


BMJ Open Detection of exhaled methane levels for monitoring trauma-related haemorrhage following blunt trauma: study protocol for a prospective observational study

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ABSTRACT

Introduction Early recognition and effective treatment of internal bleeding impose a cardinal challenge for trauma teams. The reduction of the superior mesenteric artery (SMA) blood flow is among the first compensatory responses to blood loss, thus being a promising candidate as a diagnostic tool for occult haemorrhage. Unfortunately, methods for monitoring the SMA flow have not been elaborated to date. Nevertheless, animal experiments suggest that exhaled methane (CH₄) levels correspond to the SMA perfusion. We hypothesise that real-time detection of CH₄ concentrations in the exhaled air is an applicable technique for the early recognition of haemorrhage in severely injured patients. We also hypothesise that exhaled CH₄ levels reflect the volume of blood loss more accurately than conventional markers of blood loss and shock such as shock index, haemoglobin, base deficit, lactate, end-tidal carbon dioxide and sublingual microcirculatory indices.

Methods and analysis One hundred and eleven severely injured (Injury Severity Score ≥16), intubated, bleeding patients sustaining blunt trauma will be included in this prospective observational study. Blood loss will be detected with CT and estimated with CT-linked radiologic software. Exhaled CH₄ concentrations will be monitored by attaching a near-infrared laser technique-based photoacoustic spectroscopy apparatus to the exhalation outlet of the ventilator on patient arrival. The primary outcome is the volume of blood loss. Need for massive transfusion and 24-hour mortality will constitute secondary outcomes. The relation of exhaled CH₄ to study outcomes and its performance in predicting blood loss in comparison with conventional shock markers and microcirculatory indices will be tested.

Ethics and dissemination Our protocol (ID: 5400/2021-SZTE) has been registered on ClinicalTrials.gov (NCT04987411) and complies with the Declaration of Helsinki and has been approved by the medical ethics committee at the University of Szeged (Ref.nr.:121/2021-SZTE RKEB). It is in data collection phase, the results will be shared with the scientific community through publication in a peer-reviewed journal.

Trial registration number NCT04987411; ClinicalTrials.gov, registered on 27 July 2021.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ The protocol follows the 'Strengthening the Reporting of Observational studies in Epidemiology' statement and adheres to predefined, strict methodological, temporal and numerical criteria.
- ⇒ The investigators apply near-infrared laser technique-based photoacoustic spectroscopy for the real-time monitoring of exhaled methane and estimate the volume of blood loss with the help of a CT-linked software (FSL's FSLeYes).
- ⇒ To provide a comprehensive analysis on the haemodynamic state of study participants, the investigators apply sublingual videomicroscopy besides recording conventional shock markers.
- ⇒ Correlation analysis will be performed to reveal associations between blood loss and exhaled methane concentrations, while Receiver operating characteristic (ROC)-analysis will be applied to determine whether methane levels could predict mortality and massive blood transfusion.
- ⇒ The current protocol does not allow researchers to investigate the effect of prehospital treatment and alcohol consumption on exhaled methane levels; thus, these factors may influence the results.

INTRODUCTION

Despite of the development of trauma care in the past decades, approximately one-fourth of trauma deaths may be potentially preventable through early medical and surgical interventions.¹ The majority of potentially preventable mortality in patients with trauma is related to bleeding¹; therefore, early recognition and effective treatment of internal bleeding and impending haemorrhagic shock (HS) impose a cardinal challenge for trauma teams worldwide.

HS can be defined as inadequate organ perfusion and tissue oxygenation due to blood loss; however, due to the influence of different comorbidities and compensatory capability of patients, HS may be difficult to be defined by objective criteria that can be



