Evidence based interpretation of biomarkers in patients with chest pain - WESTCOR: Study design

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	inTerpretation of Cardiac biOmarkers in patients pResenting with chest pain (WESTCOR-study) (Clinical Trials number NCT02620202) is to improve diagnostic pathways for patients presenting to the Emergency department (ED) with acute chest pain. Design: The WESTCOR-study is a two center, cross-sectional and prospective observational study recruiting unselected patients presenting to the ED with suspected non-ST elevation acute coronary syndrome (NSTE-ACS). Patient inclusion started September 2015 and we plan to include 2250 patients, finishing in 2019. The final diagnosis will be adjudicated by two independent cardiologists based on all available information including serial high sensitivity cardiac troponin measurements, coronary angiography, coronary CT angiography and echocardiography. The study includes one derivation cohort (N=985) that will be used to develop rule out /rule in algorithms for NSTEMI and NSTE-ACS (if possible) using novel troponin assays, and to validate established NSTEMI algorithms, with and without clinical scoring systems. The study further includes one subcohort (n=500) where all patients are examined with coronary CT angiography independent of biomarker status, aiming to assess the associations between biomarkers and the extent and severity of coronary atherosclerosis. Finally, an external validation cohort (N=750) will be included at Stavanger University Hospital. Prospective studies will be based on the merged cohorts. Conclusion: The WESTCOR study will provide new diagnostic algorithms for early inclusion and exclusion of NSTE-ACS and insights in the associations between cardiovascular biomarkers, CT-angiographic findings and short and long-term clinical outcomes.

Aiming towards evidence based interpretation of cardiac biomarkers in patients presenting with chest pain - the WESTCOR study: Study design

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Chest pain; acute coronary syndrome; cardiovascular biomarkers; rule in/rule out algorithms; troponin; NSTEMI; unstable angina pectoris.

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Disclosure statement

Kristin M Aakre has served on one advisory board for Roche Diagnostics. Torbjørn Omland has served on advisory boards for Abbott Diagnostics, Roche Diagnostics, and Novartis, and has received research support from AstraZeneca, Abbott Diagnostics, Roche Diagnostics, ThermoFisher, Singulex and Biomedica via Akershus University Hospital, and speaker's honoraria from Roche Diagnostics and Novartis. Øyvind Skadberg has received lecture fees from Abbott Diagnostics. Paul Collinson has served on an advisory board for Siemens Health Care.

Abstract

Objectives: The main aim of the Aiming toWards Evidence baSed inTerpretation of Cardiac biOmarkers in patients pResenting with chest pain (WESTCOR-study) (Clinical Trials number NCT02620202) is to improve diagnostic pathways for patients presenting to the Emergency department (ED) with acute chest pain.

Design: The WESTCOR-study is a two center, cross-sectional and prospective observational study recruiting unselected patients presenting to the ED with suspected non-ST elevation acute coronary syndrome (NSTE-ACS). Patient inclusion started September 2015 and we plan to include 2250 patients, finishing in 2019. The final diagnosis will be adjudicated by two independent cardiologists based on all available information including serial high sensitivity cardiac troponin measurements, coronary angiography, coronary CT angiography and echocardiography. The study includes one derivation cohort (N=985) that will be used to develop rule out /rule in algorithms for NSTEMI and NSTE-ACS (if possible) using novel troponin assays, and to validate established NSTEMI algorithms, with and without clinical scoring systems. The study further includes one subcohort (n=500) where all patients are examined with coronary CT angiography independent of biomarker status, aiming to assess the associations between biomarkers and the extent and severity of coronary atherosclerosis. Finally, an external validation cohort (N=750) will be included at Stavanger University Hospital. Prospective studies will be based on the merged cohorts.

Conclusion: The WESTCOR study will provide new diagnostic algorithms for early inclusion and exclusion of NSTE-ACS and insights in the associations between cardiovascular biomarkers, CT-angiographic findings and short and long-term clinical outcomes.

