BMJ Open

Diagnostic Potential of Plasma Biomarkers and Exhaled Volatile Organic Compounds in Predicting the Different Stages of Acute Mesenteric Ischemia: Study Protocol for a Multicentre Prospective Observational Study (TACTIC trial)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-072875
Article Type:	Protocol
Date Submitted by the Author:	17-Feb-2023
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Keywords:	SURGERY, GASTROENTEROLOGY, Clinical Trial, Decision Making, INTENSIVE & CRITICAL CARE

Diagnostic Potential of Plasma Biomarkers and Exhaled Volatile Organic Compounds in Predicting the Different Stages of Acute Mesenteric Ischemia: Study Protocol for a Multicentre Prospective Observational Study (TACTIC trial)

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ABSTRACT

Introduction: Acute Mesenteric ischemia (AMI) is a life-threatening condition with short-term mortality of up to 80% of all cases. The diagnosis of AMI has remained troublesome due to the non-specific clinical presentation, symptoms, and laboratory findings. Early unambiguous diagnosis of AMI is critical to prevent progression from reversible to irreversible transmural intestinal damage, thereby decreasing morbidity and improving survival. The present study aims to validate a panel of plasma biomarkers and investigate the potential of volatile organic compounds (VOCs) analysis in exhaled air as a tool to timely and accurately diagnose AMI.

Methods and analysis: In this international multicentre prospective observational study, 120 patients (> 18 years of age) will be recruited with clinical suspicion of AMI. Clinical suspicion of AMI is based on a combination of the following criteria: (1) clinical manifestation, (2) physical examination, (3) laboratory measurements, and (4) the physician's consideration to perform a computed tomography (CT) scan. Upon consent, the patient's characteristics, repetitive blood samples, and exhaled air will be prospectively collected. Plasma levels of mucosal damage markers intestinal fatty acid

binding protein and villin-1, as well as transmural damage marker smooth muscle 22, will be assessed by enzyme-linked immunosorbent assay. Analysis of VOCs in exhaled air will be performed by gas chromatography time-of-flight mass spectrometry to identify an AMI-specific profile. Diagnosis of mesenteric ischemia will be based on CT, endovascular and surgical reports, clinical findings, and (if applicable) verified by histopathological examination.

Ethics and dissemination: The TACTIC trial protocol was approved by the Medical Research Ethics Committee of Maastricht University Medical Centre+ and Maastricht University (METC azM/UM), the Netherlands (registration number METC19-010) and the Ethics Committee Research UZ/KU Leuven, Belgium (registration number S63500) as well as the local committees of the other Dutch participating centres. Recruitment started in July 2020 and is still ongoing.

KEYWORDS

acute mesenteric ischemia; plasma and serum biomarkers; volatile organic compounds, diagnosis.

TRIAL REGISTRATION

A summary about the study protocol and trial registration is found below (Table 1).

Table 1: Trial registration, data set (SPIRIT)	
Data category	Information
Primary registry and trial identifying number	ClinicalTrials.gov NCT05194527 https://clinicaltrials.gov/ct2/show/NCT05194527
Date of registration in primary registry	18 January, 2022
Secondary identifying numbers	NL68026.068.19/METC19-010
Source(s) of monetary or material support	The Dutch Digestive Foundation (MLDS)
Primary sponsor	Maastricht University, Universiteitssingel 50, 6200 MD, Maastricht Limburg, the Netherlands
Secondary sponsor(s)	N.A.
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Public title	The detrimental course of acute intestinal ischemia: Improvement of the diagnosis
Scientific title	Diagnostic Potential of Plasma Biomarkers and Exhaled Volatile Organic Compounds in Predicting the Different Stages of Acute Mesenteric Ischemia

Table 1: Trial registration, data set (SPIRIT)	
Data category	Information
Countries of recruitment	The Netherlands and Belgium
Health condition(s) or problem(s) studied	Acute Mesenteric Ischemia, Diagnosis, biomarkers
Intervention(s)	N.A.
Key inclusion and exclusion criteria	Ages eligible for study: ≥18 years Sexes eligible for study: both Accepts healthy volunteers: no
	Inclusion Criteria: All patients suspected with acute mesenteric ischemia based on the following; • clinical manifestation; • physical examination by the physician; • laboratory measurements; • physician's consideration to perform computed tomography (CT)-scan
	Exclusion criteria: Age ≤ 18 years
Study type	Observational
	Allocation: Prospective Cohort study
Date of first enrolment	June 2020
Target sample size	120
Recruitment status	Recruiting

Table 1: Trial registration, data set (SPIRIT)	
Data category	Information
Primary outcome(s)	The primary outcome is plasma/serum concentrations of I-FABP, VIL-1 (markers for mucosal damage), and SM22 (a marker for transmural ischemia) in patients with a clinical suspicion of AMI. The sensitivity and specificity of the described biomarkers will be determined and compared with the current gold standard
Key secondary outcomes	This study's secondary outcome is identifying specific VOC profiles in exhaled air of patients suspected of AMI. Individual compounds of these profiles will be chemically identified to discover novel pathophysiologic pathways involved in AMI

PROTOCOL VERSION

Issue date: 20-03-2020

Version: 4 (February 24, 2020)

Protocol amendment number: 1

Authors: Annet Duivenvoorden (AD) and Kaatje Lenaerts (KL)

Primary reason for amendment: Change of primary sponsor

CLINICAL TRIAL PROTOCOL GUIDELINES

The manuscript has been written according to the SPIRIT reporting (1) and STARD guidelines (2).

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This is the first observational prospective clinical study that evaluates a panel of novel biomarkers for acute mesenteric ischemia in a multicentre international clinical cohort.
- This clinical trial will increase awareness of this life-threatening condition, which will help improve clinical outcomes.

- This trial will provide the first data on breath analysis in patients suspected of acute mesenteric ischemia.
- This trial will rely on accurate clinical documentation and a high-quality biobank.
- Patient inclusion is challenging due to acute condition of most patients and low incidence of acute mesenteric ischemia.

INTRODUCTION

Background

Acute mesenteric ischemia (AMI) is a life-threatening condition caused by a sudden interruption of blood flow, resulting in decreased supply of oxygen and nutrients to a segment of the intestinal tract. Prolonged periods of AMI lead to cellular damage and, when left untreated, to necrosis of the intestinal wall, which may cause peritonitis (3, 4). The occurrence of AMI is rare, with a reported incidence between 0.09 to 0.2% (for all admissions to emergency departments) in patients with an unknown cause of abdominal pain (5-7) but strongly increases with age (8). It remains a highly underestimated clinical emergency with short-term mortality of up to 80% (9-13). The clinical presentation for AMI is marked by non-specific signs and symptoms, including abdominal pain, elevated white blood cell count, and metabolic acidosis (9, 11, 12, 14). The non-specific clinical presentation of AMI, combined with the absence of a specific serum/plasma marker, often leads to a delay in the diagnosis. Available conventional blood laboratory tests such as leucocytes, c-reactive protein, lactate, and D-dimer have a restricted specificity to aid in diagnosing AMI (15-19). Radiological imaging is one of the most commonly used non-invasive techniques for confirming

AMI. (14, 20, 21). Computed tomography can be performed quickly compared with standard laboratory tests, and when combined with contrast enhancement of the vessels, so-called CT angiography (CTA) provides a detailed visualization of the intestines and mesenteric vasculature. CTA is the current gold standard imaging modality for diagnosing AMI, with an estimated sensitivity and specificity of around 89-100% (14, 15). However, this is probably an overestimation since the study cohort primarily consisted of patients with advanced mesenteric ischemia and not early or progressive mesenteric ischemia (8). Moreover, a considerable percentage of patients with AMI present without ischemia-specific CT signs, which overlap with other acute abdominal complications (22-24). Therefore, an around-the-clock available, highly accurate, minimally invasive, and rapid diagnostic test can increase the index of suspicion for early AMI, reducing the time to adequate treatment.

