported that systemic microvascular endothelial damage was responsible for development of MOF.[7] This novel pathophysiological mechanism encompasses systemic damage to the microvascular endothelium secondary to overactivation of the sympathico-adrenal system, leading to toxic high levels of circulating catecholamines. This leads to a pro-coagulant/pro-thrombotic and damaged endothelium resulting in *both* capillary leakage and formation of microvascular thrombus, which both result in cellular hypoxia and MOF. Essential for these pathological processes is the loss of the endothelial glycocalyx (EGL); a structure consisting of proteoglycans, glycoproteins and glucosaminoglycans that binds plasma and its proteins to the endothelial surface, forming an anti-inflammatory and anticoagulant physical barrier on the luminal side of the endothelium.[8]

We studied >600 trauma patients in Copenhagen and Houston and validated that those with haemorrhagic shock had increased levels of circulating syndecan-1 (an EGL component) in plasma, increased injury severity score (ISS) and a doubling of the mortality rate (OR 2.2) compared with non-shocked patients.[9] These patients also had high levels of circulating VEGFR1/VE-cadherin and soluble thrombomodulin (sTM), markers of endothelial tight-junction disruption and pro-coagulant/thrombotic endothelial microvasculature, respectively, leading to thrombosis and MOF. Also, in patients with TBI, we found that EGL damage is significantly associated with increased intracranial pressure and incarceration[10] pointing towards a common denominating factor, namely, failure of one of the largest "organs" or structure in the body, ie the endothelium, a disease entity we entitle shock-induced endotheliopathy (SHINE). The endothelium weighs ~1 kg and has a surface area of ~5000 m^2[11] and endothelial cells form the innermost lining of all blood vessels and extends to all reaches of the vertebrate body. The endothelium establishes a unique dialogue between the underlying tissue and the flowing blood and damage to this delicate structure can be detrimental as presented above.[12,13]

In 2010, we investigated the outcome of critically ill patients needing renal replacement therapy at the University Hospital in Copenhagen. The finding was that those receiving prostacyclin (PGI_2) as anticoagulant in the dialysis filter had substantially lower 30-day mortality than patients receiving heparin (21% vs 39%), despite being more critically ill[14] and we speculated that this may be due to a spillover effect of PGI_2 to the systemic circulation. PGI_2 is an endogenous prostanoid formed and released by endothelial cells with paracrine function including vasodilation and platelet inhibition. Because of these properties, it was introduced as a pharmacological therapy in high doses in 1979 for patients with primary pulmonary hypertension and critical limb ischaemia.[15,16]

In the new millennium it was reported that PGI_2 also confers potent endothelial cytoprotection by: synthesizing endothelial glycocalyx constituent (hyaluronic acid),[17,18] acting on prostaglandin I (IP_1) receptors on endothelial progenitor cells leading to re-endothelium formation in damaged vessels[19] upregulating VE-cadherin responsible for tight-junction integrity, ie preventing capillary leakage,[20] inducing peroxisome PPAR attenuation of NF-kB and TNF activation in ischaemia-reperfusion injury which minimizes the inflammatory hit on the endothelium[21] and is protecting against ischaemia-reperfusion injury through the PGI_2-PPARα-HEME Oxygenase-1 signalling pathway that provide robust rejuvenation of the damaged endothelium.[22]

A clinical trial in healthy volunteers showed that low dose of the PGI_2 analogue iloprost did not affect blood pressure or platelet function but instead appeared to improve endothelial functionality as evaluated by soluble thrombomodulin (sTM) [EudraCT no: 2011-006200-12]. The effect of low-dose iloprost infusion (1 ng/kg/min) was, therefore, investigated in randomized, double-blind pilot trials in coronary stent (n = 18),[23] major surgery (n = 56)[24] and septic shock patients (n = 18).[25] These trials collectively documented no adverse effect on blood pressure or platelet function secondary to low-dose iloprost administration. Instead, iloprost infusion significantly improved endothelial function and integrity, measured by validated biomarkers, in all groups.

1.1 | Trial hypothesis

We hypothesize that continuous infusion of low-dose iloprost, ie 1 ng/kg/min, for 72 hours after randomization as compared to placebo in trauma patients with haemorrhagic shock-induced

endotheliopathy will increase the number of ICU-free days alive, within 28 days of admission.

2 | Material and Methods

2.1 | Trial design

This is a multicentre, randomized (1:1, iloprost: placebo), placebo controlled, blinded, investigator-initiated phase 2b trial in trauma patients with haemorrhagic shock and SHINE, investigating the efficacy and safety of continuous intravenous administration of iloprost (1 ng/kg/min) vs placebo for 72 hours, in a total of 220 patients.