Introduction

Despite reduced incidence and improved therapies non ST- elevation acute coronary syndrome (NSTE-ACS) remains one of the leading causes of death in the industrialized world [1]. Internationally do 6-10% of patients who are admitted to the ED have symptoms suggestive of acute coronary syndrome (ACS) [2, 3]. Only a minor proportion have ACS [4, 5], apparently15-20% are discharged with a final diagnosis of myocardial infarction, while another 10-15% are diagnosed with unstable angina pectoris (UAP) [6, 7]. Numerous strategies to improve the efficiency of the diagnostic pathway of patients presenting with acute chest pain have been published [8, 9, 10, 11]. While ST elevation myocardial infarction (STEMI) is identified based on specific electrocardiogram (ECG) changes, the European Society of Cardiology (ESC) currently advocates specific troponin-based algorithms for early rule out and rule in of non-STEMI (NSTEMI) [12]. Evaluation of these algorithms shows that a small percentage of NSTEMI patients mistakenly are ruled out [13], and this percentage might be unacceptable high to some clinicians [14]. Finally, UAP is diagnosed based on clinical history, laboratory results, ECG and imaging [8]. UAP is associated with a more favorable prognosis compared to NSTEMI, but still need in-hospital diagnosis and follow-up [15, 16, 17]. Faster and more accurate diagnostic pathways for UAP is beneficial from an individual patient and health care provider perspective.

Aims of the WESTCOR study

First, we will develop algorithms for rule out and rule in of NSTEMI using novel troponin assays and possibly also new myocardial necrosis biomarkers. We will investigate whether already published troponin based algorithms used alone or in combination with clinical risk scores might reduce the number of incorrectly ruled out NSTE-ACS patients, and incorrectly ruled in patients with non-coronary chest pain. Second, we will search for and validate novel

biomarkers or algorithms for diagnosing UAP. Third, we will investigate if novel biomarkers may predict significant obstructive coronary artery disease as diagnosed with coronary computed tomography angiography (CCTA). The fourth and last aim is to investigate the ability of different biomarkers to predict long-term mortality and cardiovascular risk.

Study organization and ethics

The WESTCOR study is a collaborative project including Haukeland University Hospital (Bergen) and Stavanger University Hospital *(Stavanger)*. The study is chaired by a steering committee. The Regional Committees for Medical and Health Research Ethics in Norway has approved the study and biobank (2014/1365 REK vest and 2014/1905 REK vest), and the study is registered at Clinical Trials (NCT02620202).

Materials and methods

Study design

The study is a two-center, cross-sectional prospective observational study. The study plans to include 2250 patients, divided into three different cohorts (figure 1). The WESTCOR derivation cohort (WESTCOR-D) will include approximately 1000 patients. The data will be used to develop novel algorithms (see Supplemental data, table 1) and for validation of already suggested rule out/rule in algorithms (see Supplemental data, table 2) *for* NSTEMI, NSTE-ACS and short-term major adverse cardiovascular events *(MACE) [9, 18],* and for prediction of long-term cardiovascular endpoints. The second cohort is the WESTCOR-CT cohort including 500 patients who will have a CCTA performed as part of the study protocol, unless clinically contraindicated, (constrast allergy, decompensated heart failure and a eGFR below 30 ml/min/1.73m^2). The pulse frequency needs to be below 60 beats per minute.

The data will be used for investigation and validation of novel biomarkers for diagnosing significant coronary artery stenosis and arteriosclerosis. The WESTCOR-D and WESTCOR-CT cohorts are recruited at Haukeland University Hospital using high-sensitive c-TnT (5th gen, Roche Diagnostics) as routine clinical test. The last subcohort (the WESTCOR validation cohort (WESTCOR-V)) will recruit 750 patients at Stavanger University Hospital and utilize hs-cTnI (Abbott Diagnostics) as the clinical routine test for adjudication. Finally, we will merge the three cohorts and validate different algorithms in subgroups of patients. Prediction of long-term endpoints may also be undertaken based on the total data set.

Study enrollment and bio-banking

Norway has large rural areas and a general practitioner (GP) commonly evaluates patients with acute conditions before they are referred to the ED, while other patients come directly to ED after contacting the emergency service. A Norwegian study showed that most chest pain patients who present to the GP are referred to the hospital [19]. All patients with suspected NSTE-ACS are potentially eligible for inclusion in the study (Table 1), and receive oral information about the study upon arrival. After oral consent is given blood is drawn for the biobank at arrival, and after 1 (approximately 2/3 of the patients), 3 and 8-12 hours. Troponin results obtained after 1 hour are by design not reported to the attending clinicians. Full study information and written consent are obtained when the clinical situation is stabilized. Patients who do not wish to participate after reading the study information (less than 1% of those enrolled to date) are immediately withdrawn from the study and their samples destroyed.

