DIRECTIONS OF DEVELOPMENT IN THE INITIAL MANAGEMENT OF TRAUMA-RELATED BLEEDING AND HEMORRHAGIC SHOCK

PhD Thesis

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Szeged

2023

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2023

TABLE OF CONTENTS

1 LIST OF ABBREVIATIONS

TIC – trauma-induced coagulopathy

ATLS – Advanced Trauma Life Support

VS – vital signs

BD – base deficit

HR – heart rate

SBP – systolic blood pressure

pRBC – packed red blood cells

Hb – hemoglobin

Hct – hematocrit

POC – point of care

CT – computer tomography

FAST – focused assessment with sonography in trauma

eFAST – extended focused assessment with sonography in trauma

CH_4 – methane

SMA – superior mesenteric artery

PAS – photoacoustic spectroscopy

PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analyses

OSF – Open Science Framework

TXA – tranexamic acid

SD – standard deviation

IQR – interquartile range

QUIPS – Quality In Prognostic Studies

STROBE – Strengthening the Reporting of Observational Studies in Epidemiology

ISS – injury severity score

P_aCO_2 – partial pressure of carbon dioxide

$ETCO_2$ – end-tidal carbon dioxide

ARDS – acute respiratory distress syndrome

ALI – acute lung injury

AECC – American-European Consensus Conference

ROI – region of interest

OPSI – orthogonal polarization spectral imaging

DBS – De Backer score

PVD – perfused vessel density

MFI – microvascular flow index

HI – heterogeneity index

SI – shock index

MBT – massive blood transfusion

ROTEM – rotational thromboelastometry

ADP – adenosine diphosphate

CFT – clot formation time

A10 – amplitude 10 minutes after clotting time

MCF – maximum clot firmness

LI30 – lysis index 30 minutes after clotting time

ML – maximum lysis

AUC – area under curve

MS – maximum slope

A6 – amplitude at 6 minutes

aPTT – activated partial thromboplastin time

PT – prothrombin time

INR – international normalized ratio

OxPhos – oxidative phosphorylation

2 LIST OF ORIGINAL PAPERS

2.1 List of full papers relating to the subject of the thesis

JÁVOR P, HANÁK L, HEGYI P, CSONKA E, BUTT E, HORVÁTH T, GÓG I, LUKACS A, SOÓS A, RUMBUS Z, PÁKAI E, TOLDI J, HARTMANN P. Predictive value of tachycardia for mortality in trauma-related haemorrhagic shock: a systematic review and meta-regression. BMJ Open. 2022 Oct 19;12(10):e059271. doi: 10.1136/bmjopen-2021-059271. **IF: 3.006**

JÁVOR P, RÁROSI F, HORVÁTH T, TÖRÖK L, VARGA E, HARTMANN P. Detection of exhaled methane levels for monitoring trauma-related haemorrhage following blunt trauma: study protocol for a prospective observational study. BMJ Open. 2022 Jul 6;12(7):e057872. doi: 10.1136/bmjopen-2021-057872. **IF: 3.006**

JÁVOR P, RÁROSI F, HORVÁTH T, TÖRÖK L, HARTMANN P. Mitochondrial

dysfunction in trauma-related coagulopathy - Is there causality? - Study protocol for a prospective observational study. Eur Surg Res. 2021 Dec 24. doi: 10.1159/000521670. **IF: IF: 1.114**

Cumulative IF: 7.126

2.2 List of full papers not relating to the subject of the thesis

JÁVOR P, MÁCSAI A, BUTT E, BARÁTH B, JÁSZ DK, HORVÁTH T, BARÁTH B, CSONKA Á, TÖRÖK L, VARGA E, HARTMANN P. Mitochondrial Dysfunction Affects the Synovium of Patients with Rheumatoid Arthritis and Osteoarthritis Differently. Int J Mol Sci. 2022 Jul 7;23(14):7553. doi: 10.3390/ijms23147553. **IF: 6.208**

BARÁTH B, JÁSZ DK, HORVÁTH T, BARÁTH B, MARÓTI G, STRIFLER G, VARGA G, SÁNDOR L, PERÉNYI D, TALLÓSY S, DONKA T, **JÁVOR P**, BOROS M, HARTMANN P. Mitochondrial Side Effects of Surgical Prophylactic Antibiotics Ceftriaxone and Rifaximin Lead to Bowel Mucosal Damage. Int J Mol Sci. 2022 May 3;23(9):5064. doi: 10.3390/ijms23095064. **IF: 6.208**

TÖRÖK L, **JÁVOR P**, TÖRÖK K, RÁROSI F, HARTMANN P. Early Return to Play After Anterior Cruciate Ligament Reconstruction: Is It Worth the Risk? Ann Rehabil Med. 2022 Apr;46(2):97-107. doi: 10.5535/arm.22010. **IF: 1.57*** (*not approved for scientific metric calculation by the University of Szeged)

TÖRÖK L, **JÁVOR P**, HARTMANN P, BÁNKI L, VARGA E. Should we abandon the patient-specific instrumentation ship in total knee arthroplasty? Not quite yet! BMC Musculoskelet Disord. 2021 Aug 24;22(1):730. doi: 10.1186/s12891-021-04581-2. **IF: 2.562**

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GREKSA F, BUTT E, CSONKA E, **JÁVOR P**, TUBOLY E, TÖRÖK L, SZABO A, VARGA E, HARTMANN P. Periosteal and endosteal microcirculatory injury following excessive osteosynthesis. Injury. 2021 Mar;52 Suppl 1:S3-S6. doi: 10.1016/j.injury.2020.11.053. **IF: 2.687**

JÁVOR P, VARGA E, FEKETE K, TÓTH F, HARTMANN P. Novel coronavirus and trauma surgery: successful infection control from a level I trauma centre. Eur J Trauma Emerg Surg. 2020 Aug;46(4):737-741. doi: 10.1007/s00068-020-01435-9. **IF: 3.693**

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CSONKA E, TUBOLY E, **JÁVOR P**, VARGA E. Need for a National Trauma Registry – Presentation of a mass casualty. Magyar Traumatológia Ortopédia Kézsebészet Plasztikai Sebészet. 2020;63(1-4):59-65. **IF: -**

Cumulative IF: 29.598

3 INTRODUCTION

3.1 Preventable mortality in emergency trauma care

Trauma remains the leading cause of death among people under 45 years of age; thus, optimization of trauma care, especially the reduction of mortality is of utmost importance both from socio-economical and moral aspects [1-3]. As a general principle, 3 categories of trauma-related deaths can be differentiated: non preventable, potentially preventable and definitely preventable [4,5]. In the first case, death is unavoidable as result of anatomic injuries and/or comorbid factors despite adequate care. Contrary to this, mortality is definitely preventable if it is avoidable with using the current standard practice. Situated between these two extremes, potentially preventable mortality means that although anatomic injuries are severe, death may be avoided under optimal conditions and early initiation of adequate care [1]. Since compliance with mandatory clinical protocols is mainly a matter of proper quality control, the present thesis focuses on facilitating the reduction of potentially preventable fatalities.

According to the literature, delay in hemorrhage control in the early phase of treatment is considered as the most common cause of preventable trauma-related mortality [6,7]. Furthermore, even if exsanguination can be prevented, about one-quarter of patients with severe trauma develop a clotting disorder termed trauma-induced coagulopathy (TIC) which is fatal in 30–50% of cases [8].

Considering the above statements, improving our trauma care protocols and diagnostic

methods for the timely recognition of impending hemorrhagic shock; and the more efficient management of TIC may be the most important areas of improvement in today's emergency trauma care.

3.2 Hemorrhagic shock

Hemorrhage is the most common cause of shock in trauma patients [9]. The initial assessment of circulation includes hemodynamic assessment, recognition of potential bleeding, and the determination of its site [9]. Life-threatening blood loss can occur inside or outside the body. In contrast to hemorrhaging outwards, internal bleeding may be difficult to detect promptly. As a rule of thumb, when large amount of blood loss is suspected, principally five bleeding sites must be considered: "The floor", which indicates external bleeding, and "four more", namely the thigh compartments, the pelvis, the abdomen, and the chest [10]. If bleeding into one of these major sites goes undetected, hemorrhagic shock may develop fast, leading to an unfavorable outcome.

Hemorrhagic shock can be defined as inadequate organ perfusion and tissue oxygenation as a result of blood loss [11]; however, due to the influence of different comorbidities and compensatory capability of patients, hemorrhagic shock may be difficult to define with objective criteria that are applicable to every case. The Advanced Trauma Life Support (ATLS), a widely used training program for emergency trauma care providers differentiates and characterizes four distinct severity classes of trauma-related hypovolemic shock according to the extent of blood loss, which is estimated based on vital signs (VS) and base deficit (BD) [9] (Table 1). The severity classes entail recommendations regarding blood transfusion [9]. Despite being widely implemented, the validity and applicability of the ATLS classification of hypovolemic shock in clinical practice sparks controversy [12].

Severity classes ***Estimated blood loss***		***Class I*** ***<15%***	***Class II*** ***15-30%***	***Class III*** ***31-40%***	***Class IV*** ***>40%***
Physiologic variables	HR	↔	↔/↑	↑	↑/↑↑
	SBP	↔	↔	↔/↓	↓
	GCS	↔	↔	↓	↓
	Pulse pressure	↔	↓	↓	↓
	Respiratory rate	↔	↔	↑	↑
	Urine output	↔	↔	↓	↓↓
	BD	0-2 mEq	2-6 mEq	6-10 mEq	≥10 mEq
Transfusion		Monitor	Possible	Yes	Massive transfusion

Table 1. Advanced Trauma Life Support (ATLS) classification of trauma-related hypovolemic

shock The table is based on the 10th edition of ATLS. Estimated blood loss is shown as percentage of total blood volume. HR=heart rate, SBP=systolic blood pressure, GCS=Glasgow Coma Scale, BD=base deficit

3.2.1 The role of heart rate in the recognition of bleeding and prevention of hemorrhagic shock

Regarding the detection of hemorrhagic shock, the challenge lies in identifying its impending presence in the pre-shock state. To date, the initial hemodynamic assessment of the injured relies largely on VS such as heart rate (HR) and systolic blood pressure (SBP), and metabolic markers such as BD and lactate [9,13,14].

Among these variables, HR is one of the most controversial when it comes to blood loss [15-18]. As commonly criticized, HR is not only influenced by hemodynamic changes, but also by several other factors such as anxiety, pain, and medications resulting in a low specificity for hemorrhage [15,19,20]. Furthermore, ATLS suggests the continuously increasing tendency of HR in accordance with the severity of bleeding [9]. However, in clinical reality, the HR response to hemorrhage is rather biphasic or triphasic than linear [19,21,22]. Consequently, the utility of relying on HR in the early management of bleeding trauma patients was called into doubt during the past decades [15,16,19,20].

The reliability of HR was already questioned in the early 2000s by a retrospective analysis on 14325 trauma patients. According to the results of this study, HR displayed insufficient sensitivity and specificity in predicting hypotension after trauma [20]. A few years later, a registry analysis denoted further doubts in HR, as it had performed poorly in predicting the need for an emergent intervention and administration of packed red blood cells (pRBC) in the first 24 hours post-injury [15]. In 2013, 16305 patients from the German trauma register (DGU®) were enrolled in a study and allocated into shock severity classes (I-IV) according to ATLS guidance [23]. Ultimately, no group displayed relevant tachycardia at all. According to these data, expecting tachycardia in case of hypovolemia can be misleading in many instances. Moreover, a false sense of hemodynamic stability based on normal HR can lead to fatal consequences, since the lack of tachycardia in hypoperfusion is associated with poor prognosis [24].

Despite criticism, increased HR has been known as a characteristic of hypovolemic shock for a very long time. The utility of HR as a predictor of mortality is supported by several papers [25,26]. An international, cross-sectional study using data from two large trauma cohorts was conducted to develop and validate a prognostic model to predict death due to bleeding. Although HR showed a significant relation to mortality, the curve was U-shaped

as opposed to the linear model presented by ATLS [26]. A notable limitation of previous studies is that trauma protocols have undergone several changes (such as the use of tranexamic acid (TXA) [27]) or the limitation of crystalloids in fluid resuscitation [28]), which makes recent information incomparable with data from the past.

