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Biomarkers In Prediction of Acute Mesenteric Ischaemia (BIPAMI): a prospective multicentre international study

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Abstract

Background:

Available evidence on biomarkers of acute mesenteric ischaemia (AMI) is limited due to unclear reporting of AMI mechanisms, localization and severity and random time points for biomarker sampling. The primary aim of this study was to identify a combination of biomarkers allowing accurate identification of transmural AMI, the primary determinant of outcome.

Methods:

In this prospective multicentre study, consecutive adult patients admitted with suspected AMI during one-year recruitment period were included before any AMI-directed intervention. Serial blood samples were collected during up to 72 hours from admission. Intestinal fatty acid-binding protein (iFABP), alpha-glutathione S-transferase (alpha- GST), ischaemia-modified albumin (IMA), D-lactate, signal peptide-CUB-EGF domain-containing protein 1 (SCUBE-1), lipopolysaccharide-binding protein (LBP), interleukin 6 (IL-6), procalcitonin, D-dimers, acid-base status, lactate, C-reactive protein (CRP), white blood cell count, creatinine and troponin were analysed. Biomarker accuracy was assessed by ROC analysis. Optimal biomarker combination was determined by exhaustive logistic regression modelling and cut-offs by multi-objective (Pareto) optimization. Internal validation was performed using bootstrap resampling.

Results:

Thirteen sites from six European countries included in total 256 patients, among them 86 without AMI, 158 with AMI and 12 with strangulating bowel obstruction identified only after study inclusion. No individual biomarker or biomarker combination accurately distinguished AMI from non-AMI. However, among patients with classical AMI (arterial occlusive, venous and non-occlusive mesenteric ischaemia [NOMI]), a combination of five biomarkers (IL-6, alpha-GST, CRP, SCUBE-1 and blood lactate) discriminated transmural (n=86) from non-transmural AMI (n=39) with AUC of 0.872 (0.802-0.942), positive likelihood ratio of 5.52 and negative likelihood ratio of 0.18. The presence of at least two of the following biomarkers above threshold values correctly classified 106/125 (85%) patients: IL-6 >130 ng/L, alpha-GST >2200 µg/L, CRP >180 mg/L, SCUBE-1 >22 µg/L, blood lactate >5.5 mmol/L.

Conclusions:

Although biomarkers could not reliably distinguish AMI from non-AMI in patients presenting with suspected disease, a five-biomarker panel identified transmural bowel injury with acceptable accuracy. These findings suggest that circulating biomarkers primarily reflect the extent of intestinal injury and necrosis rather than the presence of AMI itself, making them potentially valuable for identification of AMI patients with irreversible bowel damage requiring urgent intervention.

Study registration: NCT06212921 (Registration Date 19–01-2024).

Keywords: Mesenteric ischaemia, Biomarkers, Diagnosis, Blood Biomarkers, Vascular Diseases

Background

To date, no single biomarker has demonstrated sufficiently high diagnostic accuracy for AMI, as highlighted in several systematic reviews and meta-analyses [1-3]. Therefore, current research has shifted toward the evaluation of panels or combinations of biomarkers reflecting different pathophysiological pathways involved in intestinal ischaemia [4].

Among markers of enterocyte injury, intestinal fatty acid-binding protein (iFABP) has been the most extensively studied in humans, showing moderate diagnostic accuracy for AMI [5,6]. Markers of tissue injury such as alpha-glutathione S-transferase (alpha-GST) and ischemia-modified albumin (IMA) have also demonstrated moderate diagnostic performance; however, their clinical utility is limited by suboptimal specificity and heterogeneity across studies [7,8]. Inflammatory biomarkers, including interleukin-6 (IL-6) and procalcitonin (PCT), have shown relatively good performance in detecting AMI across different stages, reflecting systemic inflammatory responses associated with intestinal ischaemia [9,10].

Coagulation- and fibrinolysis-related markers, particularly D-dimers, have been associated with transmural bowel injury and may contribute to identify more advanced disease [11,12]. Other candidate biomarkers, such as signal peptide-CUB-EGF domain-containing protein 1 (SCUBE-1), a platelet-derived peptide driven by localized hypoxia in the mesenteric bed, have shown promise in experimental models of early intestinal ischaemia [13,14]. No studies of SCUBE-1 in AMI in humans are available.

Markers of intestinal barrier dysfunction, including D-lactate and lipopolysaccharide-binding protein (LBP), have demonstrated variable performance in human studies but may provide complementary information, particularly in reflecting bacterial translocation and intestinal ischaemia–reperfusion injury [6,15,16].

Importantly, previous studies have several methodological limitations, including reliance on single time-point measurements, limited evaluation of biomarker combinations, and lack of differentiation between transmural and non-transmural injury. Furthermore, some differences pathophysiological mechanism between AMI subtypes—arterial occlusive, mesenteric venous thrombosis, and non-occlusive

mesenteric ischaemia (NOMI)—may result in distinct biomarker profiles, which have not been adequately explored in clinical studies [1].
Taken together, these limitations provide a strong rationale for studies evaluating serial measurements and combinations of biomarkers reflecting multiple pathophysiological mechanisms, with the aim of improving early diagnosis of AMI and, critically, distinguishing between reversible and irreversible bowel injury.

Hypothesising that biomarkers more likely reflect the extent of bowel injury rather than vascular occlusion, we aimed to identify a combination of biomarkers that reliably detects transmural AMI [4]. As a secondary objective, we evaluated whether biomarker panels could discriminate patients with AMI from those in whom AMI was ultimately excluded.

Methods

This was a prospective multicentre study including adult patients with clinically suspected AMI, enrolled prior to initiation of any therapeutic intervention for AMI. The study period across all participating sites extended from October 14, 2024, to October 12, 2025.

Study population

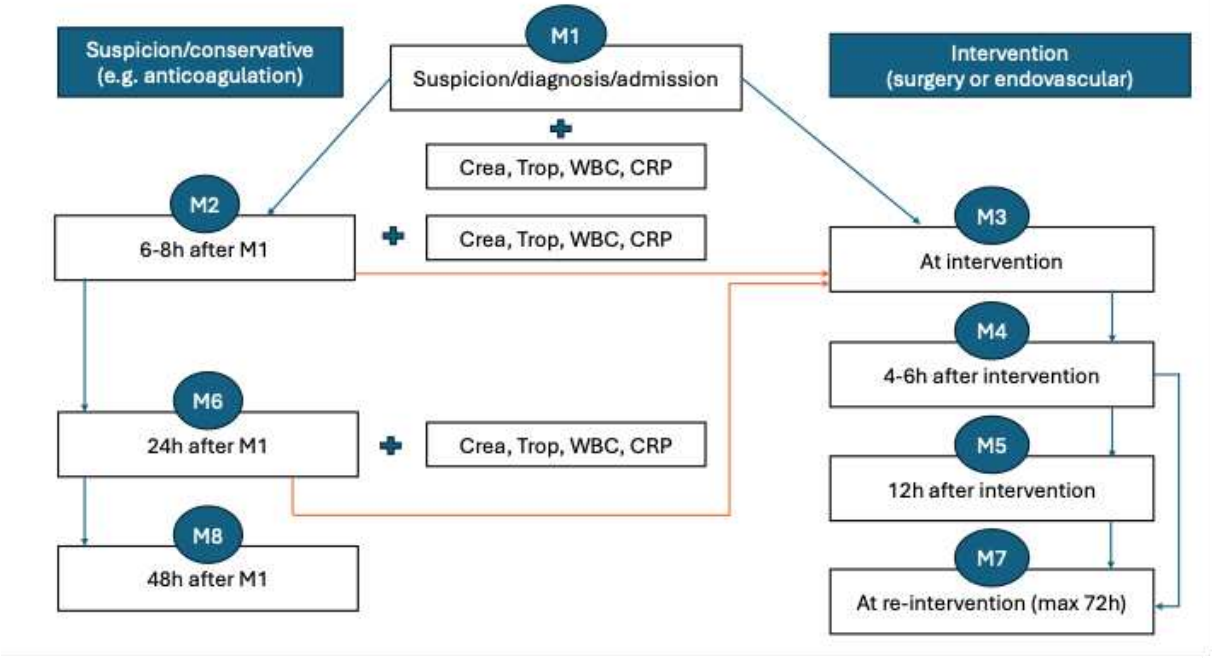
Patients were eligible if they were clinically suspected of having AMI. Patients were excluded if they were younger than 18 years, immediately allocated to palliation without any further diagnostic workup and active treatment, had a diagnosis of strangulating bowel obstruction (SBO) as a mechanism of ischaemic bowel damage confirmed before study inclusion, were referred from another hospital more than 8 hours after confirmation of the AMI diagnosis, were diagnosed having AMI at surgery without previous consideration, or declined participation in the study.