2.2 | Trial registration

EudraCT nr: 2019-000936-24 and Capital Region Ethics Committee no. H-19014482. The study is monitored by the Capital Region GCP Unit, Bispebjerg Hospital, Denmark.

2.3 | Setting

The trial will be conducted at the trauma centres at Rigshospitalet, Odense University Hospital, Århus University Hospital in Denmark and at Oslo University Hospital in Norway.

2.4 | Study population

2.4.1 | Inclusion criteria

Patients will be included in the SHINE-TRAUMA trial, if they fulfil the following criteria:

- Age ≥ 18 years
- Present with clinical signs of haemorrhagic shock (defined by systolic blood pressure < 90 mm Hg or administration of pre-hospital blood transfusion).
- Activation of local massive transfusion protocol and initiation of the first transfusion after admission.
- Can be randomized within 5 hours of injury and 3 hours of admission to the ED of the participating trial site.
- Consent is provided on behalf of incapacitated patients by a Scientific Guardian

2.4.2 | Exclusion criteria

Patients are not eligible for inclusion in this trial if they fulfil one or more of the following criteria:

- Withdrawal from active therapy
- Known hypersensitivity to Iloprost.
- Pregnancy (non-pregnancy confirmed by patient having a negative urine- or blood hCG) or being post-menopausal defined as women beyond 60 years old)
- Known severe heart failure (NYHA class IV)
- Suspected acute coronary syndrome
- Estimated weight < 40 kg

Patients enrolled in other interventional trials will not be excluded unless the protocols of the two trials collide. A co-enrolment agreement will be established between the sponsors.

2.5 | Screening

All trauma patients admitted to a participating clinical trial site is considered for participation and is screened for eligibility of enrolment by local investigators using paper CRF. The distribution of screened patients will be displayed in a Consolidated Standards of Reporting Trials (CONSORT) diagram.[23]

2.6 | Randomization

The randomisation sequence will be done in permuted blocks of variable sizes stratified per trial site using centralized, concealed allocation. The randomisation sequence will be generated 1:1 (active/placebo) using the online randomisation software 'Sealed Envelope' (https://www.sealedenvelope.com/). Once generated the randomisation sequence will be formatted and uploaded into an electronic research database REDCap to facilitate centralized, web-based allocation according to local written instruction. The randomization sequence will be printed and signed by two independent individuals and stored in a sealed envelope in the sponsor's trial master file.

The patient randomisation at each site will be done in the electronic system REDCap, where each patient will be given a unique randomisation number/Study ID number. The randomization sequence will be concealed from all clinicians, patients, investigators and statisticians and will first be shown in REDCap after completion of all trial-related procedures and statistical analyses are finalized.

2.7 | Trial intervention

A 1:1 randomized stratification for active study drug or placebo infusion for 72 hours. Patients in both randomization groups will be treated in accordance with state-of-the-art therapy. Interventions are considered emergency procedures and study drug infusion should be commenced as soon as possible after screening and randomization. Patients with active treatment will receive a low-dose prostacyclin infusion while the placebo group receives a dummy

saline infusion for 72 hours in a total of 220 patients. The active treatment (n = 110 patients) will consist of continuous administration of iv infusions of 1.0 ng/kg/min of iloprost for 72 hours. Patients in the placebo group (n = 110 patients) will receive dummy saline infusion at a volume comparable to active treatment and will otherwise be treated exactly as active *patients*. This dosing regimen was chosen since intravenous doses of prostacyclin 0.5-2.0 ng/kg/min have been reported to be successful at achieving endothelial modulating/preserving effect with no significant haemodynamic or platelet aggregation complications.[26-28]

2.8 | Outcome measures

2.8.1 | Primary outcome measure

Number of ICU-free days alive, within 28 days of admission where death during the ICU stay gives the score 0.

2.8.2 | Secondary outcome measures

- 28 and 90-day all-cause mortality
- Hospital length of stay
- Vasopressor-free days in the ICU within 28 days
- Ventilator-free days in the ICU within 28 days
- Renal replacement-free days in the ICU within 28 days
- Number of serious adverse reactions and serious adverse events (defined as ischaemic events, see below) within the first 4 days of admission

2.9 | Blinding

To circumvent selection bias, researchers and health care personnel, will be blinded to the treatment assignment. Furthermore, to avoid investigator, healthcare staff and patient performance and detection bias, patients will be randomized to receive either iloprost (Ilomedin®, Bayer AG, Leverkusen, Germany) or placebo similar in colour, consistency and volume. Blinded study and non-study personnel will record clinical data and analyse blood samples. All randomized patients who have received iloprost will continue to be included in the assessments of its safety and efficacy. Also, all analyses of the endpoints are performed by blinded personnel. We expect no loss to follow-up in hospital, provided that this study takes place in a hospital setting where the majority of the outcomes will be evaluated.