The issue described above calls for a study providing recent metadata on the prognostic value of HR in emergency trauma care.

3.2.2 Difficulties of the initial management of bleeding trauma patients

Alike HR, the remaining physiologic variables of the ATLS shock classification also react on various impacts apart from hypovolemia (e.g. pain, alcohol consumption, crystalloids, etc.) [29-31]. Furthermore, VS and metabolic markers such as BD and lactate are global markers of shock that are maintained at near-normal levels until the compensatory mechanisms of the individual patient become fully exhausted. Consequently, derangements of these indicators during blood loss may remain subtle in the pre-shock state and become apparent when the changes are already non-reversible. In contrast, hemorrhage induces early compensatory mechanisms and temporospatial differences in regional perfusion hallmarked by a redistribution of blood flow from non-vital organs (e.g. the gut and the skin) towards vital vascular beds (i.e. the coronary and cerebral areas) [32,33].

In addition to the difficulties of timely recognition of occult bleeding, the evaluation of the efficacy of initial treatment also poses a frequently occurring challenge. Increasing urinary output is a reasonably sensitive marker of improving hemodynamic status; nevertheless, underlying kidney injury, hyperglycemia, or diuretic agents can limit its accuracy [9]. Invasive monitoring methods such as pulmonary artery catheterization offer substantial benefits; however, they are hardly applicable during the initial phase of therapy due to patient positioning and time factor [34,35].

In addition to VS, and metabolic markers, hemoglobin (Hb) and hematocrit (Hct) levels are the most frequently used indicators of blood loss due to their several advantages including easy accessibility either with standard laboratory or minimally invasive point of care (POC) testing. However, their diagnostic values in the initial management of trauma patients remains controversial [36]. Initial Hb and Hct levels are influenced by many factors that are not associated with bleeding, such as the patient’s age, gender, weight, and underlying conditions including anemia [37,38]. Furthermore, the on-site Hb values are often lower due to the almost immediate fluid refilling from the interstitium to restore the intravascular

volume, early after sustaining trauma. Then, prehospital fluid resuscitation induces further hemodilution and fall in Hct and Hb. Therefore, serial measurements are recommended for the evaluation of trauma-related hemorrhage [38,39] but the results are still controversial [37,40].

Imaging modalities are important adjuncts to the initial hemodynamic assessment in trauma care. Computer tomography (CT) is a reliable method for detecting internal hemorrhage; however, requires transportation out of the emergency department resulting in unfavorable time delays. As compared to CT, ultrasound has notable advantages including bedside availability, lack of radiation, reproducibility, and low costs [41]. The focused assessment with sonography in trauma (FAST) and extended FAST (eFAST) protocols can be performed in less than 5 minutes and display high sensitivity and specificity for hemoperitoneum, hemopericardium, and hemothorax [42]. Nonetheless, eFAST is hampered by several limitations. Most importantly, the reliability of POC ultrasound depends on the experience of the user and the patient's body composition. Additionally, visualization of retroperitoneal hemorrhage and differentiation between blood and urine are hardly feasible with ultrasound [41].

Ultimately, no gold standard technique exists for diagnosing and assessing hemorrhage in severe trauma, thus decision-making is commonly based on a combination of tests, which all have their strengths and limitations. The lack of an easily accessible, highly applicable test with high sensitivity and specificity calls for further research in the diagnostics of acute blood loss.

3.2.3 Impairment of mesenteric perfusion and exhaled methane (CH_4) concentrations as markers of major bleeding

The diminution of mesenteric perfusion is among the first compensatory reactions to blood loss, thereby being a potential early clinical indicator of hemorrhage [43,44]. The quick reaction of mesenteric perfusion is regulated by finely tuned physiological reflexes and neurohumoral processes. As a primary response to bleeding, declining arterial baroreceptor filling leads to growing efferent sympathetic activation. The elevated sympathetic output is accompanied by reflex tachycardia and fluid retention through aldosterone and vasopressin, aiming to sustain blood pressure. Furthermore, the release of sympathetic mediators increases to trigger the α-adrenergic receptors of the pre- and postcapillary vessels in the microcirculatory system. Selective vasoconstriction of the afferent arterioles aims to

maintain vascular resistance, while the stimulation of α-adrenergic receptors on the efferent venules results in autotransfusion by increasing vascular and cardiac filling [45].

Arteriolar responses depend on the distribution of the vasoconstrictor α-adrenergic and the vasodilator β2-adrenergic receptor subtypes, which differs in distinct tissues. According to this, visceral perfusion becomes suppressed through the vasoconstrictive effect that is mediated by the sympathetic nervous system. Nonetheless, the abdominal organs are affected unequally by redistribution. Intestinal, gastric, and pancreatic blood supplies are more susceptible to the effects of hemorrhage compared to the liver due to the hepatic arterial buffer response [46-48]. Intestines are affected by ischemia particularly adversely and rapidly due to their unique microanatomy, where the artery and vein within the villi run parallel to each other, which results in low oxygenation in the most luminal areas of the intestine, even under optimal conditions [49,50]. The particular sensitivity of mesenteric perfusion to blood loss is demonstrated by studies on large animal models, where the superior mesenteric artery (SMA) flow displays a significant drop already at 5% loss of total blood volume; and continues to diminish in parallel with ongoing hemorrhage [43]. Considering the total circulating blood volume as 5 L for an adult, 5% loss means 250 ml of blood, which can hardly be detected with the currently used routine diagnostic tools. Unfortunately, easily applicable methods for continuous monitoring of the SMA blood flow and downstream intestinal microcirculation have not been elaborated to date.

Nevertheless, animal experiments suggest that exhaled CH_4 levels correspond to the SMA blood flow [51]. CH_4 is an intrinsically non-toxic, combustible gas produced by anaerobic bacterial fermentation [52,53]. According to the literature, CH_4 in the human body originates mainly from methanogenic intestinal microorganisms [54]. Due to its physicochemical attributes, CH_4 can enter freely to the intestinal microcirculation and systemic circulation, and as a gas with low solubility in blood, it becomes rapidly excreted by the lungs [55]. For the measurement of exhaled CH_4, gas chromatography mass spectrometry is considered as the gold standard technique; however, it does not allow continuous monitoring. Nonetheless, real-time monitoring can be conducted with selected ion flow tube-mass spectrometry, proton transfer reaction mass spectrometry, laser spectrometry, or with photoacoustic spectroscopy (PAS) based sensors [56], which offer good applicability to the clinical setting, thereby raising the possibility of a future non-invasive diagnostic and monitoring method in the management of severely injured patients.

3.3 Trauma-induced coagulopathy

Hemorrhage control often poses a great challenge for clinicians due to TIC, a condition that is present in approximately one-quarter of severely injured patients and results in mortality in 30–50% of cases [8,57]. Alterations in coagulation following severe injury were documented already in the 1960's [58]; however, a standard definition for TIC still does not exist [59]. Trauma-induced coagulopathy is characterized by dysfunctional clot formation and breakdown, impaired vascular homeostasis, and is associated with increased risk of multiple organ failure and mortality [8,60]. 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 [61-63]. 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 [64,65]. Diminished platelet functions are suggested to be consequences of injury-induced early hyperactivation [62]; nevertheless, the mediators and pathways of the process are elusive, thus being subjects for further research [57,66].

In the past decade, the presence of altered mitochondrial functions has been confirmed in the background of several diseases [67-69]. Furthermore, mitochondrial dysfunction of various cell types occurs also in trauma-related conditions such as hemorrhagic shock and traumatic brain injury [70-72]. 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 [73].

3.4 Main goals

The main goal of our studies was to contribute to the progress of emergency trauma care by disclosing areas of improvement and lay the foundations for development in the initial management of trauma-related bleeding and hemorrhagic shock. The authors consider the early detection of hemorrhage and impending hemorrhagic shock; and the more efficient management of TIC as the most prominent endeavors to decrease potentially preventable mortality.

As VS play an important role in the recognition of the pre-shock state, and the pattern of HR alterations in the ATLS shock classification is questionable, we aimed to update current knowledge on the role of HR in the initial assessment of bleeding trauma patients as first step. For this purpose, we performed a systematic review and meta-regression investigating the prognostic value of tachycardia for post-injury mortality in trauma patients with

hemorrhage (Study 1).

Thereafter, in search for a solution to the shortcomings of the currently available methods for the initial hemodynamic assessment and monitoring of trauma patients, we presented a promising new technique, the real-time monitoring of exhaled CH_4 levels. As this method has only been tested in animal models bis Dato, we aimed to provide a protocol for a prospective observational clinical study disclosing the associations between exhaled CH_4 levels and the volume of blood loss (Study 2).

Ultimately, we discussed the potential role of mitochondrial dysfunction in TIC. We intended to provide a protocol 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 (Study 3).

4 MATERIALS AND METHODS

4.1 Study 1. The predictive value of tachycardia for mortality in trauma-related hemorrhagic shock: A Systematic Review and Meta-regression

We performed a systematic review and meta-regression in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations [74]. The review protocol was registered in the Open Science Framework (OSF) system under registration DOI: 10.17605/OSF.IO/HJWYR.

4.1.1 Literature search

A systematic search of EMBASE, MEDLINE (via PubMed), Cochrane Controlled Register of Trials (CENTRAL) and Web of Science databases was performed on 1 September 2020 with the following search terms: "trauma" AND ("heart rate" OR "pulse rate" OR "tachycardia" OR "bradycardia" OR "vital sign" OR "vital signs" OR "vital parameter" OR "vital parameters") AND "mortality" AND ("bleeding" OR "haemorrhage" OR "hemorrhage" OR "haemodynamic" OR "hemodynamic"). Articles published before 2010 were excluded from our study.

4.1.2 Eligibility criteria

Records on bleeding trauma patients were considered for eligibility only if they provided initial HR values (prehospital or upon admission) in addition to mortality data covering a

time interval not exceeding 30 days from the time of injury. Only full-text articles were considered. Non-English language reports, reviews, conference abstracts and case reports with low patient number (<10) were excluded. Taking the development of trauma care in the past decade into consideration (e.g.: introduction of TXA) [27], and paradigm shift in fluid resuscitation [28]) all studies that included data on patients treated before 2010 were also excluded.

To consider a patient cohort hemorrhagic, the inclusion criteria of the individual studies had to include transfusion of blood products and/or positive FAST examination and/or hemodynamical instability after trauma and/or abdominal gunshot injury. Records on special populations such as pregnant, pediatric (<18 years of age) or geriatric (≥55 years) were not considered. Studies on patients suffering burns, traumatic spinal or- brain injuries were excluded.

With excluding special populations and pediatric and older age groups we aimed to reduce the influence of confounding factors. Since studies of geriatric trauma patients have used age cutoffs ranging from 55 to 80 years and there is no clear consensus in the literature [75,76] we decided to exclude study populations of 55 years of age or older to diminish the effects of age-related confounding factors.

4.1.3 Study selection

After having duplicates removed with the help of a reference manager software (EndNote X7), articles published before 2010 were also discarded. On the remaining studies, title and abstract screenings were performed by two review authors (P.J., I.G.). Thereafter, the full texts of the potentially eligible records were obtained and assessed based on the criteria described above. Disagreements were resolved by consensus.

4.1.4 Data extraction

The following information was extracted from the eligible studies: title, first author's name, year of publication, study design, data origin (country, hospital database/registry), data collection period, inclusion criteria, subgroups, patient number of the subgroups, total patient number, HR (mean ± standard deviation (SD) or median [interquartile range] (IQR)), phase of recording HR values (prehospital/admission), mortality within 30 days (n, %). In case of studies using overlapping data, the less comprehensive report with the smaller sample size was excluded.