Biomarker sampling and analysis

Serial blood sampling was performed according to a predefined protocol, with repeated measurements up to 72 hours in patients undergoing intervention for AMI and up to 48 hours in patients managed without specific intervention (Figure 1) [4].

Measurement points M1 and M2 were applicable for all patients; M6 and M8 for patients without interventions; M3, M4 and M5 for patients with interventions; and M7 for patients with re-intervention.

Figure 1. Biomarker measurement schedule.



Legend: Patients with and without intervention are separated after measurement point 1 (M1). Patients staying without any AMI specific intervention (endovascular or open surgical) stay on the left side (AMI patients receiving only anticoagulation follow this schedule). Orange lines represent crossing to interventional schedule of sampling when intervention is performed later. Novel biomarkers and blood gas analyses were scheduled at measurement points marked with "M". Measurements of creatinine, troponin, white blood cell count and C-reactive protein were allowed to be adapted to clinical routine (e.g. measurements from morning samples).
Crea – creatinine; CRP – C-reactive protein; Trop – Troponin; WBC – white blood cell count.

A panel of candidate biomarkers reflecting intestinal injury, ischaemia, inflammation, and barrier dysfunction was chosen based on recent systematic reviews [1,17]. iFABP, LBP, PCT, IL-6, and D-dimers in plasma and alpha-GST, IMA, SCUBE-1, and D-lactate in serum [4] were analysed in central laboratory in Tartu, Estonia. Additionally, data on commonly measured laboratory markers at site was collected, including acid-base status and lactate in blood gas analyses, C-reactive protein, white blood cell count, troponin, and creatinine. Time points for measurements were identical to novel biomarkers for fast-changing biomarkers (blood gas analyses with lactate) but allowed to be shifted to fit common clinical routine for other biomarkers measured at site.

Detailed sampling, processing, storage and shipment procedures are provided in Additional file 1 and 2 (Supplemental Tables S1 and S2). Briefly, blood was collected at predefined study time points into lithium-heparin plasma tubes and serum separator tubes. Samples were centrifuged after collection, serum was allowed to clot prior to centrifugation, and plasma and serum were aliquoted into cryotubes and stored at −80 °C until analysis. Samples were shipped on dry ice to the central laboratory in Tartu, Estonia, where frozen storage was maintained until laboratory measurements.
All plasma and serum samples were aliquoted and stored at −80 °C until analysis. Only previously unthawed aliquots were analysed. Prior to analysis, samples were thawed, vortex-mixed, briefly centrifuged and kept at 2–8 °C for a maximum of 72 h. Laboratory personnel were blinded to diagnoses and sampling time points.
Plasma iFABP and LBP, and serum alpha-GST, IMA and SCUBE1 were measured using commercially available ELISA assays (Cusabio Technology LLC, Wuhan, China) according to the manufacturers' protocols. Sample-specific dilution optimisation was performed before study measurements. Serum D-lactate was measured using the Abcam D-Lactate colorimetric assay. Assay precision was assessed using calibration standards. Mean intra-assay coefficients of variation (CV) were 5.97% (iFABP), 7.63% (LBP), 9.66% (IMA), 7.89% (alpha-GST), 6.19% (SCUBE-1), and 6.89% (D-lactate). Mean inter-assay CVs, calculated from back-calculated standard concentrations across assay batches, were 14.5%, 16.9%, 21.1%, 27.4%, 14.0%, and 21.6%, respectively. Clinical chemistry measurements (PCT, IL-6 and D-dimers) were performed in the ISO 15189-accredited United Laboratories of Tartu University Hospital using Roche Cobas analytical platforms. Additional analytical details, dilution procedures and quality-control measures are presented in Additional file 1.

Objective

Primary objective of this study was to identify a combination of biomarkers capable of identifying patients with transmural AMI prior to initiation of any treatment of AMI. Under this objective, the following comparisons were evaluated:

- Transmural AMI vs. non-transmural AMI
- Transmural AMI vs. no AMI

Secondary objective was to identify combination of biomarkers that distinguishes patients with no AMI from patients with AMI (any stage, non-transmural or transmural).

Tertiary objectives included evaluation of performance of identified biomarker combinations across different AMI subtypes (arterial occlusive, mesenteric venous thrombosis, NOMI) and locations (small bowel, large bowel) and description of patterns of individual biomarkers before and after surgery/intervention vs. without surgical or interventional treatment.

Definitions, categorization and group allocation

AMI was categorized based on the extent of bowel wall injury (transmural vs non-transmural) and underlying aetiology. AMI subtypes, allocated by local investigators, included arterial occlusive, mesenteric venous thrombosis, and non-occlusive mesenteric ischaemia (NOMI), and these subtypes were pooled under "classical AMI". Since challenges in diagnosis and categorization of AMI are well-known [18], an online real-time steering committee consultation for inclusion and categorisation was established during the whole study period. Moreover, during database check, all cases were checked one-by-one by at least two steering committee members independently, to approve allocation in AMI subtype categories as well as categorization into transmural vs. non-transmural bowel damage.

In case of uncertainty, site investigator was asked to provide additional details, and final decision was reached based on consensus between the site and central data check team.

In cases where the mechanism of AMI remained unclear or there was another specific mechanism (e.g. aortic dissection, aortic aneurysm surgery, abdominal compartment syndrome), per protocol secondary assessment and discussion with steering committee was undertaken and, if possible, patient was re-categorized based on consensus.

Additional two categories were created after locking the database:

Probable AMI (*PROBAMI)* – clinical and CT-findings strongly suggesting AMI in cases where no surgery, endoscopy or autopsy was performed. Patients who deceased with final diagnosis of AMI were considered as presumed transmural AMI and patients who

survived were considered non-transmural. Cases with successful endovascular revascularization of arterial occlusive AMI without following surgery or endoscopy were considered being confirmed as non-transmural AMI and not categorized as PROBAMI.
SBO – SBO diagnosed secondarily at surgery after initial suspicion of AMI without a diagnosis of SBO, and respectively, per protocol inclusion of patient in the study [4]. These patients were initially categorized as no AMI, but in most cases had transmural necrosis, potentially leading to contamination of the "no AMI group" with transmural bowel necrosis. These patients were removed from the "no AMI" category and recategorized into new categories "transmural SBO" and "non-transmural SBO".
All cases of recategorizations are reported.
Definitions provided in study protocol with details of respective data collection are provided in Additional file 1.

No AMI – AMI was initially suspected but excluded after inclusion of the patient in the study. Patients with diagnosis of strangulation bowel obstruction (SBO) independent of the extent of bowel insult at screening were considered ineligible for the study inclusion and were excluded per study protocol.
Non-transmural (NTM) – reversible bowel insult was confirmed at explorative surgery without the need for bowel resection or at endoscopy confirming mucosal damage, or endovascular revascularization of arterial occlusion was performed successfully (recanalization confirmed and patient survived without secondary intervention) without visual confirmation of severity of bowel insult.
Transmural (TM) – diagnosis of irreversible bowel damage was confirmed at surgery or autopsy.
Small bowel (SB) – small bowel insult with or without large bowel involvement.
Large bowel (LB) – large bowel insult without small bowel involvement.

Sample size
A sample size of 250 patients in total was estimated to enrol 160 patients with confirmed AMI, including 40 patients with NOMI, and 90 patients with suspected but not confirmed AMI, with estimation of proportions based on the AMESI study [18]. No data on anticipated differences in biomarker levels were available to estimate sample size

based on respective power calculations. Recruitment period was planned to last 8-10 months but eventually prolonged to 12 months based on interim estimation of recruitment rate.

Statistics

Data is presented in number (%), median (interquartile range, IQR) or mean (standard deviation, SD) as appropriate, with Kolmogorov–Smirnov test used for normality testing. P-values were calculated using Wilcoxon rank-sum test and adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR) procedure. Comparisons of biomarker values in the following groups are presented:

- TM vs. NTM AMI
- TM vs. no AMI
- AMI (TM or NTM) vs. no AMI

Scores with combinations of different biomarkers were created separately for all these comparisons, using complete cases with all biomarker values available.

First, receiver operating characteristic (ROC) curve analysis was performed to identify the best cut-off for each biomarker based on maximum value of the Youden index [19] at the first available measurement point (M1 to M3).

For comparisons pooling all measurement points before (incl. no intervention throughout the study) any AMI-specific surgical or endovascular intervention (M1,M2,M3,M6,M8) the cut-offs were identified similarly, whereas the maximum value from any time point for each biomarker defined categorisation for each patient to avoid several scores calculated for one individual.

