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

PhD Thesis

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2023

LIST OF ORIGINAL PAPERS

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: 1.114**

Cumulative IF: 7.126

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**

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Cumulative IF: 29.598

INTRODUCTION

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. 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. 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.

Hemorrhage is the most common cause of shock in trauma patients. Hemorrhagic shock can be defined as inadequate organ perfusion and tissue oxygenation as a result of blood loss; 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). The severity classes entail recommendations regarding blood transfusion. Despite being widely implemented, the validity and applicability of the ATLS classification of hypovolemic shock in clinical practice sparks controversy.

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. Among these variables, HR is one of the most controversial when it comes to blood loss. 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. Furthermore, ATLS suggests the continuously increasing tendency of HR in accordance with the severity of bleeding. However, in clinical reality, the HR response to hemorrhage is rather biphasic or triphasic than linear. Consequently, the utility of relying on HR in the early management of bleeding trauma patients was called into doubt during the past decades. 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. 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. A notable limitation of previous studies is that trauma protocols have undergone several changes (such as the use of tranexamic acid) or the limitation of crystalloids in fluid resuscitation), 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.

Impairment of mesenteric perfusion and exhaled methane concentrations as markers of major bleeding

The most reliable method for the detection of internal hemorrhage is computer tomography (CT); however, it requires transportation out of the emergency department resulting in unfavorable time delays. Ultimately, there is no gold standard technique for prompt diagnostics and assessment of hemorrhage in severe trauma, thus decision-making is commonly based on a combination of tests, which all have their strengths and limitations.

The diminution of mesenteric perfusion is among the first compensatory reactions to blood loss, thereby being a potential early clinical indicator of hemorrhage. The particular sensitivity of mesenteric perfusion to blood loss has been demonstrated by studies on large animal models, where the superior mesenteric artery (SMA) flow displayed a significant drop already at 5% loss of total blood volume; and continued to diminish in parallel with ongoing hemorrhage. 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 methane levels correspond to the SMA blood flow. Methane is an intrinsically non-toxic, combustible gas produced by anaerobic bacterial fermentation. Due to its physicochemical attributes, methane 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. The real-time monitoring of exhaled methane concentrations can be conducted with photoacoustic spectroscopy (PAS) based sensors, 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.

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. Alterations in coagulation following severe injury were documented already in the 1960's; however, a standard definition for TIC still does not exist. 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. 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. 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. Diminished platelet functions are suggested to be consequences of injury-induced early hyperactivation; nevertheless, the mediators and pathways of the process are elusive, thus being subjects for further research.

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

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 methane 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 methane 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).

MATERIALS AND METHODS

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

Study design, literature search and eligibility criteria

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

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.

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 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 focused assessment with sonography in trauma (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.

Study selection, data extraction

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.

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.

Risk of bias assessment and statistical analysis

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. Disagreements were resolved by consensus.

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.

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

Study design and inclusion criteria

We elaborated a protocol for a single-center, prospective observational study investigating the association of exhaled methane concentrations with the volume of blood loss in severely injured patients. 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. The study is conducted for an estimated maximum of 36 months (between 15 August 2021 and 15 August 2024).

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 methane and hemorrhage, patients with respiratory causes of methane-decrease (e.g. in case of acute lung injury or acute respiratory distress syndrome) entail exclusion.

Measurement of exhaled methane levels and estimation of blood loss volume

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 methane concentrations.

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. 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).

Videomicroscopy of the sublingual mucosa

In our study, orthogonal polarization spectral imaging (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 will be determined.

Recorded variables

Demographic data, comorbidities and variables reflecting the hemodynamic condition of patients are recorded upon arrival. Heart rate, SBP, shock index (SI), BD, lactate, hemoglobin (Hb), hematocrit (Hct), end-tidal carbon dioxide ($ETCO_2$), results of extended FAST (eFAST) and indices of sublingual microcirculation (DBS, PVD, MFI, HI) serve to provide a detailed view on the circulatory status of the patients. 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 massive blood transfusion (MBT) and 24-hour mortality is recorded. The present protocol defines MBT according to ATLS, as more than 10 units of transfused packed red blood cells (pRBC) within the first 24 hours of admission or more than 4 units in 1 hour. 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.

Outcomes and statistical analysis

The primary outcome in our study is the volume of blood loss. The association between the volume of blood loss and the concentration of methane in exhaled breath upon admission stands in the focus of our research. Additionally, exhaled methane 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. The need for MBT and 24-hour mortality will constitute secondary outcomes.

The alternative hypothesis for the primary outcome presumes an association (Pearson correlation at least 0.3 or larger) between exhaled methane 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 $\alpha=0.05$.

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. 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 methane concentrations and the need for MBT and 24-hour mortality, respectively, ROC-analysis will be applied.

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

Study design and inclusion criteria

We elaborated a protocol for a single-center, prospective observational study investigating mitochondrial dysfunction in trauma-related coagulopathy. 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. The study is conducted for an estimated maximum of 36 months (between September 2021 and September 2024).

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.

Recorded variables

Demographic data and comorbidities of the participants are documented upon admission. 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. In our research,

rotational thromboelastometry (ROTEM) is used to yield a comprehensive analysis of the hemostatic functions of study participants. ROTEM is a widely used point-of-care (POC) tool providing rapid assessment of specific clotting pathways and platelet functions through viscoelastic assays and aggregometry. 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) in INTEM, EXTEM, APTEM and FIBTEM tests; and the area under curve (AUC), maximum slope (MS), and amplitude at 6 minutes (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.

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.

Outcomes and statistical methods

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.

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 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. 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.

RESULTS

Study 1

Results of systematic search and study characteristics

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.

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.

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.

Meta-regression analyses

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.

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.

Study 2

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. The relationship between Hb and exhaled CH_4 levels of 9 severely injured individuals who received treatment at the University of Szeged between 15 August 2021 and 15 January 2022 was investigated using regression analysis. 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 significant relationship between Hb and exhaled CH_4 levels (p=0.013) 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.

Expected results of Study 2

For the primary outcome we anticipate significant association (Pearson correlation at least 0.3 or larger) between exhaled methane levels and the volume of blood loss. Exhaled methane 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.

Study 3

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. Eleven severely injured patients with hemorrhage were compared to 11 control patients with stable hemodynamic state regarding their platelet OxPhos capacity and LEAK respiration (paired t-test). The results indicate significantly diminished mitochondrial respiration in the hemorrhage group.

The association between mitochondrial OxPhos of thrombocytes and TRAPTEM AUC values of the blood samples of the same 11 bleeding trauma patients was investigated with regression analysis. 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 ($p=0.08$).

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.

DISCUSSION

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. 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. 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. 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. 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, 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. Based on these observations, the pattern of HR alterations during hemorrhage suggested by ATLS may reflect the clinical condition more accurately after minor modifications.

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.

Exhaled methane 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 methane levels and hemorrhage in severely injured patients. Our protocol is preluded by animal experiments showing promising results with regards to the capability of exhaled methane to indicate blood loss; however, human studies have not been conducted so far. Since breath analysis does not pose a risk to patients, it is feasible and necessary to conduct clinical studies. In our protocol, examinations that are not part of routine trauma care (measurement of exhaled methane concentrations, videomicroscopy of the sublingual mucosa) are non-invasive and take only minimal time to perform; thus, they do not hinder patient care, even if the patient needs emergent surgery.

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 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 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. 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. According to the literature, inhibition of the mitochondrial electron transport chain impedes on

thrombogenesis, 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. 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.

SUMMARY

- 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.