4.1.5 Risk of bias assessment

Quality In Prognostic Studies (QUIPS) tool was used separately by two authors (T.H. and Z.R.) to assess the risk of bias for each study [77]. Disagreements were resolved by consensus. QUIPS consists of six main domains: 'Study attrition', 'Study participation', 'Prognostic factor', 'Outcome measurement', 'Study confounding' and 'Statistical analysis and reporting'. A rating for each domain was assigned as carrying 'low', 'moderate' or 'high' risk of bias. Based on the ratings of the individual domains, the overall risk of bias was evaluated by each study.

4.1.6 Statistical analysis

The association between HR and mortality of trauma patients was assessed using meta-regression analysis. A result of $p<0.05$ was considered as significant. As a subgroup analysis, meta-regression was performed on trauma patients who received blood products. Statistical analyses were performed with Stata 16 (Stata Corp, College Station, TX, USA). To convert median values to means, we used the method of Xiang Wan [78].

4.2 Study 2. Detection of exhaled methane levels for monitoring trauma-related hemorrhage following blunt trauma – Protocol for a prospective observational study

We elaborated a protocol for a single-center, prospective observational study investigating the association of exhaled CH_4 concentrations with the volume of blood loss in severely injured patients. The research is currently in progress at the University of Szeged, Szeged, Hungary. Our protocol was registered to ClinicalTrials.gov on 27 July 2021 under the identification number NCT04987411, complies with the Declaration of Helsinki and follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist.

4.2.1 Patient enrollment and inclusion criteria

This prospective study involves severely injured (Injury Severity Score (ISS) ≥16) patients with hemorrhage related to blunt force trauma, aged ≥18 years, intubated on scene or upon arrival, transported directly to the Emergency Department of the University of Szeged. Bleeding is confirmed with CT. Patients with penetrating trauma or isolated traumatic brain injury are excluded from the analysis. As the present protocol aims to investigate associations between exhaled CH_4 and hemorrhage, respiratory causes of CH_4-decrease

must be recognized. For this purpose, the gradient of partial pressure of carbon dioxide (P_aCO_2) and end-tidal carbon dioxide ($ETCO_2$) is evaluated since it differs in patients with hypovolemia from patients with respiratory distress due to obstructive causes or lung injuries [79-81]. Obtaining the $PaCO_2$-$ETCO_2$ gradient does not require additional measurements since blood gas analysis and volumetric capnometry are performed routinely in ventilated patients with severe injuries. Furthermore, lung injuries are assessed with CT. The presence of acute respiratory distress syndrome (ARDS) or acute lung injury (ALI) is assessed based on the American-European Consensus Conference (AECC) Definition of ALI and ARDS [82] and the Murray Lung Injury Score [82]; and it entails exclusion from the analysis.

The study is being conducted for an estimated maximum of 36 months (between 15 August 2021 and 15 August 2024). Figure 1 (Protocol Flowchart) includes an overview on patient enrollment (A).

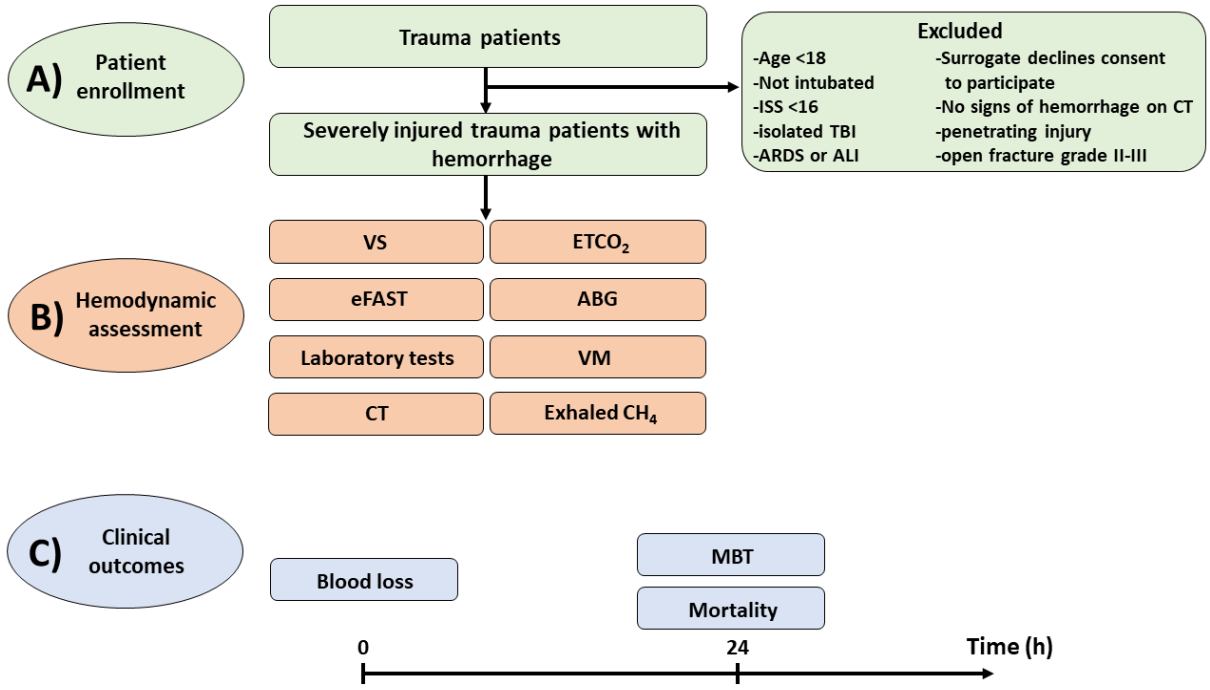

Figure 1. Protocol Flowchart. Aspects of patient enrollment and reasons for exclusion are demonstrated. Severely injured (ISS ≥16), blunt trauma patients with bleeding are enrolled into our study. CT is used to detect the presence and evaluate the severity of bleeding, and for aiding the assessment of injury severity. Signed informed consent from patients or their surrogates is required for patient enrollment. Inclusion criteria includes intubation as the exhalation outlet of the ventilator allows the attachment of the CH_4 detector apparatus, thus the continuous monitoring of CH_4 levels in breath. Patients with penetrating trauma, bleeding outwards, grade II-III fractures, isolated TBI, ARDS or ALI are excluded from the analysis. **B:** Study participants undergo a comprehensive hemodynamic assessment upon arrival, which consists of evaluation of VS (HR, SBP), $ETCO_2$, ABG (BD, lactate), laboratory tests (Hb, Hct), videomicroscopy of the sublingual mucosa using orthogonal polarization spectral imaging, eFAST, and polytrauma CT. With the help of these

parameters, a detailed dataset describing the hemodynamic state of the participants is provided. Exhaled CH_4 concentrations are monitored with a near-infrared laser technique-based PAS apparatus. **C:** Our clinical outcomes include the volume of blood that patients have already lost at the time of their arrival, the need for a MBT, and 24-hour mortality. To calculate to volume of blood loss, a CT-linked radiologic software is used. Associations between exhaled CH_4 concentrations and clinical outcomes are assessed. ISS=injury severity score, TBI=traumatic brain injury, CT=computer tomography, ARDS=acute respiratory distress syndrome, ALI=acute lung injury, VS=vital signs, HR=heart rate, SBP=systolic blood pressure, ABG=arterial blood gas, BD=base deficit, eFAST=extended focused assessment with sonography for trauma, $ETCO_2$=end-tidal carbon dioxide, VM=videomicroscopy, CH_4=methane, Hb=hemoglobin, Hct=hematocrit, PAS=photoacoustic spectroscopy

4.2.2 Measurement of exhaled CH_4 levels

In our study, a near-infrared laser technique-based PAS apparatus is attached to the exhalation outlet of the ventilator upon arrival of patients, thereby allowing the continuous monitoring of exhaled CH_4 concentrations. Photoacoustic spectroscopy is a subclass of optical absorption spectroscopy measuring optical absorption indirectly through the conversion of absorbed light energy into acoustic waves due to the thermal expansion of absorbing gas samples. The amplitude of the generated sound is directly proportional to the concentration of the absorbing gas component. The gas sample passes through the photoacoustic cell generating a photoacoustic signal, which is detected by a microphone [43].

4.2.3 Estimation of blood loss volume

CT scanning is performed on a 64-slice GE Revolution Evo scanner (GE Healthcare, Chicago, IL, USA). The polytrauma CT protocol complies with the guidelines of the European Society of Emergency Radiology [83]. Patients are positioned on the examination table with feet first, arms placed above the head if possible, unenhanced cranial CT, (un)enhanced cervical spine CT, unenhanced, arterial and venous phase imaging of the trunk (chest, upper and lower abdomen and pelvis). The protocol is tailored to the patient's need, special protocols such as urography and angiography may be employed.

The volume of the bleeding is evaluated on the unenhanced CT scans. Clinical qualitative image analysis is carried out on an eRad PACS system (version 8.1, Greenville SC, USA), on Eizo Radiforce RX850 displays (Hakusan, Ishikawa, Japan). The quantitative analysis of the volume of the bleeding is determined manually, a region of interest (ROI) is drawn on the hyperdense blood slice by slice. The volume of the bleeding is determined by multiplying the number of the voxels by the volume of a single voxel. The manual bleeding segmentation is carried out by FSL's (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki) FSLeyes

software (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLeyes).

4.2.4 Videomicroscopy of the sublingual mucosa

The direct visualization of peripheral microcirculatory networks with videomicroscopy is a suitable method for providing information on compensatory circulatory redistribution in shock, and on the therapeutic response of patients on hemodynamic resuscitation [84-87]. Videomicroscopy utilizes handheld microscopes that can detect red blood cells flowing in capillaries when placed on mucosal surfaces [84,88,89]. Multiple generations of videomicroscopic techniques including orthogonal polarization spectral imaging (OPSI), sidestream dark field imaging and incident dark field imaging are available for clinicians and researchers [84]. As videomicroscopy requires easily accessible mucosal surface, the investigation of the sublingual region is a reasonable approach if hemodynamic coherence between the microcirculatory systems of the gut and the sublingual mucosa is presumed. Although there is evidence for a relation between the two regional microcirculatory systems [86,90] reactions of the sublingual microperfusion to hemodynamic changes are considered to be significantly slower than the response of more distal gastrointestinal regions [43].

In our study, OPSI technique (Cytoscan A/R, Cytometrics) is used to visualize the microcirculation of the sublingual mucosa of the participants. The sublingual capillary network and capillary blood flow of each patient is recorded and saved to hard drive as 20 s-long video clips. The video clips are evaluated independently by two investigators and the De Backer score (DBS), perfused vessel density (PVD), microvascular flow index (MFI) and heterogeneity index (HI) of the participants are determined. De Backer score refers to capillary density and can be calculated by utilizing the principle that vessel density is proportional to the number of vessels crossing arbitrary lines [91]. Only vessels with a diameter of 20µm or less are considered as capillaries. The blood flow of the individual capillaries are characterized as continuous (continuous flow for 20s), intermittent (no flow for at least 10s), sluggish (slow blood flow), or absent (no perfusion); and PVD is calculated by multiplying vessel density by the proportion of continuously perfused vessels [92]. The MFI refers to perfusion quality and can be determined by dividing the recorded view into four quadrants, assigning a number for each quadrant based on the predominant type of blood flow (0=absent, 1=intermittent, 2=sluggish, 3=continuous) and calculating the average value from the numbers [93]. The HI describes perfusion heterogeneity by dividing the difference between the highest MFI and the lowest MFI by the mean MFI [92]. Through

providing DBS, PVD, MFI and HI, the sublingual microcirculation of each patient is described quantitatively. Disagreements between the two independent investigators are resolved by consensus.

4.2.5 Recorded variables

Demographic data and comorbidities of the participants are documented ideally upon admission. In case of an unidentified patient, surrogates must be disclosed and contacted within 24 hours to obtain demographic data and informed consent.

Variables reflecting the hemodynamic condition of patients are recorded upon arrival, as demonstrated in Figure 1 (B) and Table 2 (Documentation plan). Heart rate, SBP, shock index (SI), BD, lactate, Hb, Hct, $ETCO_2$, results of eFAST and indices of sublingual microcirculation (DBS, PVD, MFI, HI) serve to provide a detailed view on the circulatory status of the patients.