Thereafter, best biomarker combination for a score was identified by exhaustive logistic regression modelling. Best cut-offs for these biomarkers were determined using multi-objective (Pareto) optimization by simultaneously maximizing the positive and minimizing the negative likelihood ratio. Positive likelihood ratio (LR+) >5 and negative likelihood ratio (LR-) <0.2 were per protocol considered to confirm moderate diagnostic accuracy [4].

Best scores were defined by LR+ and LR-, and additionally described by area under the curve (AUC), sensitivity, specificity and accuracy. Levels of AUC interpretation were 0.50

– 0.60 = no discrimination, 0.60 – 0.70 = poor, 0.70 – 0.80 = fair, 0.80 – 0.90 = good, 0.90 – 1.0 = excellent. Bootstrap internal validation with 200 resamples was performed. Details on development of the score are presented in Additional file 2.

The best scores developed on complete-case cohort were thereafter applied to the complete cohort of "classical" AMI, full cohort (imputing 0 points for missing biomarker values), separately for each subtype of AMI and separately for localization of ischaemia in small (with or without involvement of large bowel) and large bowel. The cohort of classical AMI (arterial occlusive, venous, NOMI) excluding unclear AMI and SBO was considered of primary interest.

Sensitivity analyses included applying score to all cases with imputation of 0 points for missing biomarker values; cases excluding PROBAMI and SBO; and pooling time points before/without any AMI-specific intervention (M1,M2,M3,M6,M8).

A "leave-one-biomarker-out" analysis was performed to assess each biomarker's contribution to the score by evaluating score performance omitting biomarkers one by one.

Dynamics in time of novel biomarkers was explored with visualization of

- no AMI vs. NTM vs. TM
- no AMI vs. TM small bowel (TMSB) vs. TM large bowel (TMLB).

For this visualization, the following pooled time categories were constructed to facilitate assessment:

- before surgical or endovascular intervention (M1-3)
- after intervention (M4, M5 in the intervention group) paralleled by comparable time point in the no intervention group (M6)
- at re-intervention (M7 in the re-intervention group) paralleled by M8 in no intervention group

R Statistical Software 4.3.2 (R Core Team, Vienna, Austria, 2023) was used for statistical analyses.

Ethics

Deferred informed consent was obtained from each patient or his/her next of kin. In case of next of kin consent, the patient was approached later to confirm. Deferred

informed consent was necessary to initiate blood sampling before treatment in this emergency. The study did not have any influence on treatment decisions; the only study intervention was blood sampling. For patients who deceased before an informed consent was obtained, either patient relatives were asked for consent or local ethics committee was approached and asked for inclusion of these patients without approaching patient's relatives to avoid additional emotional burden.
Primary ethical approval was provided by the University of Tartu Ethics Committee on January 26th, 2024 (approval number 386/T-11). Each participating site obtained local ethics committee approval in accordance with institutional and national regulations.

Results

Thirteen sites from six countries (Italy = 6, Estonia = 2, Lithuania = 2, Sweden, Switzerland and Moldova) included in total 256 patients (Supplemental Figure S1 and Supplemental Table S3 in Additional file 2).

Description of study cohort

Baseline characteristics of study cohort are presented in Table 1.

Table 1. Baseline characteristics and hospital mortality of patients with AMI compared to those with suspected but not confirmed AMI.

	All patients N=256	**NoAMI N=86**	**AMI [#] N=170**	**NoAMI vs. AMI**
Ethnicity (n=256)				
African-American	1 (0.4)		1 (0.6)	
Arabian	2 (0.8)		2 (1.2)	0.726
Asian	0			
Caucasian	247 (96.5)	85 (98.8)	162 (95.3)	
Other	6 (2.3)	1 (1.1)	5 (3.0)	
Gender (M) (n=256)	135 (52.7)	44 (51.2)	91 (53.5)	0.791
Age (years) (n=256) median (IQR)	75 (66-82)	75 (65-81)	76 (66-82)	0.509
Weight (kg) (n=256) median (IQR)	75 (63-87)	71.4 (62.1–88)	75.0 (65–87)	0.612
Height (cm) (n=256) median (IQR)	168 (162-175)	169 (163–175)	168 (162–175)	0.519
BMI (n=256) median (IQR)	26.2 (22.4-30.3)	24.9 (22.2–30.1)	26.4 (22.5–30.4)	0.526
Anticoagulant drugs (n=256)				
No	182 (71.1)	62 (72.1)	120 (70.6%)	0.681
Yes	70 (27.3)	22 (25.6)	48 (28.2%)	
No information	4 (1.6)	2 (2.3)	2 (1.2)	
Antiplatelet drugs (n=256)				
No	184 (71.9)	57 (66.3)	127 (74.7)	0.363
Yes	66 (25.8)	27 (31.4)	39 (22.9)	
No information	6 (2.3)	2 (2.3)	4 (2.4)	
Patient location (n=256)				0.207

Surgical ward	39 (15.2)	10 (11.6)	29 (17.1)	
Medical ward	18 (7.0)	10 (11.6)	8 (4.7)	
ICU	59 (23.0)	22 (25.6)	37 (21.8)	
IMC/HDU	16 (6.2)	6 (7.0)	10 (5.9)	
ED	119 (46.5)	38 (44.2)	81 (47.6)	
Other	5 (2.0)		5 (2.9)	
Referral from another hospital (n=256)	49 (19.1)	8 (9.3%)	41 (24.1)	0.004
Sudden onset of symptoms with clear time point (+-2 hours) (n=256)	109 (42.6)	29 (33.7%)	80 (47.1)	0.045
Time from symptoms to suspicion of AMI (h) median (IQR) (n=109)	8.2 (4-20)	10 (3.50-19.5)	7.4 (4.4-21.0)	0.688
Approximate time from symptoms to suspicion of AMI (n=147)*				
6-12h	43 (16.8)	17 (29.8)	26 (28.9)	
12-24h	32 (12.5)	17 (29.8)	15 (16.7)	
24-48h	22 (8.6)	8 (13.8)	14 (15.6)	
>48h	36 (14.1)	11 (19.3)	25 (27.8)	
Not known	12 (4.7)	4 (7.0)	8 (8.9)	
No symptoms of AMI	2 (0.8)	0 (0.0)	2 (2.2)	0.436
APACHE II (n=254) median (IQR)	14 (10-20)	14.0 (7.0–20.0)	14.0 (10.0–20.0)	0.134
SOFA total (n=256) median (IQR)	2 (1-6)	2.5 (1.0–6.0)	2.0 (1.0–6.0)	0.900
MV at suspicion/diagnosis of AMI (n=256)	46 (18.0)	15 (17.4)	31 (18.2)	1.000
Vasopressors (n=256)	63 (24.6)	20 (23.3)	43 (25.3)	0.761
Hospital mortality n (%) (n=256)	104 (40.6)	20 (23.3)	84 (49.4)	<0.001

Legend: AMI – acute mesenteric ischaemia, BMI – body mass index, ED – emergency department, ICU – intensive care unit, IMC/HDU – intermediate care/high dependency unit, APACHE II – Acute Physiology and Chronic Health Evaluation II score, SOFA – Sequential Organ Failure Assessment Score, MV – mechanical ventilation, IQR – interquartile range.
(n=x) after the name of the variable always indicates number of patients included in the analysis for this specific variable. Data are presented in number of patients (%) if not stated otherwise.
[#] includes 12 patients with bowel insult from SBO; * only patients who had no sudden onset of symptoms.

Prevalence and outcome in patients categorized by AMI and its subtypes, different severity (NTM/TM) and localization of AMI is presented in Supplemental Table S4 (Additional file 2). The same table also presents number of cases where initial categorization was changed after profound discussion reaching final consensus between the site and data check team. The most important proportion of changes was performed in patients with SBO diagnosed during laparotomy. In most of these patients SBO led to transmural bowel necrosis (n= 9/12). Next to creation of the “SBO group” (9 patients initially categorized as no AMI and one patient as arterial embolism were recategorized as SBO, 2 patients were initially categorised as AMI SBO), 9 patients initially considered as no AMI were recategorized to AMI, six of them into NOMI and three into arterial occlusive AMI. Recategorization of AMI subtype was undertaken in six patients, with “other/unclear AMI” recategorized into NOMI in three cases, into embolic and thrombotic arterial AMI in one case each and one NOMI case recategorized into venous AMI. Remaining patients (n=6) in the “other group” had aortic or superior

mesenteric artery dissection (n=5) or tumour with mesenteric vascular compression (n=1) reported as mechanism of bowel ischaemia.