Recruitment started in September 2015. Up to October 2018 1280 patients has been enrolled at Haukeland University Hospital and 250 patients at Stavanger University Hospital, this

corresponded to 8 patients per week for Haukeland University Hospital, approximately 20-25% of the anticipated recruitment rate. Low inclusion rate is due to competing pressures on staff.

Diagnosis

Two independent cardiologists adjudicate the final diagnosis based on all available clinical, routine laboratory, ECG, ultrasound and imaging findings, including CCTA and conventional angiography. A third adjudicator resolves disagreements.

Specific diagnostic criteria are predefined for 22 different medical conditions based on current guidelines (See supplemental data). NSTEMI and UAP are defined according to the third universal definition for MI [20], and a 20-50% change in troponin concentration is regarded as a significant change as suggested by ESC in 2012 [21] (supplemental data). Clinical information needed to calculate a large number of risk scores (e.g. HEART, EDAC, GRACE, TIMI) are reterospective collected from the patients files.

Follow-up and end points

Three months after admission, all patients receives a letter inviting them to have a blood sample drawn and to fill out a questionnaire (including Seattle Angina Score, Rose Dyspnoea Score, RAND-12 and Hospital Anxiety and Depression Scale). Further follow-up is undertaken through national health care registers; the Norwegian Patient Register and Norwegian Cause of Death Registry. The following end points will be recorded 1 and 5 years after admission: total mortality, and the incidence of MACE defined as cardiovascular death, MI, UAP, stable angina (requiring hospitalization), revascularization, stroke, heart failure and cardiac arrhythmias.

Statistics

The baseline characteristics will be analyzed using ordinary descriptive statistics, including parametric (Student's t-test) and non-parametric (Mann Whitney U test) statistical tests for continuous variables and Chi-square or Fisher Exact test for categorical variables, as appropriate. The efficiency and safety of different biomarkers or biomarker panels as rule out and rule in markers will be compared using ordinary descriptive statistics. Statistical analyses will include calculation of sensitivity, specificity, positive and negative predictive value, and likelihood ratios, as well as area under the receiver operating characteristics curve (AUC-ROC). Differences in AUC-ROC will be evaluated using the Delong test. C-statistics will be used to measure the incremental prognostic information of different biomarkers by multivariate logistic and Cox proportional hazard regression analysis adjusting for established risk indices and biomarkers for prognosis. When applicable we will also calculate net reclassification index and a risk score that include established risk indices for prognosis.

Sample size and power calculations

Sample size and power calculations were targeted towards the ability of biomarkers or algorithms to diagnose NSTEMI or NSTE-ACS with a power of at least 80%. A difference between two different methods of 5% for sensitivity, 5% for specificity [22] and 0.03 in AUC was thought to be clinically meaningful. To be able to discover a 5 % difference in sensitivity or specificity, a total of 355 patients must be included (McNemar`s test). To have a power of 80% to detect a difference in AUC of 0.03 (e.g. from 0.92 to 0.95) (Delong test; rank correlation between tests set to 0.9, ratio between negative and positive subjects set to 8) 92 patients with the condition (NSTEMI or NSTE-ACS) and 736 subjects without the condition, a total of 828 patients need to be included. To do a subgroup analysis e.g. in a cohort of acute chest pain patients with chronic kidney disease (CKD) we estimated that the prevalence of NSTEMI in a

CKD population would be 35% [23]. To have a power of 80% to detect a difference in AUC of 0.03 (i.e. from 0.87 to 0.90) (Delong test; rank correlation between tests set to 0.9, ratio between negative and positive subjects set to 1.9) 141 NSTEMI and 263 patients without NSTEMI needed to be included (totally 404). If the prevalence of CKD in the total population is 18%, totally 2250 patients must be included.

Results

The first 985 of the included patients have been adjudicated, baseline characteristic are shown in table 2.

Discussion

The high sensitivity troponin assays have improved the diagnostic pathways for NSTEMI, with faster identification and better sensitivity as the main outcome [11, 24, 25, 26]. Even so, there are still important challenges that limit the efficiency of acute investigations of possible NSTE-ACS.