Controlling VS including HR and SBP is essential in the severely injured. Dividing HR with SBP displays the SI, a ratio which is commonly used in addition to traditional VS in emergency medicine. Although the SI is often in the normal range (0.5-0.7) in the compensatory phase of shock, SI >1.0 has been found to predict increased mortality risk, need for massive blood transfusion (MBT), and admission to intensive care unit [94]. Additionally, a register analysis with a large patient number found the performances of SI- and BD-based hypovolemic shock classification equal in predicting transfusion requirement [95].

Blood gas analysis is a promptly available method for acquiring BD and lactate values within minutes. Both metabolic markers are useful indicators in cases where bleeding is suspected. The current ATLS guidance on hemorrhagic shock emphasizes the importance of BD by associating explicit BD values with explicit percentages of blood loss, whilst the alterations of VS are only described subjectively, without quantification [9]. Furthermore, several studies support the superiority of BD over VS in indicating hemorrhage [23, 96]. In contrast to BD, which is a calculated metabolic marker, lactate is a direct byproduct of anaerobic metabolism during shock [97]. Although ATLS does not refer to lactate as an indicator of severity in the classification of hypovolemic shock, numerous studies reported its ability to predict mortality, massive transfusion, and the need for damage control laparotomy [98-101]. Modern blood gas analyzers often have incorporated technology allowing the measurement of Hb, nevertheless, it is also accessible through standard

laboratory testing. Low Hb or Hct are widely and interchangeably used as indicators of severe bleeding. Although their value in the early phase of hemorrhage remains controversial, most trauma patients with severe bleeding display a significant drop in Hb and Hct values within the first 30 minutes of patient arrival [36,102].

Monitoring $EtCO_2$ is indispensable in intubated trauma patients. Although capnography was used initially only for the confirmation of proper tracheal tube placement, due to its association with cardiac output it has proven to be useful in many clinical scenarios including severe trauma [103]. In addition to the role in ascertaining the effectiveness of chest compressions during cardiopulmonary resuscitation, $EtCO_2$ has been reported to reflect mortality, transfusion need, and fluid responsiveness after injury [103-105]. Similarly to CH_4, $EtCO_2$ is an easily measurable exhaled gas that provides information on the circulatory status of patients. However, according to our theory, monitoring CH_4 levels allows clinicians to detect hemorrhage in a much earlier phase, when cardiac output is still in the normal range due to compensatory mechanisms. The reduction of splanchnic perfusion is one of the earliest responses to blood loss; thus, the consecutive fall in exhaled CH_4 concentration may already indicate bleeding when $ETCO_2$ stays in the reference range.

After the primary assessment and stabilization, CT is the modality of choice as it allows the identification of the source and estimation of blood loss volume, and it can also detect small amounts of blood.

The need for MBT and 24-hour mortality is recorded. The present protocol defines MBT according to ATLS, as more than 10 units of transfused pRBCs within the first 24 hours of admission or more than 4 units in 1 hour [9]. Some studies accept other criteria such as the replacement of one entire blood volume within 24 hours, or the replacement of 50% of total blood volume within 3 hours as well [106]; however, we utilize criteria listed by ATLS due to practical considerations. It is important to mention that our institution utilizes a rotational thromboelastometry (ROTEM)-based strategy for the transfusion of blood products, which may reduce the number of pRBCs used.

In addition to the above-discussed parameters, the use of vasopressors including the type of drug, dose and time of administration is recorded since it may influence microcirculatory indices and splanchnic perfusion [107-109].

Data is stored in electronic database and supervised by the principal investigator (P.H.). The detailed documentation plan is shown in Table 2.

	Patient arrival	24 hours after arrival
Informed consent from surrogates	**X**	
Recording demographic data (age, sex) and comorbidities	**X**	
Recording VS (HR, SBP) and calculating SI	**X**	**X**
Recording $ETCO_2$	**X**	
eFAST	**X**	
CT (confirming, localizing and quantifying hemorrhage)	**X**	
Listing and assessing all injuries	**X**	
Determining ISS	**X**	
Assessment for eligibility	**X**	
Arterial blood gas (including BD and lactate)	**X**	**X**
Laboratory testing of venous blood (including Hb, Hct)	**X**	**X**
Assessment of sublingual microcirculation with VM (calculating DBS, PVD, MFI, HI)	**X**	**X**
Recording exhaled CH_4 concentration	**X**	**X**
Recording vasopressors (type, dose and time of administration)	**X**	**X**
Recording MBT		**X**
Recording 24-hour mortality		**X**

Table 2. Documentation Plan (Study 2). Key measures of the protocol and their timing are shown. Informed consent is obtained from patient surrogates upon admission. Demographic data, comorbidities are recorded. A comprehensive hemodynamic assessment is carried out upon arrival, including the evaluation of VS (HR, SBP), $ETCO_2$, arterial blood gas analysis (BD, lactate), laboratory tests (Hb, Hct), videomicroscopy of the sublingual mucosa using orthogonal polarization spectral imaging, and eFAST. Computer tomography is used to detect and assess bleeding and to aid the recognition of all injuries for ISS scoring. Vital signs, blood gas parameters, laboratory markers, and indices of sublingual microcirculation (DBS, PVD, MFI, HI) are documented at 24 hours post-admission. Exhaled CH_4 concentrations are monitored and recorded upon arrival and at 24 hours. The documentation includes MBT and mortality. VS=vital signs, VM=videomicroscopy, HR=heart rate, SBP=systolic blood pressure, SI=Shock Index, eFAST=extended focused assessment with sonography for trauma, CT=computer tomography, ISS=injury severity score, $ETCO_2$=end-tidal carbon dioxide, Hb=hemoglobin, Hct=hematocrit, BD=base deficit, MBT=massive blood transfusion, CH_4=methane, DBS=De Backer score, PVD=perfused vessel density, MFI=microvascular flow index, HI=heterogeneity index

4.2.6 Study outcomes

The primary outcome in our study is the volume of blood loss. The association between the volume of blood loss and the concentration of CH_4 in exhaled breath upon admission stands in the focus of our research. Additionally, exhaled CH_4 is compared with SI, BD, lactate, Hb, $EtCO_2$, and microcirculatory indices (DBS, PVD, MFI, HI), with respect to their ability to reflect the extent of blood loss upon patient arrival. If exhaled CH_4 displays higher predictive performance than the above-mentioned shock markers, it would strongly suggest the utility of CH_4 measurements in clinical practice considering its prompt availability, non-

invasive nature, and suitability for continuous monitoring. The need for MBT and 24-hour mortality constitute secondary outcomes.

4.2.7 Statistical methods

The alternative hypothesis for the primary outcome presumes an association (Pearson correlation at least 0.3 or larger) between exhaled CH_4 levels and the volume of blood loss. 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 α=0.05. Statistical analyses will be performed using SPSS 25.0 (IBM Corporation, Chicago, IL, USA). P-values $P < 0.05$ will be regarded as statistically significant. Normality test will be carried out with the Shapiro-Wilk test. Continuous variables will be expressed as mean ± SD, 95% confidence intervals for normally distributed variables and median and interquartile range for non-normally distributed variables respectively. Significance test for the correlation coefficient will be applied for primary and secondary analyses. Possible non-linear relationship will be analyzed using linear regression and a non-linear (polynomial regression). Regression models will be compared with F-test. To investigate the association between exhaled CH_4 concentrations and the need for MBT and 24-hour mortality, respectively, ROC-analysis will be applied.

4.3 Study 3. Protocol for a prospective observational study investigating mitochondrial dysfunction in trauma-related coagulopathy

We elaborated a protocol for a single-center, prospective observational study investigating mitochondrial dysfunction in trauma-related coagulopathy. The research is currently in progress at the University of Szeged, Szeged, Hungary. The protocol was registered to ClinicalTrials.gov on 12 August 2021 under the reference number NCT05004844, complies with the Declaration of Helsinki and follows the STROBE checklist.

4.3.1 Patient enrollment and inclusion criteria

This prospective observational study involves severely injured (ISS ≥16) patients with bleeding confirmed with CT, 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) are excluded from the final analysis. The study is conducted for an estimated maximum of 36 months (between September 2021 and September 2024). Figure 2 (Protocol Flowchart) includes an overview on patient enrollment (a).

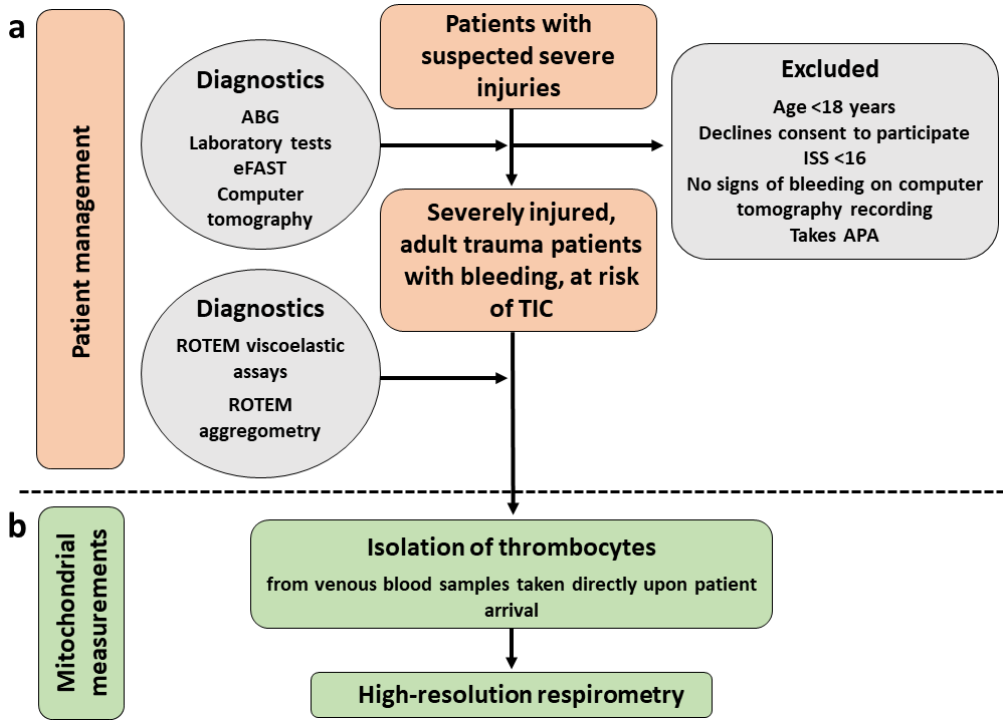

Figure 2. Protocol Flowchart. a: Selection and management of study participants. Patients transported directly to the Emergency Department of the University of Szeged with suspected severe injuries undergo comprehensive diagnostics including ABG, laboratory tests (including Hb, Hct, platelet count, activated partial thromboplastin time (aPTT), prothrombin time (PT), and international normalized ratio (INR)), eFAST, and CT. Severely injured (ISS ≥16), adult trauma patients with confirmed bleeding are enrolled into our study. Signed informed consent from patients or their surrogates is required for inclusion. Taking antiplatelet agents entails exclusion from the analysis. The participants undergo ROTEM viscoelastic tests and platelet aggregometry to provide a comprehensive description on their coagulation status. **b:** Mitochondrial measurements. We isolate thrombocytes from venous blood samples taken upon patient arrival and subjected to high-resolution fluororespirometry to measure the efficacy of platelet mitochondrial respiration (oxidative phosphorylation (OxPhos)) and coupling of the mitochondrial electron transport chain. Additionally, mitochondrial membrane potential changes and extramitochondrial Ca^{2+} movement are also measured in the samples. ABG – arterial blood gas analysis, TIC – trauma induced coagulopathy, eFAST – focused assessment with sonography in trauma, ROTEM – rotational thromboelastometry, ISS – injury severity score, APA – antiplatelet agent

4.3.2 Rotational thromboelastometry

In our research, ROTEM is used to yield a comprehensive analysis of the hemostatic functions of study participants. ROTEM is a widely used POC tool providing rapid

assessment of specific clotting pathways and platelet functions through viscoelastic assays and aggregometry [59,110].