Biomarker values

In total, biomarkers were available for M1 in 237, M2 in 144, M3 in 89, M4 in 108, M5 in 100, M6 in 111; M7 in 41 and M8 in 58 patients. In 19 patients, M1 sample was missing. In these cases, available M2 or M3 was considered M1 in respective analysis. Finally, one sample for M1 was lost for analysis.

Repeat laboratory analyses were required for 6 of 826 LBP samples, 12 of 826 iFABP samples, 14 of 827 IMA samples, 11 of 827 SCUBE1 samples, and 92 of 827 alpha-GST samples.

Median values of all measured biomarkers at all available time points are presented in Supplemental Table S5 (Additional file 2).

Areas under the curve and optimal cut-offs for biomarkers at the first measurement point for each predefined comparison are presented in Table 2 (biomarkers used in final best combinations) and Supplemental Table S6 (remaining biomarkers). Corresponding analyses using pooled measurements (M1, M2, M3, M6 and M8) are presented in Supplemental Table S7. None of the individual biomarkers met the predefined criteria for moderate diagnostic accuracy in any comparisons at any time points.

Discrimination of AMI from no AMI was limited for all biomarkers, with AUC values ranging from 0.48 to 0.67. In contrast, several biomarkers demonstrated substantially better performance for discrimination of TM versus NTM AMI. IL-6 showed the highest discriminatory ability (AUC 0.81, 95% CI 0.74-0.88), followed by alpha-GST (AUC 0.78, 95% CI 0.70-0.86), whereas CRP, lactate and SCUBE-1 achieved AUC values around 0.70. While inflammatory markers showed high sensitivity but low specificity, alpha-GST and SCUBE-1 showed the opposite pattern. The similarity of results after exclusion of PROBAMI and SBO cases suggests that the observed associations were not driven by uncertainty in AMI classification or by inclusion of bowel necrosis caused by alternative mechanisms. Overall, biomarker performance was consistently superior for discrimination of transmural bowel injury than for diagnosis of AMI itself, supporting the robustness of the findings across different case definitions.

Table 2. Areas under the curve and optimal cut-offs for biomarkers based on Youden index, for biomarkers used in final scores assessed at timepoint M1.

Dataset	Biomarker	Comparison	AUC (95% CI)	Cutoff	Sensitivity (95% CI)	Specificity (95% CI)	LR+ (95% CI)	LR- (95% CI)
Full	Alpha-GST	AMI vs No AMI	0.610 (0.535–0.685)	2217	0.469 (0.390–0.549)	0.721 (0.614–0.812)	1.68 (1.15–2.45)	0.74 (0.61–0.90)
No PROBAMI + SBO	Alpha-GST	AMI vs No AMI	0.602 (0.524–0.681)	2217	0.468 (0.379–0.559)	0.721 (0.614–0.812)	1.68 (1.14–2.47)	0.74 (0.60–0.91)
Full	Alpha-GST	TM vs NTM	0.778 (0.699–0.857)	1830	0.694 (0.599–0.778)	0.804 (0.669–0.902)	3.54 (2.00–6.25)	0.38 (0.28–0.52)
No PROBAMI + SBO	Alpha-GST	TM vs NTM	0.754 (0.661–0.848)	1830	0.702 (0.593–0.797)	0.786 (0.632–0.897)	3.28 (1.81–5.95)	0.38 (0.26–0.55)
Full	Alpha-GST	TM vs No AMI	0.691 (0.614–0.767)	2217	0.613 (0.515–0.704)	0.721 (0.614–0.812)	2.20 (1.52–3.18)	0.54 (0.41–0.70)
No PROBAMI + SBO	Alpha-GST	TM vs No AMI	0.682 (0.601–0.763)	1828	0.702 (0.593–0.797)	0.628 (0.517–0.730)	1.89 (1.39–2.57)	0.47 (0.33–0.68)
Full	Creatinine	AMI vs No AMI	0.486 (0.407–0.564)	117	0.503 (0.424-0.582)	0.548 (0.435-0.657)	1.11 (0.84-1.47)	0.91 (0.71-1.16)
No PROBAMI + SBO	Creatinine	AMI vs No AMI	0.480 (0.398-0.561)	104	0.583 (0.492-0.670)	0.464 (0.355-0.576)	1.09 (0.85-1.39)	0.90 (0.66-1.22)
Full	Creatinine	TM vs NTM	0.635 (0.547-0.723)	137	0.473 (0.377-0.570)	0.830 (0.702-0.919)	2.78 (1.49-5.21)	0.64 (0.51-0.79)
No PROBAMI + SBO	Creatinine	TM vs NTM	0.607 (0.506-0.707)	137	0.446 (0.337-0.559)	0.818 (0.673-0.918)	2.45 (1.25-4.80)	0.68 (0.53-0.86)
Full	Creatinine	TM vs No AMI	0.525 (0.441-0.608)	117	0.582 (0.484-0.675)	0.548 (0.435-0.657)	1.29 (0.97-1.71)	0.76 (0.57-1.02)
No PROBAMI + SBO	Creatinine	TM vs No AMI	0.510 (0.422-0.599)	104	0.651 (0.538-0.752)	0.464 (0.355-0.576)	1.21 (0.94-1.57)	0.75 (0.52-1.09)
Full	CRP	AMI vs No AMI	0.584 (0.507–0.661)	7.85	0.851 (0.786–0.902)	0.313 (0.216–0.424)	1.24 (1.06–1.45)	0.48 (0.29–0.77)
No PROBAMI + SBO	CRP	AMI vs No AMI	0.587 (0.507–0.666)	14.4	0.772 (0.689–0.841)	0.386 (0.281–0.499)	1.26 (1.03–1.53)	0.59 (0.39–0.90)
Full	CRP	TM vs NTM	0.712 (0.631–0.793)	89.6	0.546 (0.448–0.642)	0.792 (0.659–0.892)	2.63 (1.51–4.58)	0.57 (0.45–0.73)
No PROBAMI + SBO	CRP	TM vs NTM	0.707 (0.616–0.799)	90.0	0.554 (0.441–0.663)	0.773 (0.622–0.885)	2.44 (1.37–4.35)	0.58 (0.43–0.77)
Full	CRP	TM vs No AMI	0.648 (0.570–0.727)	179	0.398 (0.305–0.497)	0.843 (0.747–0.914)	2.54 (1.47–4.41)	0.71 (0.60–0.85)
No PROBAMI + SBO	CRP	TM vs No AMI	0.654 (0.571–0.737)	179	0.410 (0.303–0.523)	0.843 (0.747–0.914)	2.62 (1.49–4.59)	0.70 (0.57–0.86)
Full	iFABP	AMI vs No AMI	0.639 (0.569–0.709)	0.69	0.407 (0.331–0.487)	0.849 (0.755–0.917)	2.70 (1.58–4.60)	0.70 (0.60–0.82)
No PROBAMI + SBO	iFABP	AMI vs No AMI	0.633 (0.559–0.708)	0.69	0.389 (0.303–0.480)	0.849 (0.755–0.917)	2.57 (1.49–4.44)	0.72 (0.61–0.85)
Full	iFABP	TM vs NTM	0.582 (0.491–0.673)	0.21	0.640 (0.543–0.729)	0.569 (0.422–0.707)	1.48 (1.05–2.09)	0.63 (0.45–0.89)
No PROBAMI + SBO	iFABP	TM vs NTM	0.567 (0.464–0.670)	0.21	0.631 (0.519–0.734)	0.548 (0.387–0.702)	1.39 (0.96–2.02)	0.67 (0.46–1.00)
Full	iFABP	TM vs No AMI	0.659 (0.583–0.734)	0.69	0.468 (0.373–0.566)	0.849 (0.755–0.917)	3.10 (1.81–5.31)	0.63 (0.51–0.76)