In recent years, several clinical studies investigated more specific serological markers for diagnosing AMI and determining the severity of ischemic intestinal damage (25-27). One of these potential biomarkers for AMI is intestinal fatty acid binding protein (I-FABP), a small cytosolic protein that is abundantly expressed in mature enterocytes (28, 29). Upon a decrease in bowel perfusion and consequent loss of enterocyte cell

membrane integrity, a rapid release of I-FABP within the circulation is observed (25). Another mucosal marker for detecting intestinal mucosal damage is villin-1 (VIL-1), which, similar to I-FABP, is detectable in the plasma of rat and human models of mesenteric ischemia (26). As opposed to I-FABP, VIL-1 remains detectable in plasma for more extended periods after the onset of ischemic damage in rats (25, 26). These findings identify I-FABP as a potential marker for early intestinal mucosal injury and VIL-1 as a potential marker for persisting ischemic mucosal damage. Sustained periods of mesenteric ischemia can lead to ischemia of the intestinal muscle layers and, when left untreated, result in transmural ischemia. Currently, known markers of mesenteric ischemic damage focus primarily on mucosal injury, but they provide no insight regarding the possible Development of transmural ischemia. An earlier study showed that plasma levels of smooth muscle 22 (SM22) could differentiate between patients with transmural ischemia and those with mesenteric ischemia confined to the mucosal layer (27). SM22 is a small protein (22 kDa) with a high expression in intestinal smooth muscle tissue (30, 31) and is released upon sustaining ischemic damage. However, the SM22 protein is not exclusively expressed in the intestinal muscle tissue (31). Still, in combination with other specific markers for intestinal mucosal damage, such as I-FABP, it is expected to provide insight into the severity

and progression of intestinal injury in patients with AMI. Unfortunately, none of the described markers have yet to make their appearance in the clinic. Currently, there is limited knowledge of the I-FABP, VIL-1, and SM22 specificity in patients with AMI.

In recent years, analysis of volatile organic compounds (VOCs) in exhaled air to diagnose various pathologies has gained increasing interest. The exhaled air of humans consists of a broad spectrum of VOCs. The composition of these VOCs is influenced by exogenous (oral ingestion, smoking, air quality) and endogenous (activity, microbiome, hormonal) factors (32). The hundreds of VOCs in exhaled air can give valuable information about various (patho)physiologic processes. The analysis of VOCs in exhaled air is a non-invasive technique that has already been demonstrated to differentiate between multiple clinical conditions and healthy subjects, including inflammatory bowel disease and non-alcoholic steatohepatitis (33). As the pathophysiologic processes of inflammatory bowel disease and AMI share common mechanisms, it is expected that VOC profiling could aid in diagnosing AMI in a rapid and non-invasive manner in the future (34).

METHODS AND ANALYSIS

Objectives

This study aims to improve the diagnosis of patients with AMI. Our primary objective is to validate the diagnostic accuracy of a selected panel of plasma and serum biomarkers, I-FABP, SM22, and VIL-1, in patients with AMI. Furthermore, we will investigate if these markers can determine the severity of ischemic intestinal damage. The secondary objective of this study is to identify a VOC profile in exhaled breath to identify AMI non-invasively.

Study design and eligibility criteria

The current study is an international multicentre, prospective observational study aiming to include 120 patients with acute abdominal symptoms fitting to AMI. The main objective is to compare biomarker expression in 60 patients with confirmed mesenteric ischemia and 60 patients with another clinical condition. We may include a higher percentage of patients without mesenteric ischemia due to its non-specific clinical presentation and low overall incidence. Therefore, study inclusions will be finalized when 60 patients with confirmed AMI are included (5-7). Patients clinically suspected of AMI will be screened for inclusion at one of the participating centres. All study

participants must fulfill the study inclusion criteria and will be excluded from participation if they cannot provide written informed consent or do not fulfill the inclusion criteria (Text Box 1: Inclusion and exclusion criteria). Clinical study procedures are initiated when all criteria are met and informed consent is obtained from the patient or legal representative. After inclusion, blood and exhaled breath will be collected at consecutive time points (Figure 1).

Study Sponsor

The sponsor (Maastricht University, Maastricht, The Netherlands) is responsible for the study design and management and for obtaining all study authorizations (Clinical Trial Centre Maastricht and medical research ethics committee). Furthermore, the study sponsor also declares all information regarding the inclusion period, beginning and end, final study report, and trial results to these authorities. Last, all obtained study samples and study-related documents will be stored for at least 15 years after the study ends.

Study population and participating medical centres

The study population will consist of patients clinically suspected of AMI admitted at Maastricht University Medical Centre+ (MUMC+, Maastricht, The Netherlands), Amsterdam University Medical Centre (AUMC), location VUmc and AMC, Gelre Ziekenhuizen Apeldoorn (Apeldoorn, The Netherlands), Medisch Spectrum Twente, Enschede, The Netherlands, University Medical Centre Groningen, The Netherlands and University Hospitals Leuven, Belgium. The clinical course of all patients will be monitored throughout the study, and medical information will be collected, including medical history, medication, vital signs, medical imaging, and information regarding

clinical management during admission. The study has been open for inclusion since June 2020.

Clinical study procedures

Samples will be collected from included patients with a clinical suspicion of AMI at different time points with an in-hospital follow-up of a maximum of five days after inclusion (Figure 1). several baseline characteristics will be acquired at inclusion and during participation. After inclusion, blood and exhaled air samples will be collected every 60 minutes, up to 180 minutes. Re-establishing blood supply to the ischemic bowel is the primary objective in patients with AMI. Therefore, patients may undergo endovascular revascularization to restore mesenteric blood supply. Surgical resection of the necrotic bowel must occur if there are signs of non-viable tissue regions after revascularization. If the patient undergoes an endovascular or surgical intervention, pre-operative and post-operative samples will be taken. Postoperatively, the patient will be monitored for up to five days, and samples (blood and exhaled air) will be collected daily, parallel with the morning routine blood collections. In addition, patients without any treatment interventions will also be monitored for up to five days, and similar blood and air samples will be obtained identically to patients with a treatment

intervention. At the end of the study, each participant will be allocated to one of the two study groups (AMI versus non-AMI). Diagnosis of mesenteric ischemia will be based on CT, endovascular and surgical reports, clinical findings, and (if applicable) verified by histopathological examination.