The first challenge is that patients with myocardial ischemia without necrosis (UAP) cannot be accurately identified using troponin measurement or the ECG alone or in combination. The second challenge is that troponin is not a specific marker of ischemic myocardial injury. Stable increases are seen in chronic diseases like kidney disease and multi-morbid conditions. Transient increases are seen in a range of conditions including atrial fibrillation, exacerbation of chronic obstructive pulmonary disease, sepsis, acute stroke, burn injury and strenuous physical activity [27]. Many of these conditions have clinical symptoms resembling acute coronary ischemia. Consequently, large proportions of patients are in need of additional investigations (often imaging) to distinguish NSTE-ACS from non-coronary chest pain or non-

coronary myocardial injury [28, 29]. The last challenge is that although troponins are specific for myocyte necrosis in the clinical setting of coronary ischemia, they provide no information of the underlying pathophysiology causing ischemia and necrosis. Even in NSTEMI patients, troponins cannot distinguish between atherosclerosis, and other often more rare causes of ischemia like spontaneous coronary dissection, coronary spasm or oxygen supply/demand imbalance as the cause of the MI. Improved knowledge of the underling mechanisms for ischemia in general and atherosclerosis in particular is necessary to develop new and targeted treatments for both acute and stable coronary artery disease.

Different diagnostic algorithms currently suggested for early rule out/rule in of NSTEMI

A substantial number of algorithms are published for early rule out and rule in of NSTEMI (See supplemental tables, table 2A, B and C). Of these, the rule-out algorithms are the most important to validate, since early, correct discharge of non-diseased individuals will have a large impact on health care expenditure, and erroneous rule-out of NSTEMI patients may cause serious harm to the patient. An earlier study found that clinicians would accept a false rule out rate for MACE of 0.5 to 1% [14], meaning that rule-out algorithms should have a sensitivity for NSTEMI of at least 99%, and high negative predictive value. The different algorithms for ruling out NSTEMI have a sensitivity ranging from 89.5% to 100% when tested in different populations.

Lower sensitivity for ischemic coronary artery disease should be expected if troponins were used in an algorithm developed to diagnose UAP, NSTE-ACS or short-term MACE compared to diagnosing NSTEMI. However, using troponin assays with improved analytical sensitivity and/or lower analytical variation combined with optimally adapted clinical scoring systems, may show improved sensitivity.

Rule in algorithms are intend to route critically ill NSTE-ACS patients directly to coronary care units and should consequently have high specificity and positive predictive value. Usually they are less accurate compared to rule out pathways, with specificity ranging from 75% to 100% (See supplemental data, table 2C). When tested in different populations the rule out and rule in algorithms have very different efficiencies for diagnostic clarification of patients, ranging between a total of 21% to 80% [30, 31, 32]. The reason for this is probably the heterogeneity of the chest pain populations included in the different studies.

Different biomarkers used for identification of NSTEMI, UAP and coronary artery disease

High sensitivity troponin assays have been available since 2009 [33, 34], and novel assays are still released. Another recently available biomarker of myocardial necrosis is cardiac Myosin binding protein C [35]. Whether this marker is superior to troponins for diagnosing MI and confers incremental prognostic information, needs further investigation. Recently, a multimarker approach including midkine, adiponectin, apolipoprotein C-I, and kidney injury molecule–1 could predict obstructive coronary artery disease ≥ 70% stenosis with a positive predictive value of 90% [36]. Furthermore, different microRNAs have been suggested as potential diagnostic biomarkers for NSTE-ACS [37]. Analysis of components of Neutrophil Extracellular Traps has shown promising results for investigation of the pathophysiology and mechanisms that lead to atherosclerosis [38]. Measurement of these and other novel biomarkers may be possible in the WESTCOR-study.

Strengths and limitations of the WESTCOR-study

An important strength of the WESTCOR-study is that the patients have three to four troponin measurements, ensuring a minimum observational time in hospital of 8 hours, increasing the validity of the clinical diagnosis. The study closely mirrors clinical practice, by not excluding

patients with end stage renal disease or patients with more than a 12 hour history of symptoms suspicious of NSTE-ACS. Further, investigations with CCTA are scheduled in a high proportion of included patients, which also adds certainty to the clinical diagnosis and furthermore enables us to investigate biomarkers that may predict coronary artery stenosis. The follow-up blood sample and clinical data registered 3 months after admission, permits monitoring of long-term dynamics in troponin concentrations. The study takes advantage of the high quality health care registers that are available in Norway, and register follow-up data at least up to five years after inclusion.