No PROBAMI + SBO	iFABP	TM vs No AMI	0.649 (0.566–0.732)	0.69	0.440 (0.332–0.553)	0.849 (0.755–0.917)	2.91 (1.67–5.08)	0.66 (0.53–0.81)
Full	IL-6	AMI vs No AMI	0.670 (0.599–0.741)	65.9	0.796 (0.726–0.855)	0.523 (0.413–0.632)	1.67 (1.32–2.11)	0.39 (0.27–0.56)
No PROBAMI + SBO	IL-6	AMI vs No AMI	0.672 (0.597–0.747)	65.9	0.817 (0.739–0.881)	0.523 (0.413–0.632)	1.71 (1.35–2.17)	0.35 (0.23–0.53)
Full	IL-6	TM vs NTM	0.810 (0.739–0.881)	125	0.847 (0.766–0.908)	0.647 (0.501–0.776)	2.40 (1.64–3.51)	0.24 (0.15–0.38)
No PROBAMI + SBO	IL-6	TM vs NTM	0.801 (0.719–0.883)	125	0.857 (0.764–0.924)	0.643 (0.480–0.784)	2.40 (1.58–3.63)	0.22 (0.13–0.39)
Full	IL-6	TM vs No AMI	0.761 (0.692–0.829)	65.9	0.928 (0.863–0.968)	0.523 (0.413–0.632)	1.95 (1.55–2.44)	0.14 (0.07–0.28)
No PROBAMI + SBO	IL-6	TM vs No AMI	0.759 (0.687–0.831)	65.9	0.952 (0.883–0.987)	0.523 (0.413–0.632)	2.00 (1.59–2.51)	0.09 (0.03–0.24)
Full	Lactate	AMI vs No AMI	0.554 (0.477–0.631)	4.35	0.344 (0.271–0.423)	0.785 (0.678–0.869)	1.60 (1.00–2.56)	0.84 (0.71–0.98)
No PROBAMI + SBO	Lactate	AMI vs No AMI	0.543 (0.462–0.624)	2.35	0.563 (0.472–0.652)	0.570 (0.453–0.681)	1.31 (0.97–1.76)	0.77 (0.58–1.01)
Full	Lactate	TM vs NTM	0.697 (0.614–0.780)	2.58	0.651 (0.552–0.741)	0.704 (0.564–0.820)	2.20 (1.42–3.39)	0.50 (0.36–0.68)
No PROBAMI + SBO	Lactate	TM vs NTM	0.661 (0.565–0.757)	2.58	0.642 (0.528–0.746)	0.667 (0.510–0.800)	1.93 (1.24–3.00)	0.54 (0.38–0.77)
Full	Lactate	TM vs No AMI	0.618 (0.536–0.700)	2.65	0.623 (0.523–0.715)	0.620 (0.504–0.727)	1.64 (1.19–2.25)	0.61 (0.45–0.82)
No PROBAMI + SBO	Lactate	TM vs No AMI	0.596 (0.507–0.685)	2.65	0.605 (0.490–0.712)	0.620 (0.504–0.727)	1.59 (1.14–2.22)	0.64 (0.46–0.88)
Full	SCUBE-1	AMI vs No AMI	0.563 (0.489–0.637)	18.7	0.420 (0.343–0.500)	0.721 (0.614–0.812)	1.50 (1.02–2.21)	0.80 (0.67–0.97)
No PROBAMI + SBO	SCUBE-1	AMI vs No AMI	0.544 (0.465–0.623)	18.7	0.381 (0.296–0.472)	0.721 (0.614–0.812)	1.37 (0.91–2.05)	0.86 (0.71–1.04)
Full	SCUBE-1	TM vs NTM	0.707 (0.621–0.793)	19.0	0.532 (0.434–0.627)	0.843 (0.714–0.930)	3.39 (1.75–6.55)	0.56 (0.44–0.70)
No PROBAMI + SBO	SCUBE-1	TM vs NTM	0.641 (0.538–0.744)	19.0	0.464 (0.355–0.576)	0.810 (0.659–0.914)	2.44 (1.25–4.74)	0.66 (0.52–0.85)
Full	SCUBE-1	TM vs No AMI	0.631 (0.554–0.709)	18.8	0.532 (0.434–0.627)	0.721 (0.614–0.812)	1.90 (1.30–2.79)	0.65 (0.51–0.82)
No PROBAMI + SBO	SCUBE-1	TM vs No AMI	0.592 (0.506–0.678)	18.8	0.464 (0.355–0.576)	0.721 (0.614–0.812)	1.66 (1.10–2.51)	0.74 (0.59–0.94)

Legend: AMI – acute mesenteric ischaemia, PROBAMI – probable AMI, SBO – strangulating bowel obstruction, TM – transmural bowel damage, NTM – non-transmural bowel damage, alpha-GST – alpha-glutathione S-transferase (µg/L), CRP – C-reactive protein (mg/L), iFABP – intestinal fatty acid-binding protein (µg /L), IL-6 – interleukin-6 (ng/L), lactate (mmol/L), SCUBE-1 – signal peptide-CUB-EGF domain-containing protein 1 (µg/L).
Datasets: Full – patients with PROBAMI and SBO included in analysis; No PROBAMI+SBO – patients with PROBAMI and SBO excluded from analysis.

Predictive models with combinations of biomarkers

All scores presented in the main manuscript were developed and applied in complete-case cohorts comprising patients with all biomarkers available. The complete-case full cohort compiled of patients with all mechanisms of bowel insult and level of diagnostic certainty.

The primary focus was set on discrimination between transmural and non-transmural injury within the cohort of classical AMI (arterial occlusive, venous and NOMI) using the first measurement point (M1) (Table 3). Complementary analyses were performed after exclusion of PROBAMI cases and in the complete-case full cohort used for score development. The best -performing score discriminating transmural from non-transmural classical AMI combined IL-6, alpha-GST, CRP, SCUBE-1 and lactate. Using a threshold of at least two positive biomarkers, the score discriminated transmural from non-transmural classical AMI with an AUC of 0.872 (95% CI 0.802-0.942), sensitivity of 0.849, specificity of 0.846, LR+ of 5.52 (95% CI 2.63-11.58) and LR− of 0.18 (95% CI 0.11-0.30), fulfilling the predefined criteria for moderate diagnostic accuracy (Table 3). Similar performance was observed after exclusion of PROBAMI cases, indicating that the results were not driven by diagnostic uncertainty (Table 3). Bootstrap validation demonstrated moderate optimism, with an optimism-corrected AUC of 0.828. Although the optimism-corrected likelihood ratios no longer fulfilled predefined criteria for moderate diagnostic accuracy, overall discrimination remained good and consistently exceeded that achieved by any individual biomarker. A “leave-one-biomarker-out“ analysis in which the score's performance was assessed omitting biomarkers one at the time, is presented in Supplemental Table S8.

Sensitivity analyses in the entire cohort (assigning 0 score points for unavailable biomarkers), after exclusion of PROBAMI and SBO cases, and using pooled time points before/without intervention (M1,M2,M3,M6,M8) yielded broadly similar results and did not identify a superior-performing model (Supplemental Tables S9 and S10).

Table 3. Performance of the final score for discrimination of transmural intestinal damage (TM) from non-transmural intestinal insult (NTM) at M1, applied in classical AMI (arterial occlusive AMI, venous occlusive AMI, NOMI), classical AMI excluding PROBAMI and in full cohort (used for development of the score, including unclear and other mechanisms of bowel ischaemia); all presented also as optimism-corrected (internal validation using bootstrap resampling).

Biomarker	**Cut-off value**					
Alpha-GST (µg/L)	>2200					
CRP (mg/L)	>180					
IL-6 (ng/L)	>130					
Lactate (mmol/L)	>5.5					
SCUBE-1 (µg/L)	>22					
Score cut-off	>1 (>=2)					
	Performance of the score:					
	Classical AMI		**Classical AMI excluding PROBAMI**		**Complete case full cohort**	
	Apparent	Optimism corrected	Apparent	Optimism corrected	Apparent	Optimism corrected
N of patients	86 vs 39	86 vs 39	70 vs 36	70 vs 36	92 vs 47	92 vs 47
AUC (95% CI)	0.872 (0.802-0.942)	0.828 (0.766–0.899)	0.865 (0.788–0.942)	0.819 (0.749–0.895)	0.872 (0.808-0.935)	0.824 (0.758–0.896)
Sensitivity (95% CI)	0.849 (0.755-0.917)	0.802 (0.710–0.875)	0.829 (0.720–0.908)	0.778 (0.682–0.862)	0.826 (0.733-0.897)	0.776 (0.694–0.848)
Specificity (95% CI)	0.846 (0.695-0.941)	0.760 (0.655–0.865)	0.861 (0.705–0.953)	0.776 (0.656–0.881)	0.872 (0.743-0.952)	0.783 (0.679–0.875)
LR+ (95% CI)	5.52 (2.63-11.58)	3.03 (2.63–7.84)	5.97 (2.63–13.55)	3.25 (2.47–9.39)	6.47 (3.05-13.74)	3.56 (2.81–5.20)
LR- (95% CI)	0.18 (0.11-0.30)	0.27 (0.08–0.49)	0.20 (0.12–0.34)	0.31 (0.08–0.52)	0.20 (0.13-0.32)	0.30 (0.13–0.49)
Accuracy	0.85 (0.77-0.91)	0.788 (0.73–0.85)	0.840 (0.76–0.90)	0.78 (0.71–0.85)	0.84 (0.77-0.90)	0.78 (0.72–0.84)