Blood collection and biomarker analysis

In this study, obtained blood samples will be analysed for serum and plasma biomarker analysis of I-FABP, SM22, and VIL-1. Blood samples will be collected via an arterial line, an intravenous needle, or a central venous catheter. Occasionally a separate venapuncture can also be used to collect blood. Blood samples will be collected in Vacutainer tubes treated with Ethylenediaminetetraacetic acid for plasma and SST™ II Advance tubes for serum specimens. Whole blood samples will be centrifuged, and plasma/serum will be transferred to storage tubes. After processing, the samples are stored at -80°C until further analysis. I-FAPB, SM22, and VIL-1 concentrations will be determined in plasma/serum samples through enzyme-linked immunosorbent assay (ELISA). Highly specific I-FABP and SM22 ELISAs were developed and validated in our lab and selectively detect human I-FABP and human SM22 in plasma with a lower limit of detection of 12.5 pg/mL and 62.5 pg/mL, respectively (27, 35). The intra-assay

and inter-assay coefficient of variation is 4.1% and 6.2%, respectively, for IFABP ranging from 6.2% to 14.8% and 4.9% to 16.3%, respectively, for SM22 (27, 35). VIL-1 ELISA was developed at PharmAbs (KU Leuven, Leuven, Belgium) and can detect human VIL-1 with a lower detection limit of 0.78 ng/mL (Ceulemans et al. in preparation).

Exhaled breath collection and analysis

This study's second objective focuses on using VOCs in exhaled breath as a potential diagnostic tool for AMI. Exhaled breath is collected using resistance-free plastic bags (Tedlar bag, 3L, SKC Ltd, Dorset, UK) parallel to the blood samples. To collect breath samples, the patient must breathe into the valve of the Tedlar bag, which takes three to four exhalations to fill. Exhaled breath from an incapacitated patient will be collected from mechanical ventilation through a co-axial tubing system. Collected exhaled air containing VOCs is stabilized on carbon desorption tubes (SU60520-60-S, Camsco) with a Flow air sampling pump (LFS-113, 360-041-01, Sensidyne) and stored at 4°C until further analysis by gas chromatography time of flight mass spectrometry (GC-TOF-MS)(34).

Study outcomes

We hypothesize that with the use of serum/plasma biomarkers I-FABP, VIL-1, and SM22, a timely diagnosis of patients with AMI before irreversible transmural bowel damage occurs will be achieved. Through a multimodal diagnostic approach, we will be able to characterize each patient's condition and correlate these biomarkers' concentration to the disease's corresponding etiology. The primary outcome is plasma/serum concentrations of I-FABP, VIL-1 (markers for mucosal damage), and SM22 (a marker for transmural ischemia) in patients with a clinical suspicion of AMI. The sensitivity and specificity of the described biomarkers will be determined and compared with the current gold standard (14, 15). A receiver operating characteristic (ROC) curve analysis will be used to evaluate the diagnostic power of the biomarker (panel) test.

This study's secondary outcome is identifying specific VOC profiles in exhaled air of patients suspected of AMI. Individual compounds of these profiles will be chemically identified to discover novel pathophysiologic pathways involved in AMI. Furthermore, these VOC profiles will be used to investigate their potential use as a novel non-invasive diagnostic technique for AMI.

Data collection and management

Patients will receive a patient information folder and consent forms before study initiation, explaining the study procedures in detail and providing information on the study data collection, protection, and pseudonymization of their medical information. Data will be obtained by the local study teams and registered using study-specific Case Report Forms (CRFs) and CASTOR (36) electronic data capture (EDC) system, which facilitates monitoring the study progress and outcomes in real-time. To ensure the privacy of all individuals in this study, blood and breath samples, data, and results of our research will be treated confidentially and encoded accordingly. The encoding of their personal data will ensure the patient's anonymity. The source data and encoding key for the patient's personal data will only be accessible to the principal and coordinating investigator. After the termination and publication, the patients can be informed about their study results, which can be explained to them if requested on their informed consent form. With the participants' approval in this study, collected data, blood, and exhaled air will be stored for 15 years for future research purposes. All samples will be transported to the Department of Surgery (Maastricht University, Maastricht, The Netherlands), where they will be stored and analysed. All data concerning participants or their participation in this trial will be considered confidential

and handled in compliance with all applicable regulations. Only members of the study team and local investigators have access to these data.

Safety Considerations and withdrawal of participation

The study will be suspended if there is sufficient ground that continuation of the study will jeopardise the subject health or safety. The sponsor will notify the accredited Medical Ethical Board without undue delay of a temporary halt, including the reason for such an action. The study will be suspended pending a further favorable decision by the accredited board. The coordinating researcher will ensure that all subjects are kept informed during trial participation.

Patients participate in this research voluntarily. Any sign of patient resistance will lead to the discontinuation of research involving this patient. Patients withdraw their permission and leave the study at any time for any reason if they wish to do so without any consequences for their further treatment. For example, the investigator can withdraw a subject from the study for urgent medical reasons. Data obtained during participation can be used for future research purposes unless the patient or legal representative gives a written or verbal objection.

Sample size

Data from a previous study undertaken by our group was used to determine the sample size (27). Based on an effect size (medium to large) of 0.631 (calculated with mean I-FABP levels), with a power of 0.8 and a 95% confidence interval, 54 patients per group (AMI versus non-AMI) are needed for this cohort. By including 60 patients per group, possible dropouts (10%, n = 6) are considered.

Statistical analysis

Statistical analysis will be performed with SPSS software (IBM) and GraphPad Prism 8 software. All the data obtained consists of continuous and categorical variables. The data will be tested for normality using the Kolmogorov-Smirnov test. Relative changes between the two groups will be tested using a Student's T-test. Dichotomous variables will be compared using Pearson's chi-squared test. During the statistical analysis, numerical values will be reported as mean ± standard deviation or median (Interquartile range, i.e., 25th to 75th percentile). Relevant variables with a p-value <0.05 for univariate analysis were introduced into a multiple logistic regression model using confidence intervals (CI). The area under the curve (AUC) of receiver operating characteristic (ROC) curves will be used to assess the functionality of I-FABP, SM22, and VIL-1 and VOC profiles in predicting AMI. Logistic regression analyses will be

performed to investigate the most effective biomarker combination in patients with ischemia or without ischemia. Furthermore, we will assess the difference in mean I-FABP, SM22, and VIL1 plasma/serum levels between patients suffering from AMI and those diagnosed with other clinical conditions at different times. The severity of the mesenteric ischemic damage will be determined (reversible versus non-reversible mesenteric ischemic injury) by (1) levels of plasma IFABP, VIL1, and SM22 at baseline and (2) an increase of IFAPB, VIL1, and SM22 plasma levels over time as AMI progresses (until intervention).

Patient and public involvement

A patient panel from The Dutch Digestive Foundation (MLDS) reviewed the grant proposal to obtain funding. There was no patient or public involvement in the design of this clinical study.