applied to every case. Regarding the detection of HS, the challenge lies in identifying its impending presence in the preshock state. To date, the initial haemodynamic assessment of the injured relies largely on vital signs (VS) such as heart rate (HR) and systolic blood pressure (SBP) and metabolic markers such as base deficit (BD) and lactate.^{2,3} However, the specificity of VS and metabolic markers for hypovolemia remained questionable, since several factors such as medication, alcohol intoxication, administration of crystalloids (lactated Ringer or saline) or even advanced age can diminish their reliability.³⁻⁷ Furthermore, the above-mentioned parameters are global markers of shock who are influenced positively by the compensatory mechanisms of the individual patient. Consequently, derangements of these indicators during bleeding may remain subtle in the preshock state and become apparent when it is already too late. Invasive monitoring methods such as pulmonary artery catheterisation offer substantial benefits; however, they are hardly applicable during the initial phase of therapy due to patient positioning and time factor.^{8,9} Determining the 'gold standard' for prompt haemodynamic assessment and estimation of transfusion need have been the objective of numerous studies in the past decades. As a result, several markers and their combinations (such as PT_{ratio} , delta pulse pressure, massive transfusion score (MTS) and revised MTS) have been investigated and proposed as clinical guides.¹⁰⁻¹³ Additionally, due to the deeper understanding of the link between bleeding-associated mortality and coagulopathy, research efforts targeting trauma-induced coagulopathy are increasing.^{11,14} Nevertheless, haemorrhage is still responsible for most potentially preventable trauma-related mortality.¹

In contrast to global markers of hypoperfusion, regional indicators such as intestinal macroperfusion and microperfusion reflect on the early, compensatory phase of haemorrhage when blood flow becomes redistributed away from non-vital organs such as the gut and the skin to maintain adequate cerebral and coronary perfusion.^{15,16} The reduction of mesenteric perfusion is among the first compensatory responses to blood loss,¹⁷ thus being a potential revealer of occult haemorrhage and early predictor of HS. According to studies on large animal models, 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 haemorrhage.¹⁷ Unfortunately, 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 (CH_4) levels correspond to the SMA blood flow,¹⁸ making CH_4 a promising new clinical indicator of haemodynamic deterioration.

We hypothesise that real-time detection of CH_4 concentrations in the exhaled air is an applicable technique for the early recognition of haemorrhage in severely injured patients. We also hypothesise that exhaled CH_4 levels reflect the volume of blood loss more accurately than

conventional markers of blood loss and shock such as shock index (SI), haemoglobin (Hb), BD, lactate, end-tidal carbon dioxide ($EtCO_2$) and sublingual microcirculatory indices.

Methods and analysis

Study design

The present paper is a protocol for a single-centre, prospective observational study that will be conducted at the University of Szeged, Szeged, Hungary. Our protocol was registered to ClinicalTrials.gov on 27 July 2021, complies with the Declaration of Helsinki and follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist (see additional file 1).

Patient enrolment and inclusion criteria

This prospective study will involve severely injured (Injury Severity Score (ISS) ≥ 16) patients with haemorrhage related to blunt force trauma, aged ≥ 18 years, intubated on scene or on arrival, transported directly to the emergency department of the University of Szeged. Bleeding will be confirmed with CT. Patients with penetrating trauma or isolated traumatic brain injury will be excluded from the analysis. As the present study investigates associations between exhaled CH_4 and haemorrhage, respiratory causes of CH_4 -decrease must be recognised. For this purpose, the gradient of partial pressure of carbon dioxide (P_aCO_2) and $ETCO_2$ will be evaluated since it differs in patients with hypovolemia from patients with respiratory distress due to obstructive causes or lung injuries.¹⁹⁻²¹ Obtaining the P_aCO_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 will be assessed with CT. The presence of acute respiratory distress syndrome (ARDS) or acute lung injury (ALI) will be assessed based on The American-European Consensus Conference Definition of ALI and ARDS²² and the Murray Lung Injury Score²²; and it will entail exclusion from the analysis.

The study will be 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 enrolment (A).

