Mitochondrial Dysfunction in Trauma-Related Coagulopathy: Is There Causality? Study Protocol for a Prospective Observational Study

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**Abstract**

Hemorrhage control often poses a great challenge for clinicians due to trauma-induced coagulopathy (TIC). The pathogenesis of TIC is not completely revealed; however, growing evidence attributes a central role to altered platelet biology. The activation of thrombocytes and subsequent clot formation are highly energetic processes being tied to mitochondrial activity, and the inhibition of the electron transport chain (ETC) impedes on thrombogenesis, suggesting the potential role of mitochondria in TIC. Our present study protocol provides a guide to quantitatively characterize the derangements of mitochondrial functions in TIC. One hundred eleven severely injured (injury severity score ≥16), bleeding trauma patients with an age of 18 or greater will be included in this prospective observational study. Patients receiving oral antiplatelet agents including cyclooxygenase-1 or adenosine diphosphate receptor inhibitors (aspirin, clopidogrel, prasugrel, and ticagrelor) will be excluded from the final analysis. Hemorrhage will be confirmed and assessed with computer tomography. Conventional laboratory markers of hemostasis such as prothrombin time and international normalized ratio will be measured and rotational thromboelastometry (ROTEM) will be performed directly upon patient arrival. Platelets will be isolated from venous blood samples and subjected to high-resolution fluororespirometry (Oxygraph-2k, Oroboros Instruments, Innsbruck, Austria) to evaluate the efficacy of mitochondrial respiration. Oxidative phosphorylation (OxPhos), coupling of the ETC, mitochondrial superoxide formation, mitochondrial membrane potential changes, and extramitochondrial Ca\textsuperscript{2+}-movement will be recorded. The association between OxPhos capacity of platelet mitochondria and numerical parameters of ROTEM aggregometry will constitute our primary outcome. The relation between OxPhos capacity and results of viscoelastic assays and conventional markers of hemostasis will serve as secondary outcomes. The association of the OxPhos capacity of platelet mitochondria upon patient arrival to the need for massive blood transfusion and 24-h mortality will constitute our tertiary outcomes. Mitochondrial dysfunction and its importance in TIC are yet to be assessed for the deeper understanding of this common, life-threatening condition. Disclosure of mitochondria-mediated processes in this study is encouraged.

**Keywords**

Blood coagulation disorders · Blood platelet disorders · Coagulopathy · Trauma induced · Hemorrhage · Mitochondria
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thrombocytes may reveal new therapeutic targets in the management of hemorrhaging trauma patients, thereby leading to a reduction of potentially preventable mortality. The present protocol was registered to ClinicalTrials.gov on 12 August 2021, under the reference number NCT05004844.

Background

The majority of potentially preventable mortality in trauma patients is related to bleeding [1–3]. Hemorrhage control often poses a great challenge for clinicians due to trauma-induced coagulopathy (TIC), a condition that is present in one-third of bleeding trauma patients [4]. Alterations in coagulation following severe injury were documented already in the 1960s [5]; however, a standard definition for TIC still does not exist [6]. TIC is characterized by dysfunctional clot formation and breakdown, impaired vascular homeostasis and is associated with increased risk of multiple organ failure and mortality [7, 8].

Regarding the pathogenesis of TIC, the contribution of factor depletion and dysregulated fibrinolysis is clear; however, growing evidence attributes central role to altered platelet biology [9–11]. According to related studies, dysfunctional platelet aggregation can be identified with aggregometry assays in, approximately, 50% of trauma patients, entailing a higher risk for mortality [12, 13]. Diminished platelet functions are suggested to be consequences of injury-induced early hyperactivation [10]; nevertheless, the mediators and pathways of the process are elusive, thus being subjects for further research [4, 14].