Ethical and dissemination

The TACTIC study protocol was approved (September 4th, 2019) by the Medical Research Ethics Committee of MUMC+ and Maastricht University, the Netherlands (registration number METC19-010), and the Ethics Committee Research UZ/KU Leuven, Belgium (registration number S63500), as well as the local committees of the

other Dutch participating centres. This study will be conducted according to the principles of the Declaration of Helsinki (adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013) in accordance with the WMO. Trial results will be disseminated via open-access peer-reviewed scientific journals and national and international conferences.

Funding statement

This study is funded by the MLDS foundation, Amersfoort (grant number D17-14).

Acknowledgments

We have received funding from MLDS, to conduct the TACTIC trial. We want to thank S.M.J. van Kuijk for his statistical insights for the study protocol. In addition, we would like to thank Professor C.H.C De Jong for his indispensable work investigating the complex multifactorial background of AMI, which led to the discovery of new potential biomarkers for future clinical applications and improving patients' clinical outcomes.

Authors' Contributions

KL and TL originated the study. AD, KL, LC, and TL were involved in the study design. AD, KL, and TL drafted the manuscript. AD, RG, MC, RG, JV, HB, SD, FJ, LC, TL, and KL are local investigators at the participating centres. The study is supervised and coordinated by AD, KL, and TL. All authors provided essential feedback to the successive manuscript versions and approved the final version.

Conflicts of Interest

None declared.

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ABBREVIATIONS

AMI:	Acute Mesenteric Ischemia
AUC	Area Under Curve
CT(A):	Computed Tomography (angiography)
CRF	Case Report Form
EDC	Electronic Data Capture
ELISA	Enzyme-Linked Immunosorbent Assay
GS-TOF-MS:	Gas chromatography time-of-flight mass spectrometry
I-FABP:	Intestinal Fatty Acid Binding Protein
MLDS:	Maag Lever Darm Stichting
PREOP:	Pre-operative
RPF:	Reperfusion
ROC	Receiver Operating Characteristic
SM22:	Smooth Muscle 22
VIL-1:	Villin-1

VOC: Volatile Organic Compounds

FIGURES LEGENDS

Figure 1: Study outline. Patients with a clinical suspicion of acute mesenteric ischemia are considered applicable for participation if all criteria are met. Patients are screened and asked for study participation by informed consent. When consent has been received, study procedures will start. Part 1: blood and exhaled air is collected every 60 minutes (min), up to 180 min (T180) after inclusion; Part 2 is initiated if the patient receives an intervention (endovascular or surgical), with a pre-operative and post-operative sample collection. Patients that do not receive the intervention will directly move into Part 3: Follow-up. Daily samples up to five days (T5) will be retrieved during routine blood collection. In the final stage of the study (Part 4: Diagnosis), each patient will be placed in one of the two study groups (AMI versus non-AMI) based on the collected data. T: time point; PRE: pre-operative; POST: post-operative.

Inclusion criteria

- The ability to provide informed consent, either by themselves or by a legal representative
- The patients must be suspected of AMI, which is based on the following;
  - Clinical manifestation of the disease
  - The physical examination by the local physician
  - Laboratory measurements
  - Physician's consideration to perform CT(A)-scan

Exclusion criteria

- Unable to provide informed consent, either by themselves or by a legal representative
- < 18 years of age

Textbox 1: Inclusion and exclusion criteria

Textbox 1: Inclusion and exclusion criteria

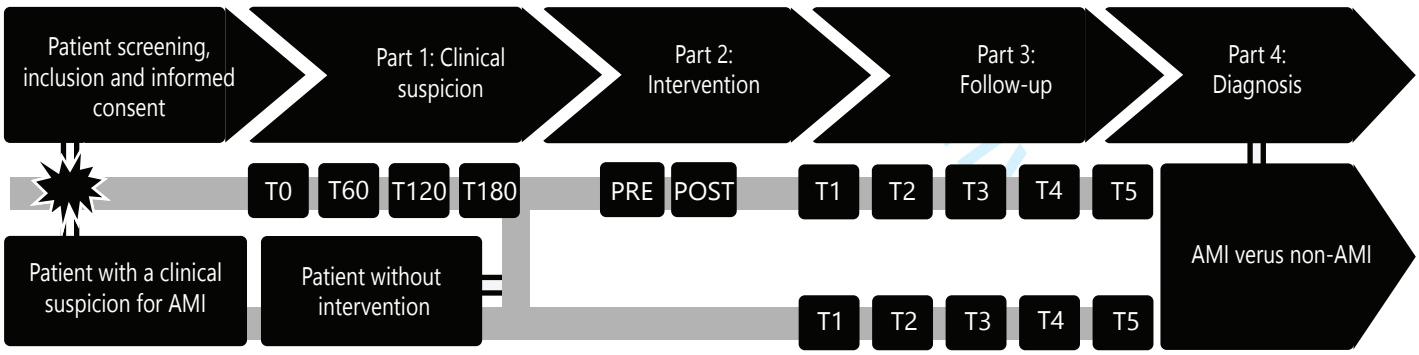

Figure 1: Study Outline

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	5
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	5-6
Protocol version	#3	Date and version identifier	7
Funding	#4	Sources and types of financial, material, and other support	5, 17
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1-2, 18
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	5
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	17, 18
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and	17, 18

		other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	8-10
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	11-12
Objectives	#7	Specific objectives or hypotheses	14-15
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	11-15
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	12

Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	11
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	13-14
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	16
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	16
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	-
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	14-15

Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	12-13
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	16
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	11-12
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	N.A.
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque,	

		sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	N.A.
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N.A.
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N.A.
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14-15

Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14-15
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	14-15
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14-16
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	15-16
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	15-16
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further	15-16

		details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	15-16
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	15-16
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	15-16
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	17-18
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	5-6

Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	11-13, 16
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N.A.
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	N.A.
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N.A.
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	N.A.

Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	17-18
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N.A.
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N.A.

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	1, 4
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	4-5
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	8-10
	4	Study objectives and hypotheses	11-12
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	11-12
Participants	**6**	Eligibility criteria	11
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	11-12
	8	Where and when potentially eligible participants were identified (setting, location and dates)	11-12
	9	Whether participants formed a consecutive, random or convenience series	11-12
Test methods	**10a**	Index test, in sufficient detail to allow replication	11-13
	10b	Reference standard, in sufficient detail to allow replication	11-13
	11	Rationale for choosing the reference standard (if alternatives exist)	11-13
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	11-13
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	11-13
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	11-13
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	11-13
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	13-17
	15	How indeterminate index test or reference standard results were handled	16-17
	16	How missing data on the index test and reference standard were handled	16-17
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	16-17
	18	Intended sample size and how it was determined	16-17
RESULTS			
Participants	**19**	Flow of participants, using a diagram	11
	20	Baseline demographic and clinical characteristics of participants	12
	21a	Distribution of severity of disease in those with the target condition	8-10, 16
	21b	Distribution of alternative diagnoses in those without the target condition	8-10, 16
	22	Time interval and any clinical interventions between index test and reference standard	N.A.
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	N.A.
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	16-17
	25	Any adverse events from performing the index test or the reference standard	16
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	7
	27	Implications for practice, including the intended use and clinical role of the index test	7-10
OTHER INFORMATION			
	28	Registration number and name of registry	5-7
	29	Where the full study protocol can be accessed	5-7
	30	Sources of funding and other support; role of funders	5-7

STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.