2.10 | Data registration and monitoring

Data will be entered into a central web-based electronic case report form (eCRF) using the data management system REDCap® software (REDCap 8.10.18—© 2019 Vanderbilt University). The eCRF is password protected, audit-trailed, encrypted and allows for detailed centralized and de-centralized surveillance of data completeness overall and at each site. Each participating trial site will only have access to their own data.

2.11 | Serious adverse reactions and serious adverse events

All serious adverse events (SAEs) will be captured as part of the daily routine in the patient electronic health record (ie ICU notes, laboratory reports) and this will allow for later inspection if needed. The investigator will record the occurrence of SARs until day 4 for all included patients in the CRF. SARs are only recorded until day 4 as no further safety concerns related to iloprost beyond day 4 is expected due to the short half-life of the study drug. Safety assessment will be done comparing safety events for iloprost vs placebo. Patients will not be withdrawn from the trial if a SAR occurs, but it will be recorded in the CRF. The volume of up to 100 ml NaCl per 24 hours does not cause any safety concerns in this population.

The following adverse reactions identified in the SmPC as having the potential to be serious adverse reactions, but as they are known reactions, they can´t be SUSARs and therefore not subject for expedited reporting. The following will be observed and recorded in the CRF until day 4:

- Bleeding events (intracerebral haemorrhage (verified by CT), bloody diarrhoea, rectal bleeding)
- Severe cardiac failure (defined as severe cardiogenic shock and ejection fraction < 20% cardiac ultrasound)
- Pulmonary embolism (symptomatic and verified by CT)
- Deep vein thrombosis (symptomatic and verified by ultrasound)

The following SAEs are of special interest in this population. These will be recorded until day 4 as for all other SAR.

- Ischaemic events (intestinal or limb ischaemia or myocardial infarction (STEMI) or cerebral ischaemia (verified by CT))

The following SAEs are clinical endpoint and will be recorded at day 28 and/or day 90 as part of endpoint assessments and not separate:

- Deaths
- Need for mechanical ventilation
- Need for renal replacement therapy
- Need for vasopressor therapy

2.12 | Approval

The trial is approved by the Danish Medicines Agency (EudraCT no. 2019-000936-24), the Committees on Health Research Ethics in

the Capital Region of Denmark (H-19014482) and the Danish Data Protection Agency (P-2019-85). All patients will be enrolled after consent from a scientific guardian who is independent of the trial.

2.13 | Statistics

The primary endpoint will be analysed using linear regression adjusted for site. Effect size will be summarized using adjusted mean differences with confidence intervals based on robust standard errors as residuals are not expected to be normally distributed. The same analysis will be employed to all continuous secondary outcomes. All binary secondary outcomes will be analysed using logistic regressions adjusted for site. Effect size will be provided as odds ratio with confidence intervals. All-cause mortality will be further illustrated using Kaplan-Meier curves.

2.13.1 | Sample size estimation

The power calculation is based on not yet published data from patients admitted to Rigshospitalet/Copenhagen University Hospital that were included in the iTACTIC trial [NTC 02593877] having the same in- and exclusion criteria as the present trial. The number of ICU-free days within 30 days from admission is chosen as the primary endpoint and a clinically relevant increase in ICU-free days within 28 days of 30% with α 0.05, power 0.85 will require 107 patients in each randomization group. We plan on including 110 patients in each group and 220 in total. The final statistical analysis plan will be published before the last patient is included in the trial and analysis of the blinded data from the randomized trial will be performed by Theis Lange, Assoc. Professor, Section of Biostatistics, Department of Public Health, University of Copenhagen.

2.14 | Populations and subgroups

Definitions of trial populations are as follows:

2.14.1 | Intention to treat

This will comprise all randomized patients (except those randomized in error who never received the trial medication). This population will be evaluated for all endpoints.

2.14.2 | Per-protocol

This is a subset of the intention-to-treat population encompassing correctly included patients who have received the trial medication according to protocol (ie 72 hours infusion of iloprost or placebo after inclusion or until dead or discharged to ward, whichever comes first). This population will be evaluated for the primary endpoint only.

2.14.3 | Safety population

This comprise all randomized patients including those who are withdrawn.

One subgroup analysis is planned: Patients with traumatic brain injury (TBI). For this sub-group analysis, effect measures on all outcomes will be computed along with *P*-values and confidence intervals. For the sub-group and patient outcome, a test for no-treatment heterogeneity will also be reported.