In the past decade, the presence of altered mitochondrial functions has been confirmed in the background of several diseases [15–17]. Furthermore, mitochondrial dysfunction of various cell types occurs also in trauma-related conditions such as hemorrhagic shock and traumatic brain injury [18–20]. As platelets are considered as central mediators in TIC, the understanding of mitochondria-mediated processes in thrombocytes may disclose new therapeutic targets in the management of severely injured patients [21]. It is already well known that the activation of thrombocytes and subsequent clot formation are highly energetic processes being tied to mitochondrial activity [22, 23]. According to the literature, inhibition of the electron transport chain impedes on thrombogenesis [24–26], suggesting the potential role of mitochondria in TIC. Furthermore, the decreased physiological function of transfused platelets is believed to be a consequence of deteriorated mitochondrial respiration occurring already after 2 days of storage in blood-banked platelets [21, 27].

We hypothesize that mitochondrial dysfunction occurs in the platelets of trauma patients with TIC. We intend to quantitatively characterize the derangements of mitochondrial functions in TIC, and assess the relation between mitochondrial respiration and clinical markers of platelet function measured with aggregometry, viscoelastic tests, and conventional laboratory analysis.

Methods

Study Design

The present paper is a protocol for a single-center, prospective observational study that will be conducted at the University of Szeged, Hungary. Our protocol complies with the Declaration of Helsinki and follows the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) checklist (see online suppl. file. 1; see www.karger.com/doi/10.1159/000521670 for all online suppl. material).

Patient Enrollment and Inclusion Criteria

This prospective observational study will involve severely injured (injury severity score ≥16) patients with bleeding confirmed with computer tomography, aged ≥18 years, transported directly to the emergency department of the University of Szeged. Patients receiving oral antiplatelet agents including cyclooxygenase-1 or adenosine diphosphate (ADP) receptor (P2Y12) inhibitors (aspirin, clopidogrel, prasugrel, and ticagrelor) will be excluded from the final analysis. The study will be conducted for an estimated maximum of 36 months (between September 2021 and September 2024). Figure 1a (Protocol Flowchart) includes an overview on patient enrollment.

Rotational Thromboelastometry

In our present paper, rotational thromboelastometry (ROTEM) will be used to yield a comprehensive analysis of the hemostatic functions of study participants. ROTEM is a widely used point-of-care tool providing rapid assessment of specific clotting pathways and platelet functions through viscoelastic assays and aggregometry [6, 28].

Viscoelastic Assays

Viscoelastic assays aid clinicians in choosing the appropriate blood products for patients with acute hemorrhage. Measurements require whole citrated blood to be transferred to a cylindrical cup, in which a pin performs an oscillating rotational movement. Until the blood remains in liquid state, the rotational movement is unrestricted. As soon as the blood starts the clotting process, the pin encounters increasing resistance due to rising clot firmness. Thus, the rotation of the pin is inversely proportional to clot firmness. An optical system detects the impedance of the rotation of the pin, and an integrated computer calculates the ROTEM curve and its numerical parameters. The instrument includes four measurement channels for four simultaneous assays from the following 5 test types: INTEM, EXTEM, APTEM,
FIBTEM, and HEPTEM. Coagulation can be activated intrinsically (INTEM) or extrinsically (EXTEM). The APTEM test uses fibrinolysis inhibitors aprotinin or tranexamic acid, thus comparing EXTEM to APTEM serves to detect hyperfibrinolysis. In the FIBTEM test, the contribution of platelets to clot formation is inhibited by cytochalasin-D; consequently, clotting depends solely on fibrin formation and polymerization. HEPTEM contains heparinase, thus serves to confirm the presence of heparin in the sample. Numerical parameters provided by ROTEM include clotting time (CT), clot formation time (CFT), speed of clot formation (α-angle), amplitude 10 min after CT (A10), maximum clot firmness (MCF), lysis index 30 min after CT (LI30), and maximum lysis (ML) [28–30].