BMJ Open

Diagnostic Potential of Plasma Biomarkers and Exhaled Volatile Organic Compounds in Predicting the Different Stages of Acute Mesenteric Ischemia: Protocol for a Multicentre Prospective Observational Study (TACTIC study)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-072875.R1
Article Type:	Protocol
Date Submitted by the Author:	16-May-2023
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Primary Subject Heading:	Diagnostics
Secondary Subject Heading:	Diagnostics, Gastroenterology and hepatology, Research methods
Keywords:	SURGERY, GASTROENTEROLOGY, INTENSIVE & CRITICAL CARE

Diagnostic Potential of Plasma Biomarkers and Exhaled Volatile Organic Compounds in Predicting the Different Stages of Acute Mesenteric Ischemia: ~~Study~~ Protocol for a Multicentre Prospective Observational Study (TACTIC study)

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ABSTRACT

Introduction: Acute mMesenteric ischemia (AMI) is a life-threatening condition with short-term mortality of up to 80% ~~of all cases~~. The diagnosis of AMI has remained troublesome due to the non-specific clinical presentation, symptoms, and laboratory findings. Early unambiguous diagnosis of AMI is critical to prevent progression from reversible to irreversible transmural intestinal damage, thereby decreasing morbidity and improving survival. The present study aims to validate a panel of plasma biomarkers and investigate volatile organic compound (VOC) profiles in exhaled air as a tool to timely and accurately diagnose AMI.

Methods and analysis: In this international multicentre prospective observational study, 120 patients (> 18 years of age) will be recruited with clinical suspicion of AMI. Clinical suspicion ~~of AMI~~ is based on: (1) clinical manifestation, (2) physical examination, (3) laboratory measurements, and (4) the physician's consideration to perform a computed tomography (CT) scan. The patient's characteristics, repetitive blood samples, and exhaled air will be prospectively collected. Plasma levels of mucosal damage markers intestinal fatty acid binding protein and villin-1, as well as

transmural damage marker smooth muscle 22, will be assessed by enzyme-linked immunosorbent assay. Analysis of VOCs in exhaled air will be performed by gas chromatography time-of-flight mass spectrometry. Diagnosis of AMI will be based on CT, endovascular and surgical reports, clinical findings, and (if applicable) verified by histopathological examination.

Ethics and dissemination: The study protocol was approved by the Medical Research Ethics Committee (METC) of Maastricht University Medical Centre+ and Maastricht University (METC azM/UM), the Netherlands (METC19-010) and the Ethics Committee Research UZ/KU Leuven, Belgium (S63500). Executive boards and local METCs of other Dutch participating centres, Gelre Ziekenhuizen (Apeldoorn), Medisch Spectrum Twente (Enschede), and University Medical Centre Groningen, have granted permission to carry out this study. Study results will be disseminated via open-access peer-reviewed scientific journals and national/international conferences.

KEYWORDS

acute mesenteric ischemia; plasma and serum biomarkers; volatile organic compounds, diagnosis.

PROTOCOL VERSION

Issue date: 20-03-2020

Version: 4 (February 24, 2020)

Protocol amendment number: 1

Authors: Annet Duivenvoorden (AD) and Kaatje Lenaerts (KL)

Primary reason for amendment: Change of primary sponsor

CLINICAL STUDY PROTOCOL GUIDELINES

The ~~manuscript~~ protocol has been reported~~written~~ according to the STROBE statement (http://www.strobe-statement.org/) ~~(1)~~ and STARD guidelines (https://www.equator-network.org/reporting-guidelines/stard/) ~~(2)~~. The checklists are given as online supplementary materials.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This is the first observational prospective clinical study that evaluates a panel of novel biomarkers for acute mesenteric ischemia in a multicentre international clinical cohort.

- This study will provide the first data on breath analysis in patients suspected of acute mesenteric ischemia.
- This study will rely on accurate clinical documentation and a high-quality biobank.
- Patient inclusion is challenging due to acute condition of most patients and low incidence of acute mesenteric ischemia.

INTRODUCTION

Background

Acute mesenteric ischemia (AMI) is a life-threatening condition caused by a sudden interruption of blood flow, resulting in decreased supply of oxygen and nutrients to a segment of the intestinal tract. Prolonged periods of AMI lead to cellular damage and, when left untreated, to necrosis of the intestinal wall, which may cause peritonitis (1, 2). The occurrence of AMI is rare, with a reported incidence between 0.09 to 0.2% (for all admissions to emergency departments) in patients with an unknown cause of abdominal pain (3-5) but strongly increases with age (6). It remains a highly underestimated clinical emergency with short-term mortality of up to 80% (7-11). The clinical presentation for AMI is marked by non-specific signs and symptoms, including abdominal pain, elevated white blood cell count, and metabolic acidosis (7, 9, 10, 12). The non-specific clinical presentation of AMI, combined with the absence of a specific serum/plasma marker, often leads to a delay in the diagnosis. Available conventional blood laboratory tests such as leucocytes, c - reactive protein, lactate, and D-dimer have a restricted specificity to aid in diagnosing AMI (13-17). Radiological imaging is one of the most commonly used non-invasive techniques for confirming AMI. (12, 18,

19). Computed tomography can be performed quickly compared with standard laboratory tests, and when combined with contrast enhancement of the vessels, so-called CT angiography (CTA) provides a detailed visualization of the intestines and mesenteric vasculature. CTA is the current gold standard imaging modality for diagnosing AMI, with an estimated sensitivity and specificity of around 89-100% (12, 13). However, this is probably an overestimation since the study cohort primarily consisted of patients with advanced mesenteric ischemia and not early or progressive mesenteric ischemia (6). Moreover, a considerable percentage of patients with AMI present without ischemia-specific CT signs, which overlap with other acute abdominal complications (20-22). Therefore, an around-the-clock available, highly accurate, minimally invasive, and rapid diagnostic test can increase the index of suspicion for early AMI, reducing the time to adequate treatment.

In recent years, several clinical studies investigated more specific serological markers for diagnosing AMI and determining the severity of ischemic intestinal damage (23-25). One of these potential biomarkers for AMI is intestinal fatty acid binding protein (I-FABP), a small cytosolic protein that is abundantly expressed in mature enterocytes (26). Upon a decrease in bowel perfusion and consequent loss of enterocyte cell

membrane integrity, a rapid release of I-FABP within the circulation is observed (23, 27). Another mucosal marker for detecting intestinal mucosal damage is villin-1 (VIL-1), which, similar to I-FABP, is detectable in the plasma of rat and human models of mesenteric ischemia (24). As opposed to I-FABP, VIL-1 remains detectable in plasma for more extended periods after the onset of ischemic damage in rats (23, 24). These findings identify I-FABP as a potential marker for early intestinal mucosal injury and VIL-1 as a potential marker for persisting ischemic mucosal damage. Sustained periods of mesenteric ischemia can lead to ischemia of the intestinal muscle layers and, when left untreated, result in transmural ischemia. Currently, known markers of mesenteric ischemic damage focus primarily on mucosal injury, but they provide no insight regarding the possible ~~Development~~ development of transmural ischemia. An earlier study showed that plasma levels of smooth muscle 22 (SM22) could differentiate between patients with transmural ischemia and those with mesenteric ischemia confined to the mucosal layer (25). SM22 is a small protein (22 kDa) with a high expression in intestinal smooth muscle tissue (28, 29) and is released upon sustaining ischemic damage. However, the SM22 protein is not exclusively expressed in the intestinal muscle tissue (29) . Still, in combination with other specific markers for intestinal mucosal damage, such as I-FABP, it is expected to provide insight into the

severity and progression of intestinal injury in patients with AMI. Unfortunately, none of the described markers have yet to make their appearance in the clinic. Currently, there is limited knowledge of the I-FABP, VIL-1, and SM22 specificity in patients with AMI.