Legend: AMI – acute mesenteric ischaemia, NOMI – non-occlusive mesenteric ischaemia, PROBAMI – probable AMI, IL-6 - interleukin-6, SCUBE-1 - signal peptide-CUB-EGF domain-containing protein 1, Alpha-GST – alpha-glutathione S-transferase, CRP – C-reactive protein, AUC – area under the receiver operating characteristic curve, LR+ – positive likelihood ratio, LR- – negative likelihood ratio

No biomarker combination achieved the predefined criteria for moderate diagnostic accuracy for discrimination of either TM AMI from no AMI or AMI vs. no AMI. The best-performing scores for these two comparisons were nearly identical, differing only in the optimal CRP cut-off (200 mg/L for TM vs. no AMI, and 170 mg/L for AMI vs. no AMI). Given the minimal difference in score composition and diagnostic performance, the same score was retained for presentation in both analyses (Table 4). Accordingly, the final score included iFABP >0.7 µg/L, creatinine >110 µmol/L and CRP >170 mg/L, with at least two positive biomarkers required for a positive result. However, acceptable discrimination was not achieved (Table 4). These findings contrast with the substantially better performance observed for discrimination of transmural versus non-transmural injury and further support the concept that circulating biomarkers are more strongly associated with the severity of bowel injury than with the presence of AMI itself.

Table 4. Performance of the final scores for discrimination of AMI (any severity) from no AMI, and TM from no AMI at M1, applied in classical AMI and in complete case full cohort (used for development of the score, including unclear and other mechanisms of bowel ischaemia).

Biomarker	**Cut-off value**			
Creatinine (µmol/L)	>110			
CRP (mg/L)	>170			
I-FABP (µg/L)	>0.7			
Score cut-off	>1 (>=2)			
Performance of the score:				
	AMI vs no AMI		**TM vs no AMI**	
	Classical AMI	Full cohort	Classical AMI	Full cohort
N of patients	125 vs 76	139 vs 76	86 vs 76	92 vs 76
AUC (95% CI)	0.626 (0.553-0.699)	0.614 (0.542-0.685)	0.698 (0.621-0.774)	0.690 (0.615-0.766)
Sensitivity (95% CI)	0.456 (0.367-0.547)	0.432 (0.348-0.518)	0.558 (0.447-0.665)	0.543 (0.436-0.648)
Specificity (95% CI)	0.789 (0.681-0.875)	0.789 (0.681-0.875)	0.789 (0.681-0.875)	0.789 (0.681-0.875)
LR+ (95% CI)	2.17 (1.35-3.49)	2.05 (1.27-3.30)	2.65 (1.65-4.26)	2.58 (1.61-4.15)
LR- (95% CI)	0.69 (0.57-0.84)	0.72 (0.60-0.87)	0.56 (0.43-0.73)	0.58 (0.45-0.74)
Accuracy	0.58 (0.51-0.65)	0.56 (0.49-0.63)	0.67 (0.59-0.74)	0.65 (0.58-0.73)

Legend: AMI – acute mesenteric ischaemia, TM – transmural bowel damage, CRP – C-reactive protein, iFABP - intestinal fatty acid-binding protein AUC – area under the receiver operating characteristic curve, LR+ - positive likelihood ratio, LR- - negative likelihood ratio

Subgroup analyses

The best identified combination discriminating TM vs NTM was applied to each AMI subtype and to small bowel and only large bowel insult separately. Overall, the score maintained good discriminative ability across most subgroups, suggesting that its performance is not restricted to a specific AMI aetiology (Table 5).

Among patients with occlusive arterial AMI, the score demonstrated good discrimination between transmural and non-transmural bowel damage, with an AUC of 0.812 (95% CI 0.709-0.914), sensitivity of 76.0%, specificity of 82.8%, LR+ of 4.41 and LR− of 0.29. These findings indicate a clinically relevant ability to identify transmural bowel injury in the most common AMI subtype.

When analysed according to the anatomical extent of bowel involvement, the score performed particularly well in patients with small-bowel AMI after exclusion of strangulating bowel obstruction (SBO) cases. In this subgroup, the AUC was 0.878 (95% CI 0.803-0.953), with sensitivity of 82.8%, specificity of 88.6%, LR+ of 7.24 and LR− of 0.19. These findings indicate strong discrimination of transmural bowel injury in small-bowel ischaemia, a classic case of AMI in the territory of the superior mesenteric artery. Our results in subtypes with smaller numbers of patients (Table 5) are presented as exploratory.

Table 5. Performance of the TM AMI vs. NTM AMI score in different AMI subtypes and separately in small bowel (with or without large bowel involvement) and in large bowel (without small bowel involvement). Complete case (all biomarkers available) analysis is presented.

Biomarker		**Cut-off value**				
Alpha-GST (µg/L)		>2200				
CRP (mg/L)		>180				
IL-6 (ng/L)		>130				
Lactate (mmol/L)		>5.5				
SCUBE-1 (µg/L)		>22				
Score cut-off		>1 (>=2)				
	Performance of the score:					
		Comparison				
	Occlusive arterial AMI	**Occlusive venous AMI**	**NOMI**	**SBO**	**SB, excluding SBO**	**LB, excluding SBO**
N of patients	50 vs 29	4 vs 3	32 vs 7	5 vs 3	58 vs 35	16 vs 4
AUC (95% CI)	0.812 (0.709-0.914)	1.000 (1.000-1.000)	0.978 (0.941-1.000)	0.767 (0.421-1.000)	0.878 (0.803-0.953)	0.758 (0.394-1.000)
Sensitivity (95% CI)	0.760 (0.618-0.869)	1.000 (0.398-1.000)	0.969 (0.838-0.999)	0.600 (0.147-0.947)	0.828 (0.706-0.914)	0.812 (0.544-0.960)
Specificity (95% CI)	0.828 (0.642-0.942)	1.000 (0.292-1.000)	0.857 (0.421-0.996)	1.000 (0.292-1.000)	0.886 (0.733-0.968)	0.750 (0.194-0.994)
LR+ (95% CI)	4.41 (1.96-9.93)	Infinity	6.78 (1.10-41.67)	Infinity	7.24 (2.86-18.35)	3.25 (0.59-18.03)
LR- (95% CI)	0.29 (0.17-0.49)	0.00 (0.00-NaN)	0.04 (0.01-0.26)	0.40 (0.14-1.17)	0.19 (0.11-0.35)	0.25 (0.08-0.80)
Accuracy	0.78 (0.68-0.87)	1.00 (0.59-1.00)	0.95 (0.83-0.99)	0.75 (0.35-0.97)	0.85 (0.76-0.92)	0.80 (0.56-0.94)

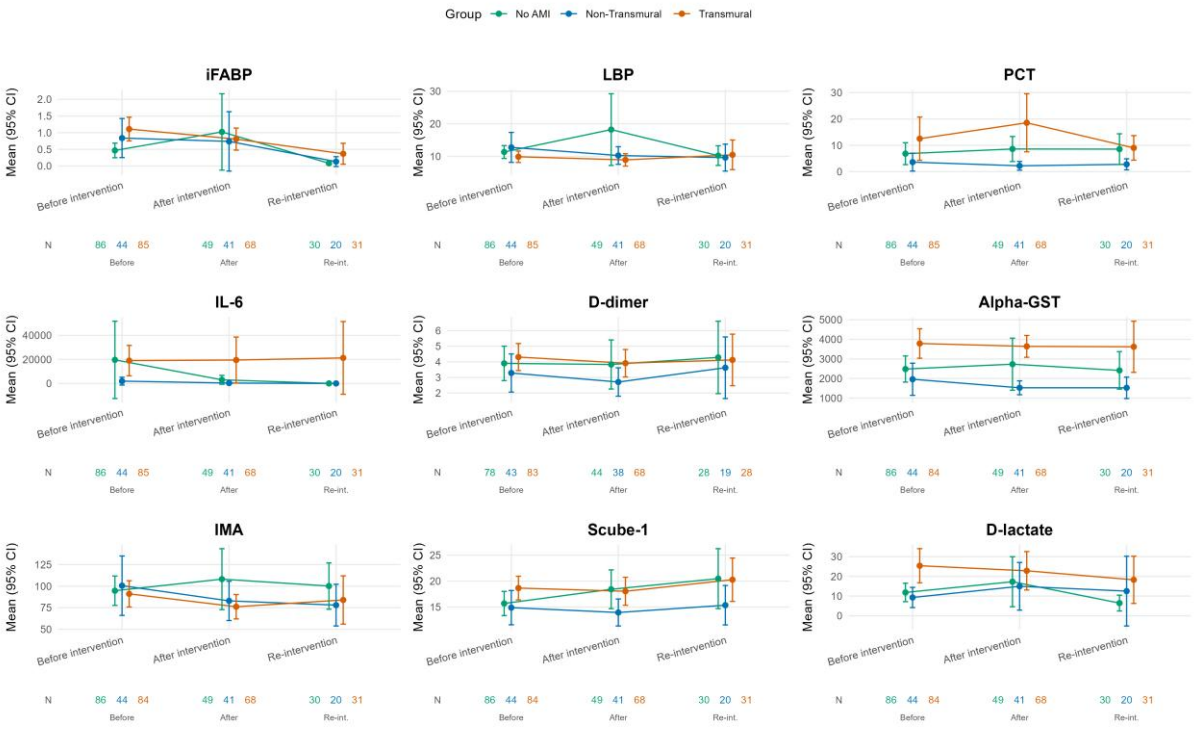
Legend: AMI – acute mesenteric ischaemia, NOMI – non-occlusive mesenteric ischaemia, LB – large bowel AMI without small bowel involvement, SB – small bowel AMI with or without large bowel involvement, SBO – strangulating bowel obstruction, TM – transmural bowel damage, IL-6 - interleukin-6, SCUBE-1 - signal peptide-CUB-EGF domain-containing protein 1, Alpha-GST – alpha-glutathione S-transferase, CRP – C-reactive protein, AUC – area under the receiver operating characteristic curve, LR+ - positive likelihood ratio, LR- - negative likelihood ratio