The limitations are that only Norwegian centers are included. A Norwegian study showed that 13% of the patients admitted to the ED had chest pain [39]. This is higher than internationally (e.g. 6-10%) [2, 3]. The reason is probably that Norwegian GPs will treat some non-cardiac acute conditions locally while most chest pain patients are referred [19], increasing the proportion of chest pain patients in Norwegian EDs. Another limitation is the relatively low inclusion rate due to ward personal not being able to priority the study during busy periods in the ER. This indicates that not all eligible patients are recruited. This is a common problem for this type of study, however the ACS rate and patient characteristics in WESTCOR are similar to comparable studies [11, 40]. The last limitation is that not all patients in the WESTCOR-CT cohort will be able to undergo CCTA since clinical contraindications prevent preforming the investigation for some individuals.

Conclusion

Most previous studies has not explored the abilities to diagnose UAP, NSTE-ACS or short-term MACE. We are conducting a cross-sectional and prospective observational study with wide inclusion criteria in order to reflect chest pain patients admitted to the ED in routine clinical practice. This study will provide new diagnostic algorithms for early inclusion and exclusion

of NSTE-ACS and insights in the associations between cardiovascular biomarkers, CT-angiographic findings and short and long-term clinical outcomes Adjudication of the NSTE-ACS diagnoses, and the ability to assess long-term prognosis utilizing one follow-up sample and high-quality health care registers are important strengths of the WESTCOR-study.

Figure legends

Figure 1: *Flow chart outlining the study design.*

Table 1. Inclusion and exclusion criteria.

INCLUSION CRITERIA
Patients admitted with chest pain suspicious of NSTE-ACS
Age >18 years
EXCLUSION CRITERIA
Patients with STEMI
Patients transferred from other wards or hospitals for second opinion
Comatose or other reasons for not being able to consent
Terminal patients, short life expectancy

Table 2. Baseline characteristics of the 985 first included patients in the WESTCOR study. Continuous variables are reported as median values (25-75 percentiles in brackets) and categorical variables as number of patients (percentages in brackets).

Baseline characteristics	**Total N=985**	**ACS n=237**	**Non ACS n=748**	**P-value**
Age in years	63 (52.0-74.0)	69 (59.0-78.0)	61 (50.0-73.0)	<0.001
Male gender	600 (60.6)	171 (72.2)	427 (57.1)	<0.001
Time from symptom onset to first troponin sample in hours	8.1 (3.4-46.0)	8.7 (3.2-47.2)	8.0 (3.4-46.1)	0.756
Risk factors				
Hypertension	409 (41.5)	120 (50.6)	289 (38.6)	0.003
Hypercholesterolemia	179 (18.5)	55(23.5)	124 (16.9)	0.002
Diabetes mellitus	121 (12.3)	51 (21.5)	70 (9.4)	<0.001
Current smoker	204 (20.7)	42 (17.5)	160 (21.7)	<0.001
History of smoking	410 (41.4)	127 (52.9)	283 (37.8)	<0.001
Family history of ischemic heart disease	192 (19.5)	42 (17.7)	150 (20.1)	0.479
Previous MI	205 (20.8)	76 (32.1)	129 (17.2)	0,001
Previous PCI	207 (21.0)	81 (34.2)	126 (16.8)	<0.001
Previous CABG	82 (8.3)	44 (18.6)	38 (5.1)	<0.001