**Aggregometry**

The ROTEM platelet module is an impedance aggregometer providing quantitative and qualitative information on platelet function in anticoagulated whole blood samples [31]. Blood is transferred into a cuvette containing a stirring pin and special electrodes. After determining an impedance baseline, platelet aggregation is initiated with aggregating agents (ADP by ADPTEM, arachidonic acid by ARATEM, and thrombin activating peptide by TRAPTEM). The increase of electrical impedance is proportional to the number of platelets coating the electrodes by aggregation. Ultimately, a special software analysis displays the results on two channels, simultaneously [32]. The numerical values characterizing platelet aggregation are area under curve (AUC), maximum slope (MS), and amplitude at 6 min (A6).

Aggregometry with ROTEM can provide information on drug-related and not-drug-related platelet dysfunction as well. In clinical practice, ADPTEM test is suitable for patients taking cyclooxygenase inhibitors such as acetylsalicylic acid. ARATEM is used for patients treated with ADP receptor antagonists such as clopidogrel, while TRAPTEM is the test of choice for patients taking GP IIb/IIIa receptor antagonists such as abciximab. Non-drug-induced platelet dysfunction may be detected on all tests; nevertheless, in case of TIC, TRAPTEM test is expected to display the derangements of platelet functions the most, prominently.

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**Fig. 1.** Protocol flowchart. a Selection and management of study participants. Patients transported directly to the Emergency Department of the University of Szeged with suspected severe injuries undergo comprehensive diagnostics including ABG, laboratory tests (including Hb, Ht, PLT, aPTT, prothrombin time (PT), and INR), extended focused assessment with sonography in trauma, and computer tomography. Severely injured (injury severity score ≥16), adult trauma patients with confirmed bleeding will be enrolled into our study. Signed informed consent from patients or their surrogates will be required for inclusion. Taking antiplatelet agents entails exclusion from the analysis. The enrolled bleeding patients will undergo ROTEM viscoelastic tests and platelet aggregometry to provide a comprehensive description on coagulative functions. b Mitochondrial measurements. Thrombocytes will be isolated from venous blood samples taken upon patient arrival and subjected to high-resolution fluororespirometry to measure the efficacy of platelet mitochondrial respiration (OxPhos) and coupling of the mitochondrial ETC. Additionally, mitochondrial membrane potential changes and extramitochondrial Ca²⁺ movement will also be measured in the samples. ABG, arterial blood gas analysis; eFAST, extended focused assessment with sonography in trauma; ISS, injury severity score; APA, antiplatelet agent; Hb, hemoglobin; Ht, hematocrit; PLT, platelet count.
Recorded Variables
Demographic data and comorbidities of the participants will be documented ideally upon admission. In case of the identity of the patient is unknown, surrogates must be identified and contacted within 24 h to obtain missing information and informed consent for study participation. Upon patient arrival, conventional laboratory tests including hemoglobin, hematocrit, platelet count, activated partial thromboplastin time (aPTT), prothrombin time (PT), and international normalized ratio (INR) will be performed. Computer tomography and extended focused assessment with sonography in trauma will be used to detect internal bleeding. ROTEM viscoelastic tests and aggregometry, and mitochondrial functional measurements will be performed. MBTs and 24-h mortality will be recorded.

Mitochondrial Functional Measurements
Platelets will be isolated from venous blood samples taken directly upon patient arrival. The efficacy of mitochondrial respiration (oxidative phosphorylation [OxPhos] and coupling of the mitochondrial electron transport chain) will be evaluated by high-resolution florespirometry (Oxygraph-2k, Oroboros Instruments, Innsbruck, Austria), after permeabilization of platelets. Mitochondrial superoxide formation, mitochondrial membrane potential changes, and extramitochondrial Ca\(^{2+}\) movement will also be assessed. Figure 1b (protocol flowchart) demonstrates an overview on mitochondrial functional measurements.

Data Management
Only investigators named in the ethical approval are entitled to handle patient data. In attempt to trace data to an individual subject, a subject identification code list will be used. A personal identification number will be generated to identify the data of the participants and it will be present on all forms and documents. Electronic case report form (eCRF) will be applied. The principal investigator will ensure that the data in the eCRF are accurate, complete, and legible. Any missing or inconsistent recordings in the eCRFs will be requested from the responsible investigator using a data query form and will be corrected for each individual subject before clean file status is declared.