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 TXA, 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, clot formation time (CFT), speed of clot formation (α-angle), amplitude 10 minutes after clotting time (A10), maximum clot firmness (MCF), lysis index 30 minutes after clotting time (LI30) and maximum lysis (ML) [110,111].

The ROTEM platelet module is an impedance aggregometer providing quantitative and qualitative information on platelet function in anticoagulated whole blood samples [112]. 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 analyses and displays the results on two channels simultaneously [113]. The numerical values characterizing platelet aggregation are area under curve (AUC), maximum slope (MS), and amplitude at 6 minutes (A6). Aggregometry with ROTEM can provide information on drug-related and not drug-related platelet dysfunction as well. In clinical practice, ARATEM test is suitable for patients taking cyclooxygenase inhibitors such as acetylsalicylic acid. ADPTEM 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.

4.3.3 Recorded variables

Demographic data and comorbidities of the participants are documented ideally upon admission. In case of the identity of the patient is unknown, surrogates must be identified and contacted within 24 hours to obtain missing information and informed consent for study participation. Upon patient arrival, conventional laboratory tests including Hb, Ht, platelet count, activated partial thromboplastin time (aPTT), prothrombin time (PT), and international normalized ratio (INR) are performed. We use eFAST and CT to detect internal bleeding. ROTEM **v**iscoelastic assays and aggregometry are carried out to assess coagulopathy. Clotting time, CFT, α-angle, A10, MCF, LI30 and ML in INTEM, EXTEM, APTEM and FIBTEM tests; and AUC, MS, and A6 in TRAPTEM test are documented. Massive blood transfusions and 24-hour mortality are recorded. We defined MBT according to ATLS, as more than 10 units of transfused pRBCs within the first 24 hours of admission or more than 4 units in 1 hour. The detailed documentation plan is demonstrated in Table 3.

	Patient arrival	24 hours after arrival
Informed consent from surrogates	**X**	
Recording demographic data (age, sex) and comorbidities	**X**	
eFAST	**X**	
Computer tomography	**X**	
Listing and assessing all injuries	**X**	
Determining ISS	**X**	
Assessment for eligibility	**X**	
ABG (including BD and lactate)	**X**	**X**
Laboratory testing (including Hb, Hct, PLT, aPTT, PT, INR)	**X**	**X**
ROTEM viscoelastic tests and aggregometry	**X**	
Isolation of thrombocytes from venous blood samples)	**X**	
High-resolution fluoroespirometry of platelet suspensions	**X**	
Recording MBT		**X**
Recording 24-hour mortality		**X**

Table 3. Documentation plan (Study 3). Key measures of the protocol and their timing are shown.

Informed consent is obtained from patients or their surrogates upon admission. Imaging modalities are used to assess bleeding and to aid the recognition of all injuries and determining ISS. Conventional laboratory tests, ROTEM viscoelastic tests and aggregometry, and mitochondrial functional measurements are performed. Massive blood transfusions and 24-hour mortality are registered. eFAST – focused assessment with sonography in trauma, ISS – injury severity score, ABG – arterial blood gas analysis, BD – base deficit, Hb – hemoglobin, Hct – hematocrit, PLT – platelet count, aPTT – activated partial, thromboplastin time, PT – prothrombin time, INR – international normalized ratio, ROTEM – rotational thromboelastometry, MBT=massive blood transfusion

4.3.4 Mitochondrial functional measurements

We isolate platelets 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 are evaluated by high-resolution fluoroespirometry (Oxygraph-2k, Oroboros Instruments, Innsbruck, Austria) after permeabilization of platelets. We also assess mitochondrial superoxide formation, mitochondrial membrane potential changes and extramitochondrial Ca^{2+} movement. Figure 1 (Protocol Flowchart) demonstrates an overview on mitochondrial functional measurements (b).

4.3.5 Outcomes

Numerical parameters of ROTEM aggregometry (AUC, MS and A6 in TRAPTEM) constitute our primary outcome. Results of viscoelastic assays (clotting time, CFT, α-angle, A10, MCF, LI30 and ML in INTEM, EXTEM, APTEM, FIBTEM) and conventional markers of hemostasis (aPTT, PT, INR) serve as secondary outcomes. The need for MBT and 24-hour mortality constitute our tertiary outcomes.

4.3.6 Statistical methods

The alternative hypothesis for the primary outcome presumes an association (Pearson correlation at least 0.3 or larger) between OxPhos capacity of platelet mitochondria and thrombocyte aggregation (indicated by AUC, MS and A6 in TRAPTEM test of ROTEM aggregometry). 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 $\alpha=0.05$. Statistical analyses will be performed using SPSS 25.0 (IBM Corporation, Chicago, IL, USA). P-values $P < 0.05$ will

be regarded as statistically significant. Continuous variables will be expressed as mean ± SD, 95% confidence intervals. Significance test for the correlation coefficient will be applied for primary and secondary analyses. To investigate the association between OxPhos capacity of platelet mitochondria and the need for MBT and 24-hour mortality, respectively, ROC-analysis will be applied. No subgroup analyses are planned.

5 RESULTS

5.1 Results of Study 1

5.1.1 Results of systematic search and selection

Two thousand and seventeen records were identified through our search strategy on 1 September 2020. One thousand three hundred seventy-three articles were screened on title. Five hundred fifty-seven abstracts were assessed, and 132 publications were enrolled into the final, comprehensive full text analysis. Ultimately, 19 records met our eligibility criteria. The flowchart of study enrollment is shown in Figure 3.

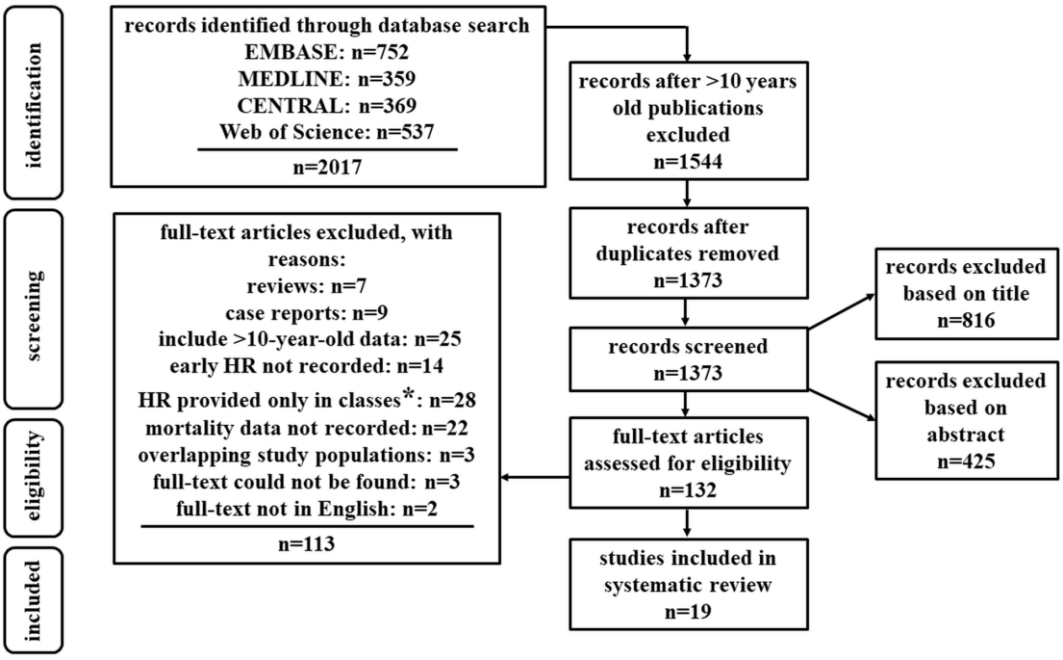

Figure 3. PRISMA flow diagram. Our search strategy resulted 2017 papers. After excluding articles published before 2010 and duplicates, 1373 papers were screened based on title and abstract. In 79 cases the title clearly indicated non-eligible study design such as review or systematic review. Twenty-four title pointed out that the paper is a case report of a sole case. In 124 cases, the title clearly indicated non-eligible study population such as pregnant or pediatric. Five hundred sixteen titles revealed that the study is not closely related to our research topic. In 73 cases the title clearly indicated an animal experiment. Twenty-one records were excluded based on abstract due to a non-eligible study design such as review or systematic review. The abstract indicated a non-eligible

study population such as pregnant or pediatric in 94 cases. In 110 cases, the abstract indicated that the study is not closely related to our research topic. Thirty-nine animal experiments were filtered out based on abstract. Eight studies did not have an English language abstract. In 112 cases, the abstract revealed that the study includes data that is more than 10 years old. Forty-one case reports with a patient number <10 were excluded based on abstract. After excluding a total of 816 papers based on title and 425 based on abstract, 132 full texts were assessed for eligibility. Reasons for non-inclusion of full-text articles are detailed above in the Figure. Ultimately, 19 studies were enrolled to our meta-regression. *heart rate (HR) was not provided in mean or median, only the number of patients in ranges of HR (e.g. 100-120 bpm) was given

5.1.2 Study characteristics

All publications processed data of trauma patients with suspected hemorrhage from the past 10 years. From 19 studies yielding 3057 patients in total, 13 records collected data retrospectively and 6 prospectively. The number of participants in each dataset ranged from 15 to 428. Ten studies enrolled patients only if they received blood products as a part of the initial management. Seven publications used hemodynamic instability identified mainly by vital parameters as inclusion criteria. One study analyzed patients with a positive result on FAST examination after blunt abdominal trauma. One research enrolled patients with abdominal gunshot injuries. Each of the inclusion criteria listed above entails a strong suspicion for significant bleeding. The main characteristics of the 19 eligible studies are summarized in Table 4. The more comprehensive description of the papers is available in the supplementary material (Supplementary Table 1).

First author, year	Country	Data collection	Patient characteristics	Patient No	HR mean ± SD (PH/AD)	Mortality n, (%)
Bohonek 2019 [114]	*Czech Republic*	*retrospective*	*received blood products*	*46*	*94.8 ± 59.0 (AD)*	*10 (21.7)*
Boudreau 2019 [115]	*USA*	*retrospective*	*received blood products*	*116*	*101.3 ± 43.0 (PH)*	*27 (23.3)*
Duchesne 2019 [116]	USA	retrospective	hemodynamic instability	279	120.6 ± 27.7 (AD)	89 (32.0)
Montazer 2019 [117]	Iran	prospective	hemodynamic instability	400	110.0 ± 14.0 (AD)	67 (16.7)
Priestley 2019 [118]	*USA*	*retrospective*	*received blood products*	*283*	*104.0 ± 24.0 (PH)*	*88 (31.1)*
Barmparas 2018 [119]	*USA*	*retrospective*	*received blood products*	*120*	*101.1 ± 39.7 (AD)*	*59 (49.2)*
Chaochankit 2018 [120]	*Thailand*	*retrospective*	*received blood products*	*15*	*113.0 ± 22.1 (AD)*	*12 (80.0)*
Moore 2018 [121]	USA	prospective	hemodynamic instability	125	110.0 ± 15.9 (PH)	16 (12.8)
Ng 2018 [122]	Canada	retrospective	hemodynamic instability	117	112.0 ± 35.0 (AD)	22 (19.0)
Guo 2017 [123]	China	prospective	hemodynamic instability	428	111.3 ± 17.9 (AD)	104 (23.4)
Heidari 2017 [124]	Iran	prospective	blunt abdominal trauma with positive FAST	168	105.3 ± 23.4 (AD)	57 (33.9)
Luehr 2017	*USA*	*retrospective*	*received blood products*	*115*	*133.3 ± 21.4 (PH)*	*20 (17.4)*