Previous peripheral vascular disease	22 (2.2)	12 (5.1)	10 (1.3)	0.001
Previous Stroke	27 (2.7)	9 (3.8)	18 (2.4)	0.381
Baseline drugs				
Statins	382 (38.8)	115 (48.5)	267 (35.7)	<0.001
Diuretics	177 (18.0)	50 (21.1)	127 (17.0)	0.150
ACE inhibitor/A2 blocker	331 (33.6)	94 (39.7)	237 (31.7)	0.067
Beta-blocker	339 (34.4)	104 (43.9)	235 (31.4)	0.002
Aspirin	342 (34.7)	123(51.9)	219 (29.3)	<0.001
Oral Anticoagulant	118 (11.9)	22 (9.3)	96 (12.8)	0.142
Antithrombotic agents	72 (7.4)	30 (12.5)	42 (5.7)	<0.001
Baseline measurements				
BMI, kg/m^2	26.3 (24.2-29.5)	25.8 (24.1-29.1)	26.6 (24.2-29.7)	0.222
HEART score	4.0 (3.0-5.0)	6.0 (5.0-7.0)	3.0 (2.0-4.0)	<0.001
HbA1c, %	5.6 (5.4-5.9)	5.8 (5.3-5.9)	5.6 (5.3-5.9)	<0.001
eGFR, ml/min/1.73m^2	85.4 (70.3-97.1)	79.5 (64.0-79.6)	86.3 (72.0-98.5)	<0.001
cTnT, ng/L	7.0 (3.0-18.0)	22.0 (9.0-63.0)	6.0 (3.0-12.0)	<0.001

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SUPPLEMEMENTAL DATA

TABLES

Table 1. Examples of how different pre-specified single sample concentrations may be tested as rule out algorithm for NSTEMI using a novel high sensitive troponin assay. Percentage of patients ruled out for NSTEMI are calculated for each concentration (first column) and each diagnosis (first row). The Limit of Detection (LoD) rounded to the nearest whole number. The concentration yielding the lowest percentage of ruled out NSTEMI patients and the highest percentage of ruled out non-cardiac chest pain patients will be the most favourable.

Concentration tested	**NSTEMI**	**UAP**	**Non-ACS cardiac disease**	**Non-cardiac chest pain**	**Other diseases**	**Total**
LoD						
LoD + 1 ng/L						
LoD + 2 ng/L						
LoD + … continuing up to 99th percentile of the assay or further as applicable						

Table 2A.The table shows currently suggested single sample and two sample (i.e. 1 and 3 hour) rule out algorithms for AMI, acute coronary syndrome and 30-day MACE.

Rule-out method	**Assay**	**Criteria**	**Sensitivity, %**	**NPV, %**	**Primary outcome**	**Study**	**Ref.**
cTn_{0h} / cTn_{3h}	Abbott Roche	TnI_{0-3} <26 ng/L TnT_{0-3} <14 g/L	Not given	98-100	Rule out ACS	2015 ESC Guidelines	[1]
cTn_{0h} / cTn_{3h}	Abbott Roche	TnI_{0-3} < 26 ng/L TnT_{0-3} < 14 ng/L	93.2 94.8	99 99	Rule out AMI	ADAPT/ ADP/ EDACS/ RING	[2]
cTn_{0h} / cTn_{3h}	Abbot	Time from symptom onset > 2 h and TnI_0 <5 ng/L and $TnI_{\Delta 0-3}$ <3 ng/L	99.7	99,5	Rule out AMI and 30- day MACE	High-STEACS	[3]

cTn_{0h} / cTn_{1h}	Abbot Roche	Time from symptom onset > 3 h and TnI_0 <2 ng/L or TnI_0 <5 ng/L and $TnI_{\Delta 0\text{-}1}$ <2 ng/L Time from symptom onset > 3 h TnT_0 <5 ng/L or TnT_0 <12 ng/L and $TnT_{\Delta 0\text{-}1}$ <3 ng/L	98.8 97.1	99.8 99.5	Rule out AMI	ADAPT/ ADP/ EDACS/ RING	[4]
cTn_{0h} / cTn_{1h}	Abbot	TnI_0 <5 ng/L and $TnI_{\Delta 0\text{-}1}$ <2ng/L TnI_0 <3 ng/L and $TnI_{\Delta 0\text{-}1}$ <5 ng/L	98,8 97.8-100	99,6 98.8-100	Rule out AMI	APACE *	[5]
cTn_{0h} / cTn_{1h}	Siemens	TnI_0 <5 ng/L and $TnT_{\Delta 0\text{-}1}$ <2ng/L	100	100	Rule out AMI	APACE*	[6]
cTn_{0h} / cTn_{1h}	Abbott	Time from symptom onset > 3 h and TnI_0 <2 ng/L Or TnI_0 <5ng/L and $TnI_{\Delta 0\text{-}1}$ <2ng/L	98.4 98.4	99.5 99.5	Rule out AMI	APACE	[7]