Measurement of exhaled CH_4 levels

For the measurement of exhaled CH_4 concentrations, gas chromatography mass spectrometry is considered as the gold-standard technique²³; however, it does not allow continuous monitoring. 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.²⁴ PAS 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

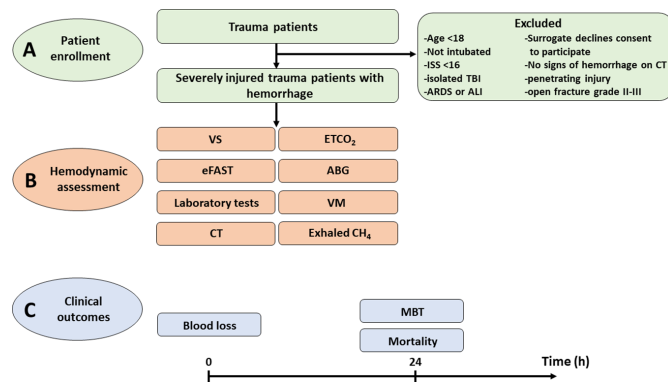


Figure 1 Protocol flowchart. (A) Aspects of patient enrolment and reasons for exclusion are demonstrated. Severely injured (ISS \geq 16), blunt trauma patients with bleeding will be enrolled into our study. CT will be used to detect the presence and evaluate the severity of bleeding, and for aiding the assessment of injury severity. Signed informed consent from patients or their surrogates will be required for patient enrolment. Inclusion criteria include intubation as the exhalation outlet of the ventilator allows the attachment of the CH₄ detector apparatus, thus the continuous monitoring of CH₄ levels in breath. Patients with penetrating trauma, bleeding outwards, grade II and III fractures, isolated TBI, ARDS or ALI will be excluded from the analysis. (B) Study participants will undergo a comprehensive haemodynamic assessment on arrival, which consists of evaluation of VS (HR, SBP), ETCO₂, ABG (BD, lactate), laboratory tests (Hb, Hct), VM of the sublingual mucosa using orthogonal polarisation spectral imaging, eFAST, and polytrauma CT. With the help of these parameters, a detailed dataset describing the haemodynamic state of the participants will be provided. Exhaled CH₄ concentrations will be monitored with a near-infrared laser technique-based PAS apparatus. (C) Our clinical outcomes will 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 will be used. Associations between exhaled CH₄ concentrations and clinical outcomes will be assessed. ABG, arterial blood gas; ALI, acute lung injury; ARDS, acute respiratory distress syndrome; BD, base deficit; CH₄, methane; eFAST, extended focused assessment with sonography for trauma; ETCO₂, end-tidal carbon dioxide; Hb, haemoglobin; Hct, hematocrit; HR, heart rate; ISS, Injury Severity Score; PAS, photoacoustic spectroscopy; SBP, systolic blood pressure; TBI, traumatic brain injury; VM, videomicroscopy; vs, vital signs.

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.¹⁷

In our study, a near-infrared laser technique-based PAS apparatus will be attached to the exhalation outlet of the ventilator on arrival of patients, thereby allowing the continuous monitoring of exhaled CH₄ concentrations.

Estimation of blood loss volume

CT scanning will be performed on a 64-slice GE Revolution Evo scanner (GE Healthcare, Chicago, Illinois). The polytrauma CT protocol complies with the guidelines of

the European Society of Emergency Radiology.²⁵ Patients will be 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 will be tailored to the patient's need, special protocols such as urography and angiography may be employed.

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

Videomicroscopy of the sublingual mucosa

The direct visualisation of peripheral microcirculatory networks with videomicroscopy (VM) is a suitable method for providing information on compensatory circulatory redistribution in shock, and on the therapeutic response of patients on haemodynamic resuscitation.^{26–29} VM uses handheld microscopes that can detect red blood cells flowing in capillaries when placed on mucosal surfaces.^{26 30 31} Multiple generations of videomicroscopic techniques including orthogonal polarisation spectral imaging (OPSI), sidestream dark field imaging and incident dark field imaging are available for clinicians and researchers.²⁶ As VM requires easily accessible mucosal surface, the investigation of the sublingual region is a reasonable approach if haemodynamic 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,^{28 32} reactions of the sublingual microperfusion to haemodynamic changes are considered to be significantly slower than the response of more distal gastrointestinal regions.¹⁷