Dynamics of biomarkers in time

To explore dynamics of biomarkers we visualized the values pooled into time periods before and after intervention, and at re-intervention, coupled with comparable time-based measurement points in patients without AMI or without interventions for non-transmural damage of other than arterial occlusive aetiology.

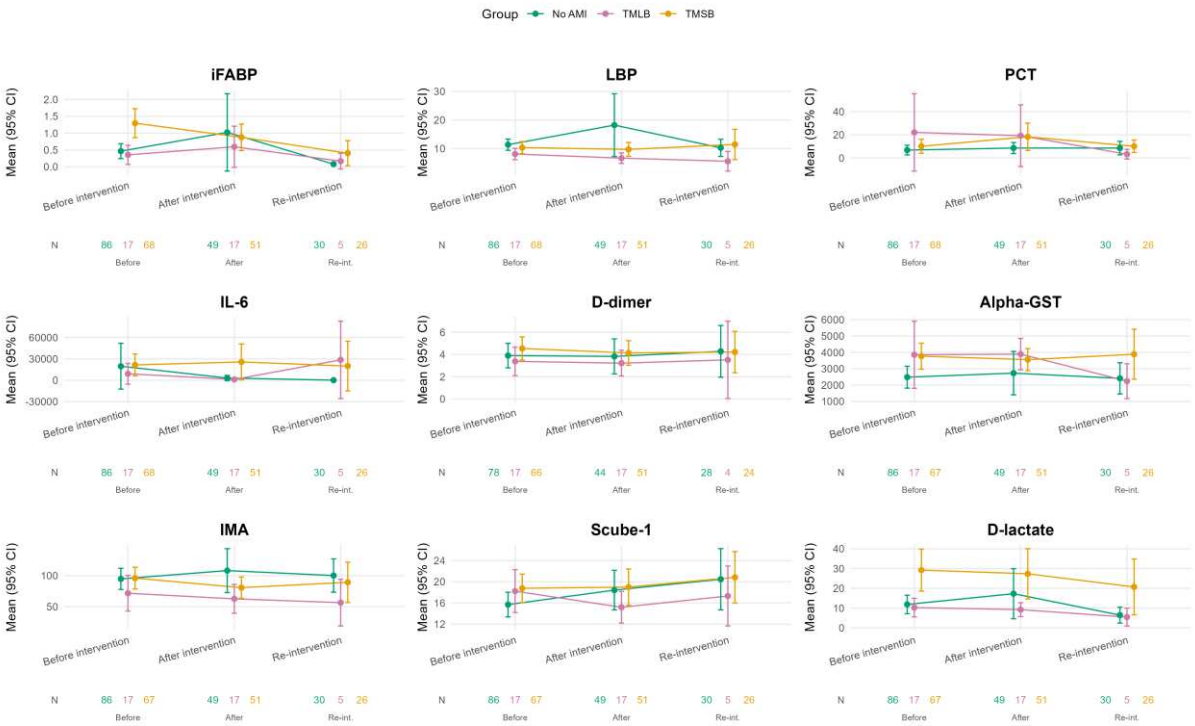
Figure 2 presents dynamics of centrally measured "novel" biomarkers in subgroups of no AMI, NTM AMI and TM AMI and Figure 3 present subgroups of no AMI, TM AMI in small bowel and TM AMI in large bowel. Supplemental Figures S2 and S3 present dynamics of routine biomarkers in similar subgroups, respectively. Comparison of biomarker levels at three time periods in subgroups of no AMI, TM AMI in small bowel and TM AMI in large bowel is presented in Supplemental Table S11.

Figure 2. Dynamics of biomarkers in patients without AMI vs. non-transmural AMI vs. transmural AMI.



Legend: patients are categorized into no AMI vs. non-transmural damage vs. transmural damage in pooled time points indicating "before intervention (M1, M2 and M3)"; "after intervention (M4 and M5)" and "at reintervention (M7)" for patients with AMI and intervention, and parallel time points M1 and M2; M6; and M8 for patients without AMI and with non-transmural AMI without intervention. Only centrally analysed novel biomarkers are presented and SBO and PROBAMI excluded. Data are presented in mean (95% confidence interval).

Figure 3. Dynamics of biomarkers in patients without AMI vs TMSB AMI vs. TMLB AMI.



Legend: patients are categorized into no AMI vs. transmural small bowel AMI (TSMB) vs. transmural large bowel AMI (TMLB)in pooled time points indicating "before intervention (M1, M2 and M3)"; "after intervention (M4 and M5)" and "at reintervention (M7)" for patients with AMI and intervention, and parallel time points M1 and M2; M6 and M8 for patients without AMI and with non-transmural AMI without intervention. Only centrally analysed novel biomarkers are presented and SBO and PROBAMI excluded. Data are presented in mean (95% confidence interval).

Discussion

This international multicentre prospective study evaluated a broad panel of routine and novel biomarkers in patients presenting with clinical suspicion of acute mesenteric ischaemia (AMI). A five biomarkers score combining IL-6, alpha-GST, SCUBE-1, CRP and lactate identified substantially well transmural bowel injury, whereas no individual biomarker or biomarker combination reliably discriminated AMI from non-AMI among patients presenting with suspected disease.

This study reflects several problems repeatedly encountered in research on AMI, such as highly variable and often not precisely identifiable time from onset of ischaemia to diagnostics, capacity to compensate hypoperfusion via collateral vessels differing between subjects and mechanisms, uncertainty in categorisation of particular subtype of AMI, and frequently absent ascertainment of diagnosis histologically. These problems

can only be solved with adopting a unified nomenclature, such as suggested by a recent multidisciplinary consensus process [20].

Our study once again demonstrated that just selecting one mechanism of AMI as a research target does not solve the issue of the control group. Differently from several previous studies on biomarkers [6,21], the control group (no AMI) was comprised from patients with actual suspicion of AMI with similar clinical presentation. This control group is adequate from viewpoint of clinical practice, as these patients need to undergo differential diagnosis of AMI. On the other hand, non-occlusive hypoperfusion of the bowels due to severe illness cannot be reliably excluded in these patients, potentially contaminating the group. That ICU patients have very high levels of e.g. iFABP without developing clinically manifested AMI has been shown previously [22-24]. Also in the current study, the no AMI group had on some occasions higher biomarker levels than non-transmural AMI group.

Inclusion of different AMI subtypes in this study allowed more detailed overview of this complex condition of AMI, and is important from practical point of view, because laboratory samples are usually prescribed and obtained before performing a CT-angiography. However, arterial occlusive AMI probably remains the most straight-forward subtype of AMI allowing reliable confirmation of vascular pathology with CT-scan and documentation of precise time of reperfusion. Definitive confirmation of non-transmural AMI in case of venous or non-occlusive aetiology remains difficult, with endoscopy being an option, but not always indicated and dependent on location of insult [25-27]. We therefore believe that future progress in assessment of other subtypes (venous AMI and NOMI) is largely dependent of arterial occlusive AMI, where translation of evidence should always be considered. Accordingly, studies selecting only patients with confirmed arterial occlusive AMI will likely not bring clarity and potentially even lead to increased ignorance towards NOMI as a “difficult to assess” condition.