cTn_{0h}	Abbott	TnI_0 <4 ng/L and glucose <6.6mmol/L	100	100	Rule out AMI within 7 days of presentation	ROMI-3	[8]
		TnI_0 <5 ng/l and glucose <6.6 mmol/L	99.2	99.7			
		TnI_0 <5 ng/l and glucose <6.6 mmol/L And HbA1c >6,5%	99.2	99.7			
		TnI_0 <5 ng/L	97.0	99.2			
cTn_{0h}	Roche	TnT_0 <24 ng/L and glucose <5.6 mmol/L.	99.2	99.6			
		TnT_0 <24 ng/L and glucose <5.6 mmol/L and HbA1c <6.5%	99.2	98.3			
		TnT_0 <14 ng/L	92.5	98.3			

		TnT_0 <14ng/L and glucose <5.6mmol/L	100	100			
cTn_{0h}	Roche	TnT_0 <3 ng/L TnT_0 <5 ng/L TnT_0 <14 ng/L	97,4 97,4 89,5	96,9 96,9 96,5	Rule out AMI	Meta analysis	[9]
cTn_{0h}	Abbot	TnI_0 <5 ng/L	98	99.5	Index Myocardial Infarction or Cardiac Death at 30 Days	Meta Analysis	[10]
cTn_{0h}	Abbot	TnI_0 <LOD (2ng/L) TnI_0 <5 ng/L	100 97.1	100 99.1	Rule out AMI	APACE	[7]

cTn_{0h}	Abbott	TnI_0 <LoD (1,9ng/L)	98,8	99,6	Rule out AMI 30- day MACE	UTROPIA / High-STEACS	[11]
		TnI_0 <LoD (1,9ng/L) + normal ECG	99,4	99,6			
		High-STEACS <(5ng/L)	94,7	98,9			
		High-STEACS < (5ng/l) + normal ECG	99,5	98,8			

*Validation cohort

Table 2B. The table includes currently suggested rule out algorithms combining biomarkers and clinical risk scores for AMI, 30 days MACE and acute coronary syndrome.

Rule-out method	**Assay**	**Criteria**	**Sensitivity, %**	**NPV, %**	**Primary outcome**	**Study**	**Ref.**
cTn<LOD and TIMI score at 0 hours	Roche	$TnT_0 \leq$ LoD (<5 ng/L) and TIMI score=0	98.7	99.6	30-day MACE	ADAPT/ IMPACT/ ADAPT-ADP/ EDACS-ADP/ TRUST	[12]
		$TnT_0 \leq$ LoD (<5 ng/L) and TIMI≤1	98.4	99.5			
		$TnT_0 \leq$ LoD (<5 ng/L) and TIMI≤2	97.4	99.3			
	Abbot	$TnI_0 \leq$ LoD (2 ng/L) and TIMI score=0	98.5	99.5			
		$TnI_0 \leq$ LoD (2 ng/L) and TIMI≤1	98.2	99.6			
		$TnI_0 \leq$ LoD (2ng/L) and TIMI≤2	97.7	99.4			

cTn_{0h} combined with five different risk scores	Roche	Hs-TnT_0 ≤14 ng/L (99 percentile)	83.5	98.3	AMI within 30 days	Post hoc analysis of TRUST	[13]
		m-Goldman Score 0 and hs-TnT_0 ≤14 ng/L	98.7	99.0			
		m-Goldman Score≤1 and hs-TnT_0 ≤14 ng/L	98.7	99.7			
		TIMI score 0 and hs-TnT_0 ≤14 ng/L	100	100			
		TIMI score ≤1 and hs-TnT_0 ≤14 ng/L	94.9	99.2			
		GRACE score <60(Incorporates hs-TnT)	100	100			
		GRACE score <80(Incorporates hs-TnT)	92.3	98.0			
		HEART score ≤2(Incorporates hs-TnT)	98.7	99.2			