In our study, OPSI technique (Cytoscan A/R, Cytometrics) will be used to visualise the microcirculation of the sublingual mucosa of the participants. The sublingual capillary network and capillary blood flow of each patient will be recorded and saved to hard drive as 20 s-long video clips. The video clips will be 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. DBS refers to capillary density and can be calculated by using the principle that vessel density is proportional to the number of vessels crossing arbitrary lines.³³ Only vessels with a diameter of 20 μ m or less will be considered as capillaries. The blood flow of the individual



capillaries will be characterised as continuous (continuous flow for 20 s), intermittent (no flow for at least 10 s), sluggish (slow blood flow) or absent (no perfusion); and PVD will be calculated by multiplying vessel density by the proportion of continuously perfused vessels.³⁴ 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.³⁵ The HI describes perfusion heterogeneity by dividing the difference between the highest MFI and the lowest MFI by the mean MFI.^{17 34} Through providing DBS, PVD, MFI and HI, the sublingual microcirculation of each patient will be described quantitatively. Disagreements between the two independent investigators will be resolved by consensus.

Recorded variables

Demographic data and comorbidities of the participants will be documented ideally on 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 haemodynamic condition of patients will be recorded on arrival, as demonstrated in figure 1B and table 1 (Documentation plan). HR, SBP, SI, BD, lactate, Hb, haematocrit (Hct), ET_{CO}₂, results of extended focused assessment with sonography for trauma and indices of sublingual microcirculation (DBS, PVD, MFI, HI) will 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 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.³⁶ Additionally, a register analysis with a large patient number found the performances of SI-based and BD-based hypovolemic shock classification equal in predicting transfusion requirement.³⁷

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 Advanced Trauma Life Support (ATLS) guidance on HS emphasises the importance of BD by associating explicit BD values with explicit percentages of blood loss, while the alterations of VS are only described subjectively, without quantification.² Furthermore, several studies support the superiority of BD over VS in indicating haemorrhage.^{38 39} In contrast to BD, which is a calculated metabolic marker, lactate is a direct byproduct of anaerobic metabolism during shock.⁴⁰ Although ATLS does not refer to lactate as an indicator of severity in the classification of

Table 1 Documentation plan

	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 ET _{CO} ₂	X	
eFAST	X	
CT (confirming, localising and quantifying haemorrhage)	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

Key measures of the protocol and their timing are shown. Informed consent will be obtained from patient surrogates on admission. Demographic data, comorbidities will be recorded. A comprehensive haemodynamic assessment will be carried out on arrival, including the evaluation of VS (HR, SBP), ET_{CO}₂, arterial blood gas analysis (BD, lactate), laboratory tests (Hb, Hct), VM of the sublingual mucosa using orthogonal polarisation spectral imaging, and eFAST. CT will be 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) will also be documented at 24 hours post-admission. Exhaled CH₄ concentrations will be monitored and recorded on arrival and at 24 hours. The documentation will include MBT and mortality. BD, base deficit; CH₄, methane; DBS, De Backer score; eFAST, extended focused assessment with sonography for trauma; ET_{CO}₂, end-tidal carbon dioxide; Hb, haemoglobin; Hct, haematocrit; HI, heterogeneity index; HR, heart rate; ISS, Injury Severity Score; MBT, massive blood transfusion; MFI, microvascular flow index; PVD, perfused vessel density; ROC, Receiver operating characteristic; ROTEM, rotational thromboelastometry; SBP, systolic blood pressure; SI, Shock Index; VM, videomicroscopy; VS, vital signs.

hypovolemic shock, numerous studies reported its ability to predict mortality, massive transfusion and the need for damage control laparotomy.^{41–44} Modern blood gas analysers 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 haemorrhage remains controversial, most trauma patients with severe bleeding display a significant drop in Hb and Hct values within the first 30 min of patient arrival.^{45 46}

Monitoring EtCO₂ 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.⁴⁷ In addition to the role in ascertaining the effectiveness of chest compressions during cardiopulmonary resuscitation, EtCO₂ has been reported to reflect mortality, transfusion need and fluid responsiveness after injury.^{47–50} Similarly to CH₄, EtCO₂ is an easily measurable exhaled gas that provides information on the circulatory status of patients. However, according to our theory, monitoring CH₄ levels allows clinicians to detect haemorrhage 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 CH₄ concentration may already indicate bleeding when EtCO₂ stays in the reference range.