In recent years, analysis of volatile organic compounds (VOCs) in exhaled air to diagnose various pathologies has gained increasing interest. The exhaled air of humans consists of a broad spectrum of VOCs. The composition of these VOCs is influenced by exogenous (oral ingestion, smoking, air quality) and endogenous (activity, microbiome, hormonal) factors (30). The hundreds of VOCs in exhaled air can give valuable information about various (patho)physiologic processes. The analysis of VOCs in exhaled air is a non-invasive technique that has already been demonstrated to differentiate between multiple clinical conditions and healthy subjects, including inflammatory bowel disease and non-alcoholic steatohepatitis (31).

In 2011, a pilot study in the analysis of VOCs in rats following acute superior mesenteric artery (SMA) occlusion showed that they were able to identify a small cluster of VOCs that increase during ischemic bowel injury (32). Other studies, have explored the possibility of monitoring exhaled methane (CH4) concentrations in order

to detect SMA malperfusion (33). This is now being investigated in a prospective observational study in patients with trauma-related haemorrhage following blunt trauma (34). Based on these findings we could speculate that CH4 concentrations could be relevant in our future measurements and analyses. As the pathophysiologic processes of inflammatory bowel disease and AMI share common mechanisms, it is expected that VOC profiling could aid in diagnosing AMI in a rapid and non-invasive manner in the future (35).

METHODS AND ANALYSIS

Objectives

This study aims to improve the diagnosis of patients with AMI. Our primary objective is to validate the diagnostic accuracy of a selected panel of plasma and serum biomarkers, I-FABP, SM22, and VIL-1, in patients with AMI. Furthermore, we will investigate if these markers can determine the severity of ischemic intestinal damage. The secondary objective of this study is to identify a VOC profile in exhaled breath to identify AMI non-invasively.

Study design and eligibility criteria

The current study is an international multicentre, prospective observational study aiming to include 120 patients with acute abdominal symptoms fitting to AMI. The main objective is to compare biomarker expression in 60 patients with confirmed mesenteric ischemia and 60 patients with another clinical condition. We may include a higher percentage of patients without mesenteric ischemia due to its non-specific clinical presentation and low overall incidence. Therefore, study inclusions will be finalized when 60 patients with confirmed AMI are included (3-5). Patients clinically suspected of AMI will be screened for inclusion at one of the participating centres. All study

participants must fulfil the study inclusion criteria and will be excluded from participation if they cannot provide written informed consent or do not fulfil the inclusion criteria (Text Box 1: Inclusion and exclusion criteria). Patients are eligible for study participation if they have a clinically suspicion of AMI, which is based on (1) the clinical manifestation of the disease, (2) physical examination by the local physician, (3) laboratory measurements and (4) the physician's consideration to perform a CT(A)-scan. If all criteria are met, the physician will contact the local research team and the patient or legal representative will be asked for informed consent to participate in the study. Clinical study procedures are initiated when all criteria are met and informed consent is obtained from the patient or legal representative. After inclusion, blood and exhaled breath will be collected at consecutive time points (Figure 1).

Study Sponsor

The sponsor (Maastricht University, Maastricht, The Netherlands) is responsible for the study design and management and for obtaining all study authorizations (Clinical Trial Centre Maastricht and medical research ethics committee). Furthermore, the study sponsor also declares all information regarding the inclusion period, beginning and end, final study report, and study results to these authorities. Last, all obtained

study samples and study-related documents will be stored for at least 15 years after the study ends.

Study population and participating medical centres

The study population will consist of patients clinically suspected of AMI admitted at Maastricht University Medical Centre+ (MUMC+, Maastricht, The Netherlands), Amsterdam University Medical Centre (AUMC), location VUmc and AMC, Gelre Ziekenhuizen Apeldoorn (Apeldoorn, The Netherlands), Medisch Spectrum Twente, Enschede, The Netherlands, University Medical Centre Groningen, The Netherlands and University Hospitals Leuven, Belgium. The clinical course of all patients will be monitored throughout the study, and medical information will be collected, including medical history, medication, vital signs, medical imaging, and information regarding clinical management during admission. The study has been open for inclusion since June 2020.

Clinical study procedures

Samples will be collected from included patients with a clinical suspicion of AMI at different time points with an in-hospital follow-up of a maximum of five days after inclusion (Figure 1). several baseline characteristics will be acquired at inclusion and

during participation. After inclusion, blood and exhaled air samples will be collected every 60 minutes, up to 180 minutes. Re-establishing blood supply to the ischemic bowel is the primary objective in patients with AMI. Therefore, patients may undergo endovascular revascularization to restore mesenteric blood supply. Surgical resection of the necrotic bowel must occur if there are signs of non-viable tissue regions after revascularization. If the patient undergoes an endovascular or surgical intervention, pre-operative and post-operative samples will be taken. Postoperatively, the patient will be monitored for up to five days, and samples (blood and exhaled air) will be collected daily, parallel with the morning routine blood collections. In addition, patients without any treatment interventions will also be monitored for up to five days, and similar blood and air samples will be obtained identically to patients with a treatment intervention. At the end of the study, each participant will be allocated to one of the two study groups (AMI versus non-AMI). Diagnosis of mesenteric ischemia will be based on CT, endovascular and surgical reports, clinical findings, and (if applicable) verified by histopathological examination.

Blood collection and biomarker analysis

In this study, obtained blood samples will be analysed for serum and plasma biomarker analysis of I-FABP, SM22, and VIL-1. Blood samples will be collected via an arterial line, an intravenous needle, or a central venous catheter. Occasionally a separate venapuncture can also be used to collect blood. Blood samples will be collected in Vacutainer tubes treated with Ethylenediaminetetraacetic acid for plasma and SST™ II Advance tubes for serum specimens. Whole blood samples will be centrifuged, and plasma/serum will be transferred to storage tubes. After processing, the samples are stored at -80°C until further analysis. I-FAPB, SM22, and VIL-1 concentrations will be determined in plasma/serum samples through enzyme-linked immunosorbent assay (ELISA). Highly specific I-FABP and SM22 ELISAs were developed and validated in our lab and selectively detect human I-FABP and human SM22 in plasma with a lower limit of detection of 12.5 pg/mL and 62.5 pg/mL, respectively (25, 36). The intra-assay and inter-assay coefficient of variation is 4.1% and 6.2%, respectively, for IFABP ranging from 6.2% to 14.8% and 4.9% to 16.3%, respectively, for SM22 (25, 36). VIL-1 ELISA was developed at PharmAbs (KU Leuven, Leuven, Belgium) and can detect human VIL-1 with a lower detection limit of 0.78 ng/mL. VIL-1 is detectable in plasma, however earlier studies showed a better detection in serum compared to plasma (Ceulemans et al in preparation).