Another challenging aspect is SBO, known to be a confusing condition when AMI is concerned [28,29]. While it appears easy to claim separation of SBO, it appears difficult to apply for all cases in practice. Our results suggest that SBO should be clearly reported as a separate entity in all studies addressing AMI (independent if and how included in analyses). While common concern is that SBO is contaminating the AMI group, our study shows that the risk of contaminating the no AMI group is at least as relevant.

Searching or testing biomarkers potentially identifying transmural intestinal damage while including SBO with transmural necrosis in the control (no AMI) group is obviously not meaningful. Whether and how studies assessing biomarkers discriminating transmural necrosis from no transmural necrosis in patients with SBO could be translated to AMI patients, remains to be clarified.

In current study, IL-6, alpha-GST, SCUBE-1, CRP and lactate were included in the score for best performance in discrimination of transmural versus non-transmural AMI. While CRP and lactate are routine tests, the other three are not. IL-6, CRP and lactate can be considered systemic, non-specific and non-novel, reflecting severe tissue injury / necrosis and inflammation rather than bowel damage specifically [30,31]. The enzyme alpha-GST is present in enterocytes and other tissues but has been very little investigated in AMI in humans. Two small studies showed promising results [7,32], in the prospective follow-up study [7], accuracy was increased from 74% for alpha-GST to 80% by combining alpha-GST with lactate or WBC. In the present study, alpha-GST performed similarly in discrimination of transmural from non-transmural intestinal damage but not for AMI vs. no AMI.

Alpha-GST has been suggested as potential biomarker of median arcuate ligament syndrome causing chronic mesenteric ischaemia and of liver dysfunction in septic patients [33,34].

SCUBE-1 has not been investigated previously in patients with acute mesenteric ischaemia. This marker showed elevated levels in patients with acute coronary syndrome and ischemic stroke compared to healthy controls or patients with chronic coronary artery disease [35] but was not higher in patients with confirmed acute appendicitis compared to healthy controls [36].

Previously probably most widely studied [1] and suggested biomarker iFABP showed highest specificity but very low sensitivity in discriminating AMI from no AMI. That a potential of this biomarker was not confirmed, is in line with some recent studies [6]. Confusing results may be attributed to patient selection, but also measurement methods [37]. However, based on our observation, diagnostic value of iFABP may differ in small bowel and large bowel injury, concordant with its higher expression in small compared to large bowel, making detailed reporting essential if any continuing attempts regarding this biomarker will be undertaken. This consideration is rather hypothesis-

generating than directly answerable in current study. However, our results presented in Figure 2 support a rationale for further investigations in this field.
IL-6 has shown conflicting results regarding its diagnostic performance in AMI. Prospective studies have shown both positive [38] and negative [39] results. In the current study, IL-6 showed the strongest discrimination between TM vs. NTM but not for AMI vs. no AMI. Inflammatory markers should probably not be expected to perform well as early markers of mesenteric vascular obstruction. However, the latter could unfortunately not be accurately identified with any of biomarkers or combinations in current study.

A prospective manner, serial measurements, case-by-case re-assessment for confirmation of categorization and large variety of different biomarkers were applied to achieve high-quality evidence compared to available earlier studies [1,4]. Despite intrinsic difficulties in variability of AMI mechanisms, clinical presentation and final confirmation remained, our study confirms the absence of a robust single biomarker and also of an ideal combination of biomarkers as a sole diagnostic tool. However, a combination of different tools may still improve accuracy and combined clinical [40], radiological [41] and biomarker assessment should be studied in the future.

Our final results fall short of reliable detection on one hand, but on the other hand provide a potential tool to assess presence of transmural necrosis with a combination of biomarkers. After validation in future studies, this tool could facilitate management decisions, refining estimation of probability of transmural necrosis. Importantly, in clinical practice no decision should be based on biomarker values alone, but different assessments should be combined. Awareness and appropriately raised suspicion remain the key that should trigger CTA to confirm or exclude vascular occlusion and assess bowel-specific features. However, using a combination of biomarkers could be included in estimation of severity of bowel damage and thereby assist in decision-making regarding primary management strategy (endovascular vs open surgical) in the future. Randomized controlled studies comparing these two initial strategies could be planned based an available information, randomizing patients within the grey zone (insufficient clarity regarding transmural damage).

Beyond initial assessment to confirm presence and severity of insult, biomarker dynamics after revascularization of arterial occlusive AMI or during application of supportive or conservative therapy for venous AMI or NOMI could be helpful to estimate treatment effect, differentiate reperfusion from ongoing ischaemia and facilitate decisions for surgical (re)-exploration. The current study cannot provide answers in this regard but may serve as hypothesis-generating basis for planning of future studies.

This study has several limitations. First, the cohort is of high heterogeneity, and patient numbers remain limited for detailed assessment of subgroups and single time points. Second, categorization of patients needed re-allocation, and despite being per protocol, could have potentially increased risk of bias. Third, the lack of standardized tool to confirm non-transmural bowel insult without intervention precludes certainty between differentiation of no AMI from transmural AMI. Fourth, the control group remained small compared to some previous investigations [42], suggesting that not all patients with AMI suspicion were included, potentially introducing selection bias and reducing generalizability.

Importantly, there are also several strengths of this study. It is the largest prospective international multicentre study with serial measurements of biomarkers and achieved its a priori estimated sample size target [4]. Non-routine biomarkers were analysed in one central laboratory to avoid variability in biomarker assays [43]. The control group was well matched to the AMI group based on strong suspicion of AMI in patients with severe illness and consequent high mortality similar to previous reports [44]. Different subtypes and diagnostic certainty were considered in main and sensitivity analyses.

Conclusions

This study suggests that circulating biomarkers primarily reflect the severity of intestinal injury rather than the presence of AMI itself. While no biomarker combination reliably distinguished AMI from non-AMI, a combination of IL-6, alpha-GST, SCUBE-1, CRP and lactate showed good discriminatory ability for identification of transmural bowel injury. Future biomarker research should therefore focus on assessing bowel viability and irreversible intestinal damage rather than solely on diagnosing AMI.

List of abbreviations

Alpha-GST – alpha-glutathione S-transferase

AMI – acute mesenteric ischaemia

APACHE II – Acute Physiology and Chronic Health Evaluation II score

AUC – area under the receiver operating characteristic curve

BMI – body mass index

CI – confidence interval

CRP – C-reactive protein

ED – emergency department

ICU – intensive care unit

iFABP – intestinal fatty acid-binding protein

IL-6 – interleukin-6

IMA – ischaemia-modified albumin

IMC/HDU – intermediate care/high dependency unit

IQR – interquartile range

LB – large bowel ischaemia without small bowel involvement

LBP – lipopolysaccharide-binding protein

LR+ – positive likelihood ratio

LR- – negative likelihood ratio

M1,2 etc. – measurement point 1,2 etc

MV – mechanical ventilation

NOMI – non-occlusive mesenteric ischaemia

NTM – non-transmural bowel damage

PCT – procalcitonin

PROBAMI – probable AMI

SCUBE-1 - signal peptide-CUB-EGF domain-containing protein 1

SB – small bowel ischaemia with or without large bowel involvement

SBO – strangulating bowel obstruction

SOFA – Sequential Organ Failure Assessment Score

TM – transmural bowel damage

Declarations

Ethics approval and consent to participate

Primary ethical approval was provided by the University of Tartu Ethics Committee on January 26th, 2024 (approval number 386/T-11). Each participating site obtained local ethics committee approval in accordance with institutional and national regulations. Deferred informed consent from patients or patients' next of kin was obtained.

Consent for publication

Not applicable

Competing interests

ARB reports holding grants from the Estonian Research Council (PRG1255 from 2021-2025 and PRG3121 ongoing since 2026). The other co-authors declare no conflicts of interest.

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Authors' contributions

ARB, KT, SA, AB, DC, KK, MK, VM, MM, KK, JS and DV contributed to design and planning of the study, MK and HS performed statistical analyses, ARB, KT and VM drafted the manuscript and supplementary material, all co-authors contributed to data acquisition or data check and reviewed and approved the manuscript.

Data availability

Data will be made available on reasonable request and regulated with data sharing agreement.

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Additional files

Additional file 1 describes definitions and data collection, including sampling details. Additional file 2 includes supplemental tables and figures on laboratory measurement procedures; study flow chart; patient numbers for each site; information on AMI subtypes, severity, localisation and re-classification; biomarker values; sensitivity analyses; and exploratory data on biomarker dynamics.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1BIPAMIMethodsFINAL.docx
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