[125]						
Naumann 2017 [126]	*UK*	*retrospective*	*received blood products*	*17*	*108.0 ± 16.2 (AD)*	*3 (17.6)*
Savage 2017 [127]	*USA*	*retrospective*	*received blood products*	*330*	*108.2 ± 55.3 (AD)*	*82 (24.8)*
Day 2016 [128]	*USA*	*retrospective*	*received blood products*	*116*	*98.0 ± 24.0 (PH)*	*13 (11.0)*
Ordoñez 2016 [129]	Colombia	retrospective	hemodynamic instability	171	112.6 ± 23.5 (AD)	26 (15.2)
Shah 2015 [130]	Pakistan	retrospective	isolated abdominal gunshot wound	70	99.8 ± 30.3 (AD)	11 (15.7)
Thurston 2015 [131]	South Africa	prospective	hemodynamic instability	50	123.3 ± 13.1 (AD)	11 (22.0)
Sisak 2013 [132]	*Australia*	*prospective*	*received blood products*	*91*	*100.0 ± 30.1 (AD)*	*13 (14.0)*

Table 4. Baseline characteristics of the included studies. The majority of the papers enrolled trauma patients who received blood products (italics) and/or showed signs of hemodynamic instability. Hemodynamic instability was defined by vital parameters in most cases. Most of the data was collected retrospectively. The number of participants in each dataset ranged from 15 to 428. There was a significant heterogeneity in mortality between datasets. The need for massive transfusion was accompanied by a prominently high mortality rate. A mean HR > 120 bpm did not entail an outstanding mortality rate. *only cohort B consisted of trauma patients with active bleeding HR=heart rate, SD=standard deviation, PH=prehospital, AD=upon admission, FAST=focused assessment with sonography for trauma

5.1.3 Study quality

The methodological quality of the enrolled papers was investigated with QUIPS tool. The domain ‘Study attrition’ was not suitable for the retrospective studies. In 5 prospective studies, a moderate risk for study attrition bias was identified. All papers were judged to carry a low risk of bias in ‘Study participation’ and ‘Prognostic factor measurement’ domains. In contrast, almost half of the records were accompanied by a moderate risk of bias with regards to ‘Study confounding’, since the role of important confounders was not clarified in these reports. The results of the QUIPS assessment are shown in Figure 4.

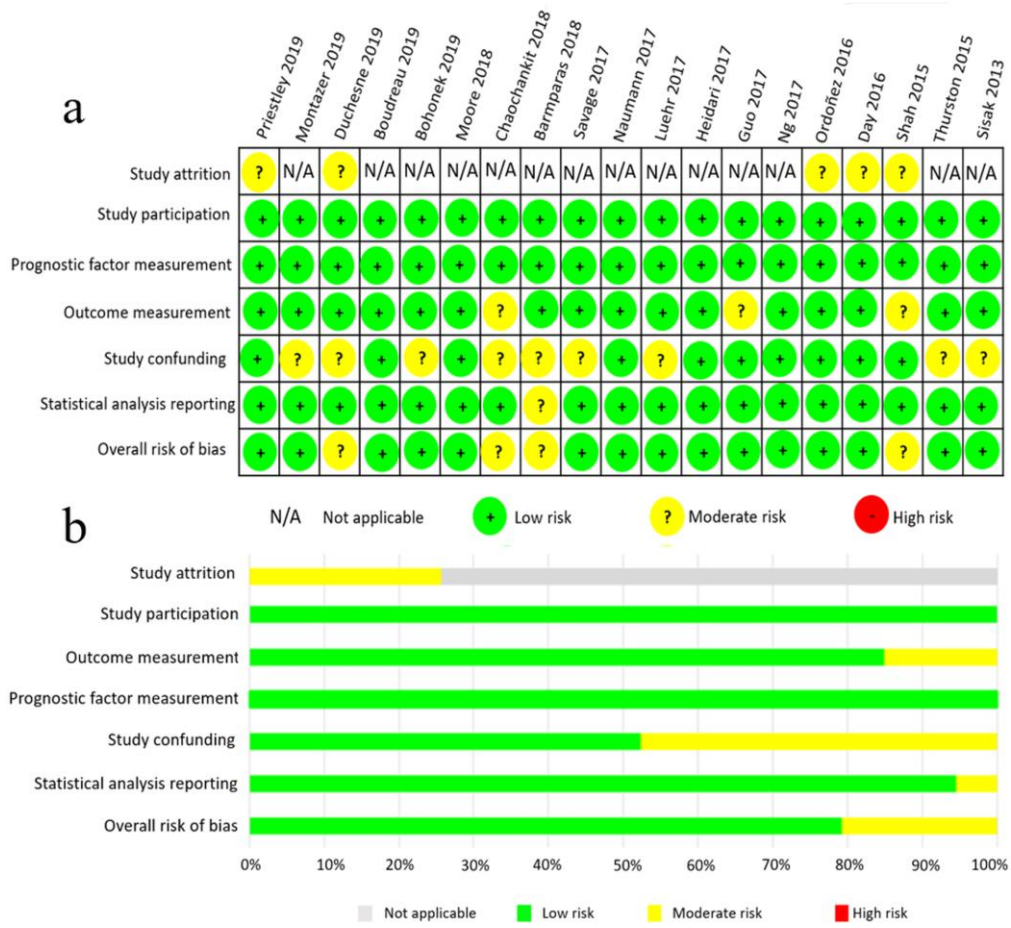

Figure 4. Risk of bias assessment. a: The figure shows the risk of bias in the 6 main domains of the Quality In Prognostic Studies (QUIPS) assessment, in each paper. 'Study attrition' was not suitable for the retrospective studies. In 5 prospective studies, there was a moderate risk for study attrition bias. All studies were judged to carry a low risk of bias in 'Study participation' and 'Prognostic factor measurement' domains. 'Study confounding' was the worst rated domain: a moderate risk appeared in almost half of the records, in which the role of important confounders was not reported thoroughly. Based on the assessment of the 6 main domains, the overall risk of bias was determined for each study. **b**: The summarized risk of bias is illustrated in percentages in the main domains

5.1.4 Primary meta-regression

Our primary meta-regression investigated the relation between HR and mortality in trauma patients with hemorrhage based on all 19 datasets. We found no significant relation between HR and the outcome (p=0.847); thus, a linear association could not be confirmed. The results with the regression line are demonstrated in Figure 5.

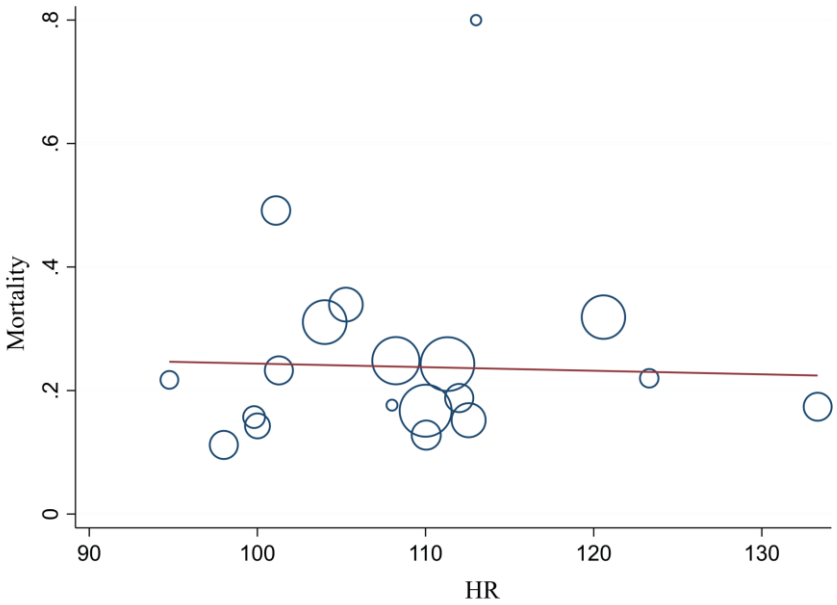

Figure 5. Relation between HR and mortality of bleeding trauma patients. Linear association between HR and mortality could not be identified. HR=heart rate

5.1.5 Subgroup analysis

Due to the relative heterogeneity of the patient enrollment criteria of the individual papers, a subgroup of 10 studies utilizing the use of blood products in the initial management as inclusion criteria was formed and analyzed separately. Again, our findings demonstrated no significant relation and linear association between HR and mortality rate (Figure 6).

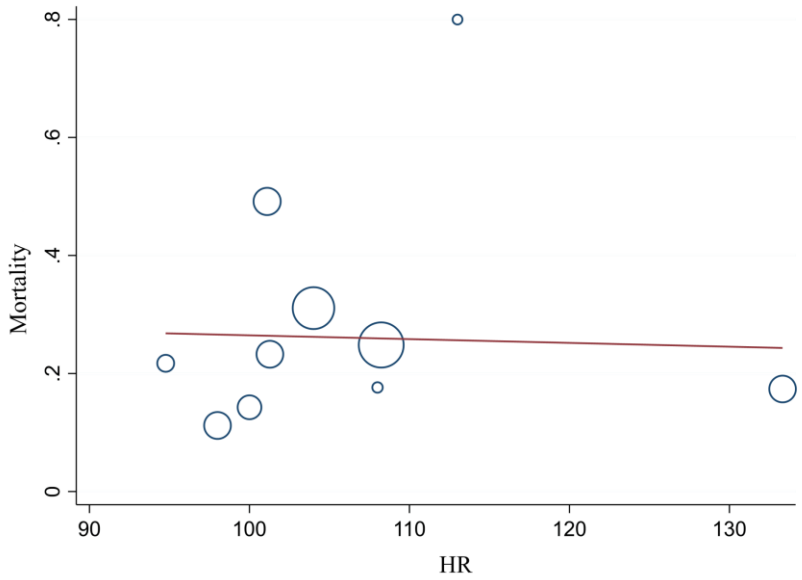

Figure 6. Subgroup analysis of studies on trauma patients who received blood products. Linear association between early HR and mortality rate of patients could not be identified. HR=heart rate

5.2 Study 2

5.2.1 Preliminary data

Study 2 is a protocol for a prospective clinical study that is currently in progress. Therefore, the results of the analysis cannot be shared at this moment. Hereby, we would like to present some preliminary data related to our research.

Figure 7 presents the PAS recording of a 45-year-old male suffering multiple severe injuries in a road traffic accident in October 2021. He was transported from the scene directly to the Emergency Department of the University of Szeged. During the first 3 hours of in-hospital care, the patient received 3*2 units of pRBCs. The increase of exhaled CH_4 levels after each transfusion depicts a clear trend.

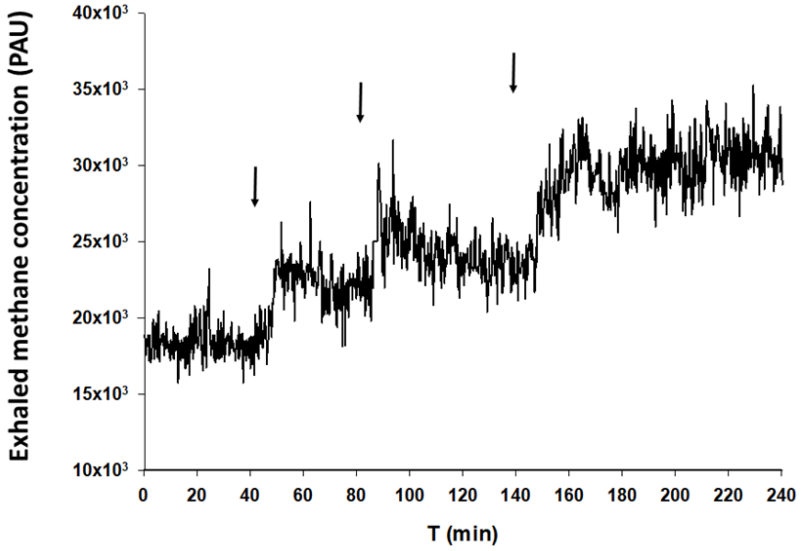

Figure 7. Representative photoacoustic spectroscopy (PAS) record of a severely injured patient. The arrows indicate administrations of 2 units of pRBCs. Each transfusion is followed by an increase of exhaled CH_4.