Exhaled breath collection and analysis

This study's second objective focuses on using VOCs in exhaled breath as a potential diagnostic tool for AMI. Exhaled breath is collected using resistance-free plastic bags (Tedlar bag, 3L, SKC Ltd, Dorset, UK) parallel to the blood samples. To collect breath samples, the patient must breathe into the valve of the Tedlar bag, which takes three to four exhalations to fill. Exhaled breath from an incapacitated patient will be collected from mechanical ventilation through a co-axial tubing system. Collected exhaled air containing VOCs is stabilized on carbon desorption tubes (SU60520-60-S, Camsco) with a Flow air sampling pump (LFS-113, 360-041-01, Sensidyne) and stored at 4°C until further analysis by gas chromatography time of flight mass spectrometry (GC-tof-MS) (35). The GC-tof-MS analysis was performed as described previously (37).

Study outcomes

We hypothesize that with the use of serum/plasma biomarkers I-FABP, VIL-1, and SM22, a timely diagnosis of patients with AMI before irreversible transmural bowel damage occurs will be achieved. Through a multimodal diagnostic approach, we will be able to characterize each patient's condition and correlate these biomarkers' concentration to the disease's corresponding etiology. The primary outcome is

plasma/serum concentrations of I-FABP, VIL-1 (markers for mucosal damage), and SM22 (a marker for transmural ischemia) in patients with a clinical suspicion of AMI. The sensitivity and specificity of the described biomarkers will be determined and compared with the current gold standard (12, 13). A receiver operating characteristic (ROC) curve analysis will be used to evaluate the diagnostic power of the biomarker (panel) test.

This study's secondary outcome is identifying specific VOC profiles in exhaled air of patients suspected of AMI. Individual compounds of these profiles will be chemically identified to discover novel pathophysiologic pathways involved in AMI. Furthermore, these VOC profiles will be used to investigate their potential use as a novel non-invasive diagnostic technique for AMI.

Data collection and management

Patients will receive a patient information folder and consent forms before study initiation, explaining the study procedures in detail and providing information on the study data collection, protection, and pseudonymization of their medical information. Data will be obtained by the local study teams and registered using study-specific Case Report Forms (CRFs) and CASTOR (38) electronic data capture (EDC) system,

which facilitates monitoring the study progress and outcomes in real-time. To ensure the privacy of all individuals in this study, blood and breath samples, data, and results of our research will be treated confidentially and encoded accordingly. The encoding of their personal data will ensure the patient's anonymity. The source data and encoding key for the patient's personal data will only be accessible to the principal and coordinating investigator. After the termination and publication, the patients can be informed about their study results, which can be explained to them if requested on their informed consent form. With the participants' approval in this study, collected data, blood, and exhaled air will be stored for 15 years for future research purposes. All samples will be transported to the Department of Surgery (Maastricht University, Maastricht, The Netherlands), where they will be stored and analysed. All data concerning participants or their participation in this study will be considered confidential and handled in compliance with all applicable regulations. Only members of the study team and local investigators have access to these data.

Safety Considerations and withdrawal of participation

Patient safety and treatment is always prioritized and is not influenced by the study. The study will be suspended if there is sufficient ground that continuation of the study

will jeopardise the subject health or safety. The sponsor will notify the accredited Medical Ethical Board without undue delay of a temporary halt, including the reason for such an action. The study will be suspended pending a further favorable decision by the accredited board. The coordinating researcher will ensure that all subjects and (if applicable) legal representatives are kept informed during study participation.

Patients participate in this research voluntarily. Any sign of patient resistance will lead to the discontinuation of research involving this patient. Patients or legal representative can withdraw their permission and leave the study at any time for any reason if they wish to do so without any consequences for their further treatment. For example, the investigator can withdraw a subject from the study for urgent medical reasons. Data obtained during participation can be used for future research purposes unless the patient or legal representative gives a written or verbal objection.

Sample size

Data from a previous study undertaken by our group was used to determine the sample size (25). Based on an effect size (medium to large) of 0.631 (determined by Cohen's d formula based on the difference in mean I-FABP levels), with a power of 0.8 and alpha 0.05/3 (corrected for multiple testing due to analysis of three primary

outcome parameters), 54 patients per group (AMI versus non-AMI) are needed for this cohort. By including 60 patients per group, possible dropouts (10%, n = 6) are considered.

Statistical analysis

Statistical analysis will be performed with SPSS software (IBM) and GraphPad Prism 8 software. All the data obtained consists of continuous and categorical variables. The data will be tested for normality using the Kolmogorov-Smirnov test. Relative changes between the two groups will be tested using a Student's T-test. Dichotomous variables will be compared using Pearson's chi-squared test. During the statistical analysis, numerical values will be reported as mean ± standard deviation or median (Interquartile range, i.e., 25th to 75th percentile). Relevant variables with a p-value <0.05 for univariate analysis will be introduced into a multiple logistic regression model using confidence intervals (CI).). The area under the receiver operating characteristics curve (AUC-ROC) will be calculated and is used to determine diagnostic utilities (sensitivity, specificity, positive predictive value and negative predictive value) of the biomarkers I-FABP, SM22, and VIL-1 to discriminate between AMI or non-AMI patients. Statistical analysis of VOCs expression profiles will be performed according

to the published standards by Horvath et al. for the exhaled breath analysis (39). Logistic regression analyses will be performed to investigate the most effective biomarker combination in patients with ischemia or without ischemia. Furthermore, we will assess the difference in mean I-FABP, SM22, and VIL1 plasma/serum levels between patients suffering from AMI and those diagnosed with other clinical conditions at different times. The severity of the mesenteric ischemic damage will be determined (reversible versus non-reversible mesenteric ischemic injury) by (1) levels of plasma IFABP, VIL1, and SM22 at baseline and (2) an increase of IFAPB, VIL1, and SM22 plasma levels over time as AMI progresses (until intervention).

Patient and public involvement

A patient panel from The Dutch Digestive Foundation (MLDS) reviewed the grant proposal to obtain funding. There was no patient or public involvement in the design of this clinical study.

Ethics and dissemination

The TACTIC study protocol was approved by the Medical Research Ethics Committee (METC) of Maastricht University Medical Centre+ and Maastricht University (METC azM/UM), the Netherlands (registration number METC19-010) and the Ethics Committee Research UZ/KU Leuven, Belgium (registration number S63500). Executive boards and local METCs of the Dutch participating centres, Gelre Ziekenhuizen (Apeldoorn), Medisch Spectrum Twente, (Enschede) and University Medical Centre Groningen) have granted permission to carry out this study according to the regulations of The Central Committee on Research Involving Human Subjects (CCMO, The Hague, The Netherlands). The study has been registered at ClinicalTrials.gov (NCT05194527).