After the primary assessment and stabilisation, 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 will be recorded. The present study 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.² 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 well⁵¹; however, we use criteria listed by ATLS due to practical considerations. It is important to mention that our institution uses 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 will be recorded since they may influence microcirculatory indices and splanchnic perfusion.^{52–54}

Data will be stored in electronic database and supervised by the principal investigator (PH). The detailed documentation plan is shown in [table 1](#).

Patient and public involvement

Patients and public were not specifically involved in designing the protocol and choosing the methods and outcome measures.

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 CH₄ in exhaled breath on admission stands in the focus of our research. Additionally, exhaled CH₄ will be compared with SI, BD, lactate, Hb, EtCO₂ and microcirculatory indices (DBS, PVD, MFI, HI), with respect to their ability to reflect the extent of blood loss on patient arrival. If exhaled CH₄ displays higher predictive performance than the above-mentioned shock markers, it would strongly suggest the utility of CH₄ measurements in clinical practice considering its prompt availability, non-invasive nature and suitability for continuous monitoring. The need for MBT and 24-hour mortality will constitute secondary outcomes.

Statistical methods

Hypothesis

The alternative hypothesis for the primary outcome presumes an association (Pearson correlation at least 0.3 or larger) between exhaled CH₄ levels and the volume of blood loss.

Sample size calculation

Sample size calculation was performed with G*Power V.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

Statistical analyses will be performed using SPSS V.25.0 (IBM Corporation, Chicago, Illinois). 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 \pm SD, 95% CIs for normally distributed variables and median and IQR 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 analysed using linear regression and a non-linear (polynomial regression). Regression models will be compared with F test. To investigate the association between exhaled CH₄ concentrations and the need for MBT and 24-hour mortality, respectively, receiver operating characteristic (ROC)-analysis will be applied.

Summary

CH₄ is an intrinsically non-toxic, combustible gas.^{55 56} According to a widely accepted perspective, CH₄ in the human body originates mainly from anaerobic methanogenic intestinal microorganisms.⁵⁷ Due to its physicochemical properties, it can enter freely to the mesenteric microcirculation, and exhaled CH₄ concentrations may correspond to the perfusion rate of the gastrointestinal tract.⁵⁸ The reduction of mesenteric blood flow is among



the first compensatory responses to haemorrhage; therefore, it may indicate occult bleeding and impending HS earlier than conventional markers of blood loss such as VS, Hb, lactate or BD. Furthermore, it may allow the better monitoring of early therapeutic responses as a promptly available, non-invasive, highly sensitive method. To the best of our knowledge, the present study is the first protocol for investigating the associations of exhaled CH₄ levels and haemorrhage in severely injured patients.

Ethics and dissemination

Our protocol complies with the Declaration of Helsinki and has been approved by the local medical ethics committee at the University of Szeged (Regional and Institutional Review Board of Human Investigations) under reference number 121/2021-SZTE RKEB. The examinations that are not part of routine trauma care (measurement of exhaled CH₄, VM of the sublingual mucosa) are non-invasive and take only minimal time to perform; thus, they will not hinder patient care, even if the patient needs emergent surgery.

Our research studies patients who may be incapable of providing informed consent due to their condition; however, in these cases, surrogates are provided a consent form and information sheet, and they have the opportunity to consult one of the investigators. The information sheet contains information about the rationale, design, methods, outcomes and dissemination of the study.

Personal information, photographs or other material that might identify the participants will not be published. Personal data will not be given out without the permission of our patients or their surrogates.

Currently, the study is in recruitment phase. On 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. The results will also be presented at national and international conferences. In case of a significant association between exhaled CH₄ levels and blood loss volume, the Hungarian Trauma Society and leading trauma surgeons of the country will be contacted to initiate a national multicentre study.

Contributors Conceptualisation: PH, PJ; methodology: PH, PJ; statistical methods, sample size calculation: FR; writing—original draft preparation: PJ; writing review and editing: PH, EV, TH; stylistic and grammatical revision: TH; supervision: PH, LT, EV; funding acquisition: PH.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Our study is a protocol; thus, it does not contain research data. An ethics and dissemination plan for data sharing after the completion of the research has been included in the manuscript.

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