Figure 8 illustrates the association between Hb levels and exhaled CH_4 concentrations measured on admission, based on the data of 9 severely injured individuals who received treatment at the University of Szeged between 15 August 2021 and 15 January 2022. The mean ISS of the patients was 32.3 ± 12.1 SD. The mean age was 41.5 ± 11.8 SD. The mechanism of injury was road traffic accident in 6 and fall in 3 cases. All patients sustained blunt trauma accompanied by internal bleeding confirmed with CT, and none of them had a previous history of anemia. The relationship between Hb and exhaled CH_4 levels suggests that CH_4-monitoring has a clinical value in the early management of trauma patients. Nonetheless, completing our research is necessary to confirm this theory.

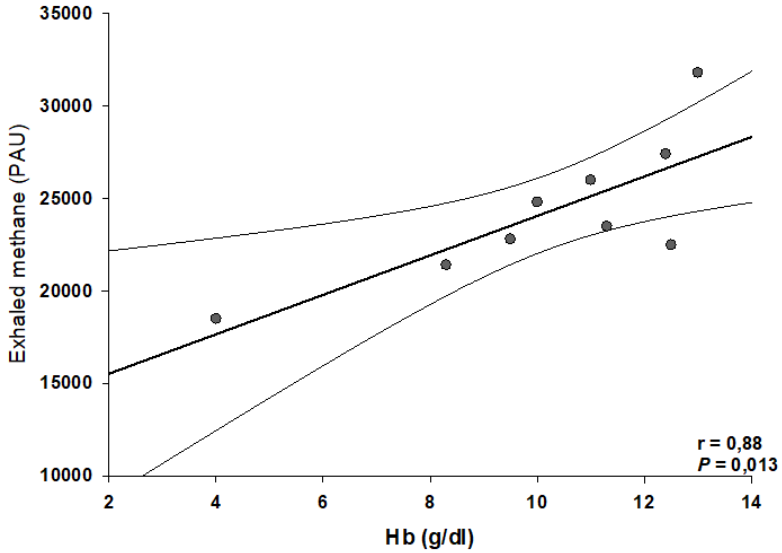

Figure 8. Association between Hb levels and exhaled CH_4 concentrations of severely injured patients (n=9). Black scatters show individual data. The plot demonstrates a regression line (straight black line) and the corresponding r value as an indicator of the strength of linear association, and the p value of significance.

5.2.2 Expected results of Study 2

For the primary outcome we anticipate significant association (Pearson correlation at least 0.3 or larger) between exhaled CH_4 levels and the volume of blood loss. Exhaled CH_4 concentrations are presumed to outperform SI, BD, lactate, Hb, $EtCO_2$, and microcirculatory indices (DBS, PVD, MFI, HI) regarding their association with the volume of blood loss. For our secondary outcomes we expect an AUROC at least 0.7 for both MBT and 24-hour mortality.

Upon completion of the research, the results will be reported according to the STROBE guidelines and will be shared with the scientific community through publication in a peer-reviewed journal.

5.3 Study 3

5.3.1 Preliminary data

Study 3 is a protocol for a prospective clinical study that is currently in progress. Therefore, the results of the analysis cannot be shared at this moment. Hereby, we would like to present some preliminary data related to our research. Figure 9 shows the clotting time, MCF and ML values in EXTEM, the clotting time and MCF values in FIBTEM, and the AUC in TRAPTEM test by 11 severely injured patients with hemorrhage compared to 11 control patients with stable hemodynamic state. All patients received treatment at the

University of Szeged between 15 August 2021 and 15 January 2022. The mean ISS of the hemorrhage group was 31.5 ± 11.1 SD. The mean ISS of the control group was 12.3 ± 5.2 SD. The mean age was 38.7 ± 12.7 SD in the hemorrhage group and 49.9 ± 9.8 SD in the control group. Regarding the mechanism of injury, road traffic accident was dominant in the hemorrhage group and fall in the control group.

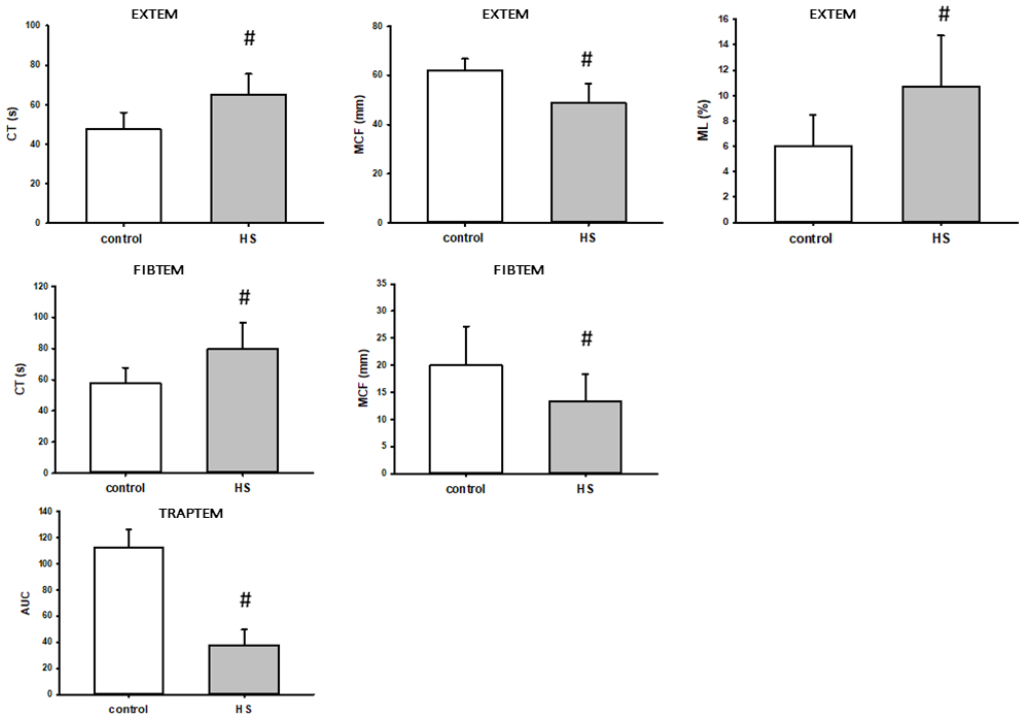

Figure 9. Viscoelastic tests and platelet function. The diagrams demonstrate results of viscoelastic assays and TRAPTEM platelet function test by 11 severely injured patients with hemorrhage compared to 11 control patients with stable hemodynamic state. CT, MCF and ML values in EXTEM, CT and MCF values in FIBTEM, and the AUC in TRAPTEM test are displayed. Data are presented as means ± SEM. #P<0.05 vs. Control. CT=clotting time, MCF=maximum clot firmness, ML=maximum lysis, HS=hemorrhagic shock, SEM=standard error of the mean

Figure 10 presents a representative record of mitochondrial oxygen consumption of thrombocytes isolated from the blood sample of a severely injured patient with hemorrhage, and comparisons of OxPhos capacity and LEAK respiration data of the 11 bleeding trauma patients and the 11 control participants. The results indicate significantly diminished mitochondrial respiration in the hemorrhage group.

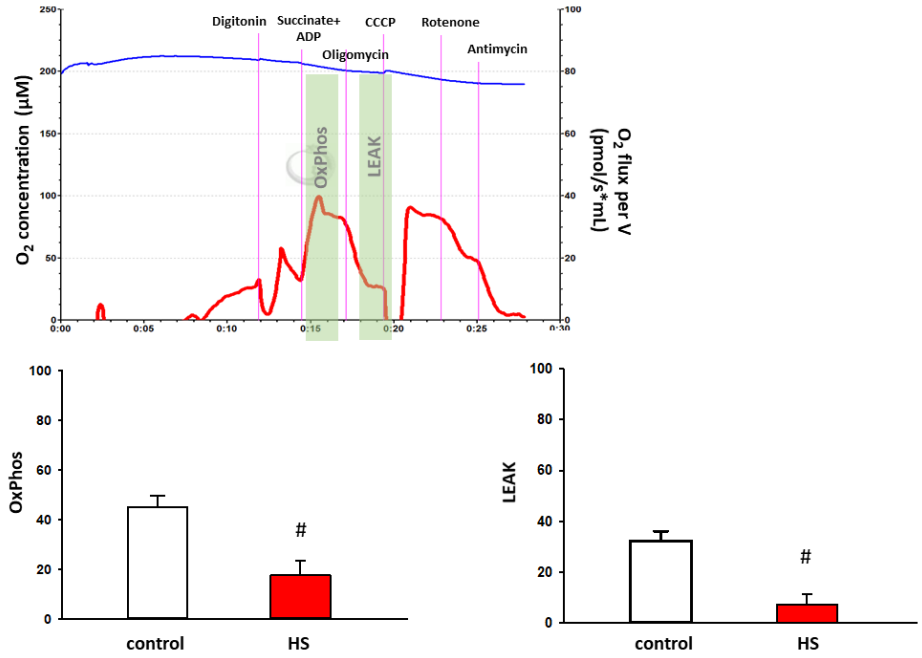

Figure 10. Oxygen consumption of platelet isolates (pmol/s/mL^{-1}). The upper chart demonstrates a representative record of mitochondrial oxygen consumption of thrombocytes isolated from the blood sample of a severely injured patient, measured with high-resolution respirometry. The blue line represents the instantaneous oxygen concentration in the respiration chamber, while the red line indicates the simultaneous oxygen consumption of the sample. The lower left-hand chart shows OxPhos capacity, and the lower right-hand chart demonstrates LEAK respiration data of patients with hemorrhagic shock (red columns, n=11) and control patients with stable hemodynamic state (white colums, n=11). Data are presented as means ± SEM. #P<0.05 vs. Control (paired t-test). OxPhos=oxidative phosphorylation, SEM=standard error of the mean

Figure 11 displays the association between mitochondrial OxPhos of thrombocytes and TRAPTEM AUC values of the blood samples of the same 11 bleeding trauma patients. Although the relation between OxPhos capacity of isolated mitochondria and the AUC in TRAPTEM test did not reach the level of significance, a clear trend can be observed.

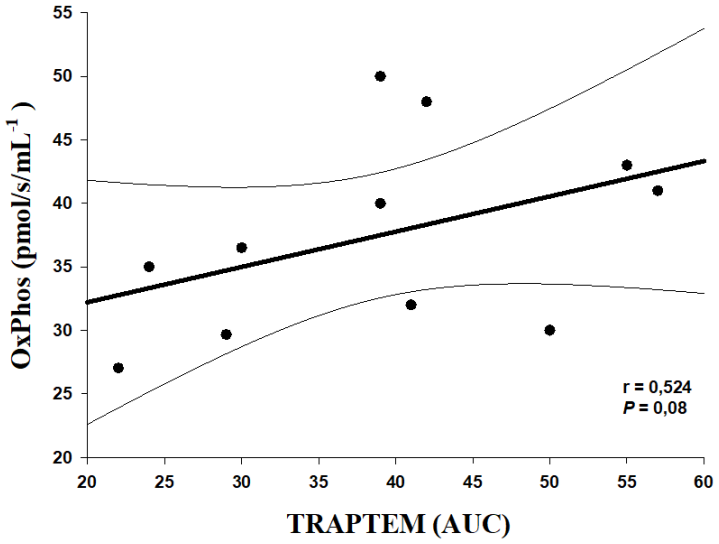

Figure 11. Association between OxPhos (pmol/s/mL^{-1}) and TRAPTEM AUC of severely injured patients (n=11). Black scatters show individual data. The plot demonstrates a regression line (straight black line) and the corresponding r value as an indicator of the strength of linear association, and the p value of significance.