This study will be conducted according to the principles of the Declaration of Helsinki (adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and

amended by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013) in accordance with the WMO. Study results will be disseminated via open-access peer-reviewed scientific journals and national and international conferences.

Funding statement

This study is funded by the MLDS, Amersfoort (grant number D17-14).

Acknowledgments

We want to thank S.M.J. van Kuijk for his statistical insights regarding the study protocol. In addition, we would like to thank Professor C.H.C De Jong for his indispensable work investigating the complex multifactorial background of AMI, which led to the discovery of new potential biomarkers for future clinical applications and improving patients' clinical outcomes.

Authors' Contributions

KL and TL originated the study. AD, KL, LC, ~~and~~ TL, and DMIS were involved in the study design. AD, KL, and TL drafted the manuscript. AD, RG, MC, RG, JD, JV, HB, SOD~~D~~, FSJ~~J~~, LC, TL, and KL are local investigators at the participating centres. The

study is supervised and coordinated by AD, KL, and TL. All authors provided essential feedback to the successive manuscript versions and approved the final version.

Conflicts of Interest

None declared.

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ABBREVIATIONS

AMI:	Acute Mesenteric Ischemia
AUC	Area Under Curve
CT(A):	Computed Tomography (angiography)
CRF	Case Report Form
EDC	Electronic Data Capture
ELISA	Enzyme-Linked Immunosorbent Assay
GS-TOF-MS:	Gas chromatography time-of-flight mass spectrometry
I-FABP:	Intestinal Fatty Acid Binding Protein
MLDS:	'Maag Lever Darm Stichting' (Dutch Digestive Foundation)
PREOP:	Pre-operative
RPF:	Reperfusion
ROC	Receiver Operating Characteristic
SM22:	Smooth Muscle 22
VIL-1:	villin-1

VOC: Volatile Organic Compounds

FIGURES LEGENDS

Figure 1: Study outline. Patients with a clinical suspicion of acute mesenteric ischemia are considered applicable for participation if all criteria are met. Patients are screened and asked for study participation by informed consent. When consent has been received, study procedures will start. Part 1: blood and exhaled air is collected every 60 minutes (min), up to 180 min (T180) after inclusion; Part 2 is initiated if the patient receives an intervention (endovascular or surgical), with a pre-operative and post-operative sample collection. Patients that do not receive the intervention will directly move into Part 3: Follow-up. Daily samples up to five days (D5) will be retrieved during routine blood collection. In the final stage of the study (Part 4: Diagnosis), each patient will be placed in one of the two study groups (AMI versus non-AMI) based on the collected data. T: time point; PRE: pre-operative; POST: post-operative.

Text box 1: Inclusion and exclusion criteria

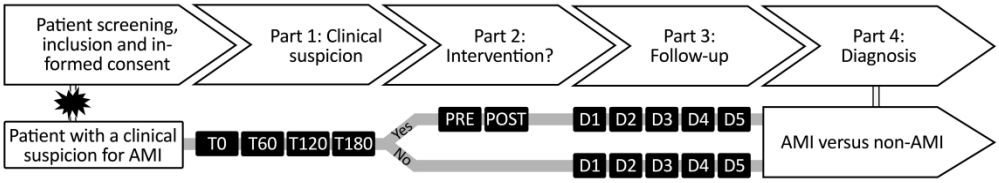
Inclusion criteria

- The ability to provide informed consent, either by themselves or by a legal representative
- The patients must be suspected of AMI, which is based on the following;

- **Clinical manifestation of the disease** such as sudden abdominal pain, nausea, vomiting, abdominal distension, diarrhoea, haematochezia, haematemesis, tenderness and signs of peritonitis
- **Physical examination** by the local physician such as body temperature, heart rate, blood pressure
- **Laboratory measurements** such as white blood cell count, lactate, pH, CRP
- Physician's consideration to perform CT(A) scan

Exclusion criteria

- Unable to provide informed consent
- < 18 years of age



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Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	1, 4
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	4-5
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	7-9
	4	Study objectives and hypotheses	10-11
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	10-11
Participants	**6**	Eligibility criteria	10
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	10-11
	8	Where and when potentially eligible participants were identified (setting, location and dates)	10-11
	9	Whether participants formed a consecutive, random or convenience series	10-11
Test methods	**10a**	Index test, in sufficient detail to allow replication	10-12
	10b	Reference standard, in sufficient detail to allow replication	10-12
	11	Rationale for choosing the reference standard (if alternatives exist)	10-12
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	10-12
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	10-12
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	10-12
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	10-12
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	12-16
	15	How indeterminate index test or reference standard results were handled	12-16
	16	How missing data on the index test and reference standard were handled	12-16
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	12-16
	18	Intended sample size and how it was determined	12-16
RESULTS			
Participants	**19**	Flow of participants, using a diagram	10
	20	Baseline demographic and clinical characteristics of participants	11
	21a	Distribution of severity of disease in those with the target condition	7-19, 15
	21b	Distribution of alternative diagnoses in those without the target condition	7-19, 15
	22	Time interval and any clinical interventions between index test and reference standard	N.A.
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	N.A.
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	15-16
	25	Any adverse events from performing the index test or the reference standard	15
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	6
	27	Implications for practice, including the intended use and clinical role of the index test	6-9
OTHER INFORMATION			
	28	Registration number and name of registry	5, 17
	29	5, 17	5, 17
	30	Sources of funding and other support; role of funders	5, 17

STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	4-5	
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	7-9	
Objectives	3	State specific objectives, including any prespecified hypotheses	7-10	
Methods				
Study design	4	Present key elements of study design early in the paper	10	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	10-18	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants		(A) Patients clinically suspected of AMI will be screened for inclusion at one of the participating centres. All study participants must fulfil the study inclusion criteria and will be excluded from participation if they cannot provide written informed consent or do not fulfil the inclusion criteria (Text Box 1: Inclusion and exclusion criteria). Patient are eligible for study participation if they have a clinically suspicion of AMI, which is based on (1) the clinical manifestation of the disease, (2) physical examination by the local physician, (3) laboratory measurements and (4) the physician's consideration to perform a CT(A)-scan. If all criteria are

			met, the physician will contact the local research team and the patient or legal representative will be asked for informed consent to participate in the study. Clinical study procedures are initiated when all criteria are met and informed consent is obtained from the patient or legal representative. After inclusion, blood and exhaled breath will be collected at consecutive time points
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	13-14, 16
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*10-16*
Bias	9	Describe any efforts to address potential sources of bias	*10-16*
Study size	10	Explain how the study size was arrived at	15

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	16
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	16
		(*b*) Describe any methods used to examine subgroups and interactions	16
		(*c*) Explain how missing data were addressed	16
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	16
		(*e*) Describe any sensitivity analyses	16
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	10,15
		(c) Consider use of a flow diagram	10,11
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	N.A.
		(b) Indicate number of participants with missing data for each variable of interest	16
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	10-12
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*10-14*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	N.A
		(*b*) Report category boundaries when continuous variables were categorized	N.A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N.A.

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N.A
Discussion			
Key results	18	Summarise key results with reference to study objectives	N.A.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	6
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	6
Generalisability	21	Discuss the generalisability (external validity) of the study results	6
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.