Commitment to Data Sharing
Our data management procedure complies with the FAIR principles (F: findable, A: accessible, I: interoperable, R: reusable). The source data and the individual patient records are stored in a secure hospital information system (MedSolution). The eCRFs in pdf format are sent to the Regional and Institutional Review Board of Human Investigations at the University of Szeged (Contact: office.rkeb@med.u-szeged.hu) with the annual periodic report of the study. These documents are not public, but the Regional and Institutional Review Board of Human Investigations at the University of Szeged reserves the right to decide in each individual case if the original eCRFs can be shared upon request. The study protocol is already entered prospectively into publicly accessible trial regis-
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necessary or inappropriate blood products, thus resulting 
vide only limited information leading to the use of un-
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Sample Size Calculation
Sample size calculation was performed with G*Power version 3.9.1.7 software. The estimation was based on the significance test for the correlation coefficient. We expect the magnitude of the correlation coefficient to be at least 0.3. Thus, 111 subjects are needed to reject the null hypothesis that this correlation coefficient equals zero with the probability (power) of 0.95. The significance level is \( \alpha = 0.05 \).

Discussion

TIC is a commonly occurring, severe condition con-
tributing significantly to trauma-related mortality. De-
spite intensive research focus, the pathophysiology of 
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necessary or inappropriate blood products, thus resulting 
in suboptimal treatment and additional costs. Thrombo-
elastometry (viscoelastic assays and aggregometry) over-
comes several pitfalls of conventional laboratory tests; 
however, it also has its limitations. Experts claim that 
thromboelastometry is not performed on activated endo-
thelium with physiological shear stress; therefore, it can 
hardly reflect in vivo clot formation, accurately [6]. Ulti-
mately, in clinical reality, the transition from hypocoagu-
ability to hypercoagulability is often impossible to detect, 
making it difficult to provide adequate therapy.

Disclosing and characterizing platelet mitochondrial 
dysfunction in TIC is necessary to deepen our under-
standing of the condition. As clot formation is tied to mi-
 tochondrial activity, further investigations may lead to 
new diagnostic tools and therapeutic targets in the man-
agement of TIC.

Trial Status

The study protocol has been registered to ClinicalTri-
als.gov under the identification number NCT05004844, 
on 12 August 2021. The study is currently in recruitment 
phase and can be identified unequivocally by the combi-
nation of ethical approval, protocols registration, and 
version numbers: 5500/2021-SZTE/NCT05004844/v1. 
Patient recruitment has started on 01 September 2021 
and is expected to last till 01 September 2024.

Statement of Ethics

Our protocol complies with the Declaration of Helsinki and has 
been approved by the local medical ethics committee at the Univer-
sity of Szeged (Regional and Institutional Review Board of Human 
Investigations, Chairman: Prof. Dr. Tibor Wittmann) under refer-
ce number 5500/2021-SZTE. Our research studies patients that 
may be incapable of providing informed consent due to their con-
dition; however, in these cases surrogates will be provided a consent 
form and information sheet describing the study in brief, and they 
will have the opportunity to consult one of the investigators. Per-
sonal information, photographs, or other material that might iden-
tify the participants will not be published. Personal data will not be 
given out without the permission of our patients or their surrogates.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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References

Author Contributions

Conceptualization: P.H., P.J.; Methodology: P.H., P.J.; Statistical methods, sample size calculation: F.R.; Writing – Original Draft Preparation: P.J.; Writing Review & Editing: P.H., T.H.; Stylistic and grammatical revision: T.H.; Supervision: P.H., L.T.; Funding Acquisition: P.H.

Data Availability Statement

Not applicable. The study is in recruitment phase. Upon completion of the research, the final data set will be primarily available to the principal investigator (P.H.). The results will be shared with the scientific community through publication.

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