2.15 | Trial organization and management

The SHINE-TRAUMA trial is performed at the Trauma Centers at Rigshospitalet, Odense University Hospital, Århus University Hospital and Oslo University Hospital and the SHINE Group at Rigshospitalet. The Management Committee encompasses the local principle investigators and a clinical data and regulatory manager at SHINE Group and is responsible for the overall management and coordination, which will be supervised by the Steering Committee. Site investigators will manage and coordinate the trial at the sites. The principal investigator is responsible for data collection and maintenance of trial documents. Co-enrolment of participants in other interventional trials have to be approved by the SHINE-TRAUMA steering committee but is generally appreciated.

2.16 | Data sharing

The trial results will be submitted to a peer-reviewed open source international clinical journal. De-identified data will be made publicly available 12 months after 1-year follow-up of the last randomized patient according to the recent ICMJE recommendations.[25]

2.17 | Finances

The research project is investigator initiated by the trial sponsor Pär I. Johansson who has received unrestricted research grants of € 878 000 from the Novo Nordisk Foundation and €156 000 from the Danish Regions. The amount is paid to and administered by Rigshospitalet. The respective trauma centres involved in the trial also support the project with personnel and laboratory facilities.

Neither patients nor health personnel will receive any remuneration from participating in the trial. The Novo Nordisk Foundation and the Danish Regions have no influence on the design, the conduct or the results of the trial.

3 | DISCUSSION

3.1 | Intervention

The introduction of haemostatic resuscitation including early aggressive administration of plasma and platelet in addition to RBC at a 1.1.1 ratio together with goal-directed pro-haemostatic and anti-fibrinolytic pharmacological interventions as exemplified by the Copenhagen Concept has dramatically reduced bleeding-related mortality in trauma.[29] Unfortunately, the reduction in early mortality has been accompanied by an increase in deaths due to MOF. We have identified a novel unifying pathophysiological mechanism entitled shock-induced endotheliopathy (SHINE) as the main cause for systemic endothelial damage associated with capillary leakage and microvascular thrombus formation resulting in impaired oxygen availability for the cells, ultimately leading to organ failure and death.[30]

The rationale for this trial is that MOF in trauma patients is caused by shock-induced endotheliopathy and that an endothelial cytoprotective effect of iloprost has been identified.[23-25] The 1 ng/kg/min dose of Iloprost necessary for the endothelial cytoprotective effect is well below the dose necessary for its vasodilatory and platelet inhibiting effects contributing to the safety of administering the intervention in shocked trauma patients. The sample size of 220 shocked trauma patients is based on the incidence of MOF in a recently finalized randomized clinical trial entitled iTACTIC trial [NTC 02593877] having the same in- and exclusion criteria as the present trial.

3.2 | Outcome

The present trial focus on an intervention with the potential to mitigate development and progression of MOF and, therefore, the primary outcome measure has been chosen from the sepsis literature as alive and free from the need for intensive care from randomization to day 28.[31] The reason for using this surrogate endpoint is firstly that it increases the likelihood of a fair evaluation of the interventional effect by considering that a proportion of the patients die of other complications than organ failure where the intervention is not expected to have any effect. Secondly, this approach enables us to evaluate the novel intervention more rapidly have since fewer patients need to be included in the study to reach the statistical power needed. Hereby, a potentially beneficial or harmful effect of the intervention can be provided for this category of patients who have a high unmet medical need.

3.3 | Strength

The SHINE-TRAUMA trial is an investigator-initiated, randomized placebo-controlled trial of iloprost as compared to placebo in shocked trauma patients with SHINE. The trial design is based on a stringent methodology, which includes concealed group allocation, blinding to the patient, clinical staff, the investigators, the outcome assessors and the trial statistician. The trial is GCP monitored. Sample size estimations and trial design are based currently on best evidence.

3.4 | Limitations

Development of multi-organ failure in trauma patients is a multicellular event where cells in the circulating blood, the endothelium and the cells of the vital organs are involved. The current trial is limited in its scope to investigate the potential role of the endothelial cell in the process leading to MOF and, hence, the current trial can only assess the potential effect of iloprost for this purpose here. Furthermore, although SHINE-TRAUMA is a multicentre trial, it only includes Scandinavian trauma centres and the sample size is limited to show effects on ICU-free days within 28 days only.

4 | Perspective

The trial is conducted with a stringent methodology, which complies with international guidelines for clinical trials and good clinical practice. Being a phase 2B trial and using a surrogate primary endpoint ensures both that the correct number of patients are included and that the results become rapidly available to the scientific and regulatory communities.

5 | Trial status

The trial is currently recruiting at 4 active trial sites. Patient inclusion was initiated on 21 May 2019 and the trial is expected to end in 2022.

CONFLICT OF INTEREST

PIJ is co-inventor of a patent covering the use of low-dose prostacyclin to critically ill patients with SHINE. All other declare that they have no competing interests.

AUTHOR CONTRIBUTION

The manuscript was written by Pär I Johansson and all authors reviewed and approved the final version.

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