5.3.2 Expected results of Study 3

For the primary outcome we expect significant association (Pearson correlation at least 0.3 or larger) between OxPhos capacity of platelet mitochondria and thrombocyte aggregation indices AUC, MS and A6 in TRAPTEM test. For our secondary outcomes (results of viscoelastic assays (clotting time, CFT, α-angle, A10, MCF, LI30 and ML in INTEM, EXTEM, APTEM, FIBTEM) and conventional markers of hemostasis (aPTT, PT, INR)) we anticipate weaker, although probably still significant relations to OxPhos capacity of platelet mitochondria than in case of thrombocyte function indices. As results of our ROC-analyses, we expect an AUROC at least 0.7 for both MBT and 24-hour mortality.

Upon completion of the research, the results will be reported according to the STROBE guidelines and will be shared with the scientific community through publication in a peer-reviewed journal.

6 DISCUSSION

6.1 The predictive value of tachycardia for mortality in trauma patients with hemorrhage

Study 1 was designed to investigate and update current knowledge on the relation between

HR and mortality in bleeding trauma patients. We identified 19 studies providing early HR and mortality data on trauma patients with hemorrhage from the past 10 years through a comprehensive database search. Due to the relative heterogeneity of the patient enrollment criteria of the individual papers, a subgroup of 10 records was created. Each of these 10 studies provided data on trauma patients who received blood products. Meta regressions were conducted on the data of all records and the subgroup, respectively.

No significant relation was found between HR and mortality in our meta regressions. This result supports the evidence provided by studies doubting the value of HR in the initial assessment of potentially bleeding trauma patients. Additionally, our findings raise further concerns over the depicted pattern of HR-alterations in the ATLS classification of hypovolemic shock.

Heart rate is an easily accessible vital parameter that indubitably reacts to circulatory volume depletion [16,17]. However, the complexity of this reaction seems to contain too many possibilities for misinterpretation to be used in the simplified scheme presented by ATLS. The current classification of hypovolemic shock suggests that HR increases continuously parallel to the severity of bleeding. The increase can stagnate between class I-II and III-IV according to ATLS [9]. This scheme seems to be incongruent with the existing literature on the physiology of HR change during intravascular volume depletion. The HR response tends to follow a biphasic or triphasic pattern instead of continuous increase [19,21,22]. If it comes to a decrease or stagnation in HR value, it is likely to occur at two separate stages of hemorrhage. First, due to increased vagal activity caused by a Bezold-Jarisch-like reflex just around 30% blood loss [16,21], between shock classes II and III, where ATLS suggests a clear increase in HR. Secondly, at the end stage of hemorrhage, bradycardia appears preceding cardiac arrest [11,26,133]. Based on these observations, the pattern of HR alterations during hemorrhage suggested by ATLS may reflect the clinical condition more accurately after minor modifications (Table 5).

Severity classes *Estimated blood loss*		*Class I* *<15%*	*Class II* *15-30%*	*Class III* *31-40%*	*Class IV* *>40%*
Physiologic variables	HR	↔	↔/↑	↑	↑/↑↑
	HR*	**↔**	**↑**	**↔/↑**	**↓/↑**
	SBP	↔	↔	↔/↓	↓
	GCS	↔	↔	↓	↓
	Pulse pressure	↔	↓	↓	↓
	Respiratory rate	↔	↔	↑	↑
	Urine output	↔	↔	↓	↓↓

	BD	0-2 mEq	2-6 mEq	6-10 mEq	≥10 mEq
Transfusion		Monitor	Possible	Yes	Massive transfusion

Table 5. Advanced Trauma Life Support (ATLS) classification of hypovolemic shock including suggested modifications in the pattern of heart rate (HR) derangements. The table is based on the 10th edition of ATLS. Estimated blood loss is shown as percentage of total blood volume. *The suggested modifications are highlighted in bold: possible stagnation in HR value is indicated around 30% blood loss due to increased vagal activity. The possibility of bradycardia in profound bleeding in Class IV is highlighted. HR=heart rate, SBP=systolic blood pressure, GCS=Glasgow Coma Scale, BD=base deficit

Despite criticism, HR is a promptly available vital sign that may lead physicians in the right direction in a relatively high percentage of cases when it comes to the initial management of potentially bleeding trauma patients. However, the question remains if it is effective enough to be taken into consideration when we can also rely on parameters with higher sensitivity and specificity for bleeding – such as BD. Multiple studies have presented the inferiority of HR as compared to other predictors included in the ATLS criteria such as SBP, Glasgow Coma Scale and BD [134,135]. Based on these concerns, the role of HR in the classification of hypovolemic shock and in the initial hemodynamic assessment of severely injured patients may be subject to re-evaluation.

Study 1 focuses on injury-related severe hemorrhage, a condition carrying high clinical importance. In the previous decades, trauma care has gone through remarkable development. On that note, we decided to use scientific data only from January 2010 – September 2020 (date of database search). The included papers were judged to carry a relatively low risk of bias.

Naturally, this study also has its limitations. Although mortality is a highly objective outcome and we included patients only with significant hemorrhage, the direct cause of death may be difficult to determine in some cases. Although studies on special populations have been excluded from our analysis, it is important to emphasize that the presence of potential confounding factors affecting HR values could not be ruled out completely. Prehospital measures may have affected the HR values registered upon admission. There is a notable difference in patient number among some of the included studies. The characteristics of the patient population by the individual records show a significant heterogeneity. To minimize this, a subgroup analysis was performed on patients who received blood products during initial in-hospital trauma care. These limitations prevented us from performing an adequate meta-analysis; however, we believe that we managed to raise attention on a clinically important issue.

In conclusion, the validity of relying on HR in the initial assessment of hypovolemic shock

seems to be obvious, but in fact, its usefulness is questionable due to unsatisfactory sensitivity and specificity. The complexity of HR response during hemorrhage leads to the possibility of misinterpretation, false sense of hemodynamic stability and consequent delay in adequate therapy. Further research is required to reappraise HR as a physiologic variable in the ATLS classification of hypovolemic shock. As a reaction frequently associated with bleeding, tachycardia should raise suspicion for hemorrhage, but it might not be appropriate as one of the determining factors of therapeutic decisions, such as administration of blood products. In addition to the literature demonstrating the multi-phasic response of HR to bleeding, our study presents the lack of linear association with mortality. Considering these, modifying the pattern of HR derangements in the ATLS shock classification may make this pragmatic guide more precise.

6.2 Exhaled CH_4 levels for monitoring trauma-related hemorrhage following blunt trauma

To the best of our knowledge, this study is the first protocol for investigating the associations of exhaled CH_4 levels and hemorrhage in severely injured patients. Our protocol is preluded by animal experiments showing promising results with regards to the capability of exhaled CH_4 to indicate blood loss; however, human studies have not been conducted so far.

The sensitivity of exhaled CH_4 for alterations in mesenteric macro-and microperfusion during controlled, graded hemorrhage and subsequent fluid resuscitation was tested recently in Vietnamese minipigs (n=6). The performance of this new method was also compared with the efficacy of sublingual microcirculatory monitoring. The SMAs of the anesthetized, intubated, ventilated animals were accessed from median laparotomy to record blood flow. To provide access to the ileal mucosa for microcirculatory measurements, a 5-cm incision was performed with diathermy 15 cm orally from the ileo-cecal junction. The open mucosal and serosal surfaces were rinsed constantly with saline. Vital signs were monitored continuously during the procedure. CH_4 concentrations were obtained by attaching a near-infrared laser technique-based PAS apparatus to the exhalation outlet of the ventilator. Hemorrhage was induced and divided into 7 phases, followed by gradual fluid resuscitation in five steps, until 80% of the baseline mean arterial pressure value was reached. Each bleeding and resuscitation interval was started with microcirculatory recordings at the ileal mucosal and serosal surfaces and at the sublingual area with incident dark field imaging

technique (using CytoCam Video Microscope System; Braedius Medical, Huizen, The Netherlands). To quantitatively characterize microcirculation, DBS, MFI, and HI were calculated. The researchers found that diminution in SMA flow and ileal microperfusion were followed rigorously by changes of exhaled CH_4 levels, and they developed earlier than systemic hemodynamic responses. In contrast, sublingual microcirculation was unable to follow the alterations of mesenteric perfusion [43].

These results raise the possibility of a future non-invasive diagnostic and monitoring method in the management of severely injured patients; however, several questions need to be addressed. Although swine is considered as the most appropriate animal species for cardiovascular research due to their cardiac anatomy and hemodynamic resemblance to humans [136], it is important to emphasize that the intestinal vascular anatomy and mesenteric perfusion of pigs differ considerably from humans [137]. Since breath analysis does not pose a risk to patients, it is feasible and necessary to conduct human studies. In our protocol, examinations that are not part of routine trauma care (measurement of exhaled CH_4 concentrations, videomicroscopy of the sublingual mucosa) are non-invasive and fast; thus, they do not hinder patient care, even if the patient needs emergent surgery.

6.3 Mitochondrial dysfunction in trauma-related coagulopathy

Trauma-induced coagulopathy is a commonly occurring, severe condition contributing significantly to trauma-related mortality. Despite of intensive research focus, the pathophysiology of TIC is still not completely understood; consequently, delivering the efficient therapy often poses a challenge for clinicians. The assessment of the coagulation status of trauma patients is complex. Conventional laboratory markers of hemostasis such as aPTT, PT and INR reflect only a small portion of the coagulation cascade [138] and may overlook clinically significant coagulopathies leading to time loss and/or the use of inappropriate or unnecessary blood products, resulting in suboptimal treatment and additional costs. Thromboelastometry (viscoelastic assays and aggregometry) overcomes several pitfalls of conventional laboratory tests; however, it also has its limitations. Measurements are performed on 37 °C; therefore, the risk of coagulopathy may be underestimated if the patient is hypothermic. Furthermore, the consumption of alcohol also seems to hamper the results of ROTEM tests. Experts also claim that thromboelastometry is not performed on activated endothelium with physiological shear stress; therefore, it can hardly reflect in vivo clot formation accurately [59]. Ultimately, in the clinical setting, the

transition from hypocoagulability to hypercoagulability is often impossible to detect, making it difficult to provide adequate therapy.

Study 3 is the first protocol aiming to disclose and characterize platelet mitochondrial dysfunction in TIC. The protocol utilizes venous blood samples taken for routine laboratory tests to isolate platelets and perform high-resolution respirometry; thus, it does not interfere with patient care. It is well-known that the activation of thrombocytes and subsequent clot formation are highly energetic processes being tied to mitochondrial activity [139,140]. According to the literature, inhibition of the mitochondrial electron transport chain impedes on thrombogenesis [111,141,142], 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 [73,143]. Based on these findings, initiating clinical research characterizing platelet mitochondrial dysfunction in TIC is a reasonable next step that may lead to new therapeutic targets.

7 SUMMARY OF NEW FINDINGS

- We showed that heart rate does not increase in parallel with the mortality rate of bleeding trauma patients. Based on literature review and our results, we suggested minor modifications in the ATLS classification of hypovolemic shock.
- We presented a promising new method, the monitoring of exhaled CH_4 concentrations for the hemodynamic assessment of trauma patients with potential hemorrhage. We provided a protocol for a prospective clinical study, and we demonstrated the association between Hb levels and exhaled CH_4 concentrations in a case series of severely injured patients.
- We referred to severe trauma-induced coagulopathy as one of the most challenging conditions in the management of bleeding trauma patients. We have confirmed the coexistence of mitochondrial dysfunction and coagulopathy in a series of trauma patients. We initiated further research to characterize the role of mitochondrial dysfunction in trauma-induced coagulopathy. We elaborated a protocol for a prospective clinical study.

8 ACKNOWLEDGEMENTS

I am highly grateful to Dr. Petra Hartmann for her unlimited daily assistance in conducting

the studies and writing the publications. I am grateful to Prof. Dr. Endre Varga for providing the opportunity to carry out my research under his valuable scientific guidance at the Department of Traumatology of the Faculty of Medicine at the University of Szeged. I am also thankful to Dr. Tamara Horváth and Dr. Ferenc Rárosi for aiding me in my research with their expertise.

9 FUNDING STATEMENT

Hungarian Academy of Sciences János Bolyai Research Scholarship, grant BO/00605/21/5 (P.H.). National Research, Development, and Innovation Office (NFKIH) grant FK138839 (P.H.).

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11 ANNEX