		HEART score ≤3(Incorporates hs-TnT)	93.7	98.3			
		Vancouver Chest Pain Rule (Incorporates hs-TnT)	100	100			
cTn_{0h} combined with five different risk scores	Abbot	hs-TnI_0 ≤26.2 ng/L (99 percentile)	62.1	96.9	AMI within 30 days	Post hoc analysis of TRUST	[13]
		m-Goldman Score 0 and hs-TnI_0 ≤26.2 ng/L	98.5	99			
		m-Goldman Score ≤1 and hs-TnI_0 ≤26.2 ng/L	92.8	98.7			
		TIMI score 0 and hs-TnI_0 ≤26.2 ng/L	95.5	99.0			
		TIMI score ≤1 and hs-TnI_0 ≤26.2 ng/L	87.9	98.3			
		GRACE score <60 (Incorporates hs-TnI)	98.5	98.9			
		GRACE score <80(Incorporates hs-TnI)	89.4	97.5			

		HEART score ≤2 (Incorporates hs-TnI)	98.5	99.1			
		HEART score ≤3(Incorporates hs-TnI)	97.0	99.3			
		Vancouver Chest Pain Rule (Incorporates hs-TnT)	100	100			
cTn_{0h} / cTn_{2h} combined with different risk scores	Abbot	ADAPT Pathway $cTnI_{0\text{-}2h}$ ≤18 ng/L No new ischemia on ECG TIMI Score ≤1	92.8	99.1	30 day ACS	ADAPT IMPACT	[14]
		EDACS Pathway $cTnI_{0\text{-}2h}$ ≤18 ng/L No new ischemia on ECG EDACS Score <16	92.1	99.0			

		HEART Pathway $cTnI_{0\text{-}2}$ ≤18 ng/L HEART Score 0-3	95.0	99.2			
		Vancouver Chest Pain Rule	98.6	99.6			
		NOT rule $cTnI_{0\text{-}2h}$ ≤18 ng/L No new ischemia on ECG NOT Score =0	99.3	99.8			
cTn_{0h} / cTn_{2h} combined with different risk scores	Abbot	ADAPT Pathway $cTnI_{0\text{-}2h}$ ≤18 ng/L No new ischemia on ECG TIMI Score ≤1	96.9	99.7	30 day acute MI	ADAPT IMPACT	[14]

		EDACS Pathway $cTnI_{0-2h}$ ≤18 ng/L No new ischemia on ECG EDACS Score <16	97.9	99.8			
		HEART Pathway $cTnI_{0-2h}$ ≤18 ng/L HEART Score 0-3	97.9	99.8			
		Vancouver Chest Pain Rule	100	100			
		NOT rule $cTnI_{0-2h}$ ≤18 ng/L No new ischemia on ECG NOT Score =0	100	100			

Table 2C. The table includes currently suggested single sample and two sample (i.e. 1 and 3 hour samples) rule in algorithms for AMI, 30 days and all-cause mortality. NC: not calculable. URL: Upper reference limit.

Rule-in method	**Assay**	**Criteria**	**Specificity, %**	**PPV, %**	**Primary outcome**	**Study**	**Ref.**
cTn_{0h} / cTn_{2h}	Roche	$TnT_{0/2}$ >53 ng/L or $TnT_{\Delta 0\text{-}2}$ >10 ng/L	99	85	30 days all-cause mortality	APACE*	[15]
cTn_{0h} / cTn_{1h}	Siemens	TnI_0 >107 ng/L and $TnI_{\Delta 0\text{-}1}$ >19 ng/L	95.6	70.4	Rule in AMI	APACE*	[6]
cTn_{0h} / cTn_{1h}	Abbot Roche	TnI_0 >52 ng/L or $TnI_{\Delta 0\text{-}1}$ >6 ng/L TnT_0>52 ng/L or $TnT_{\Delta 0\text{-}1}$ >5 ng/L	NC NC	75-80 75-80	Rule in AMI	ESC guide lines	[1]
cTn_{0h} / cTn_{1h}	Roche	TnT_0>52 ng/L or $TnT_{\Delta 0\text{-}1}$ >5 ng/L	96.1	77.2	Rule in AMI	TRAPID-AMI	[16]

cTn_{0h}	Abbot	TnI_0 >64 ng/L	97.5	72.8	Rule in AMI within 7 days	ROMI-3	[8]
		TnI_0 >99 ng/L	99	85.3			
		TnI_0 >82 ng/L and glucose >11 mmol/L	99.9	93.8			
	Roche	TnT_0 >206 ng/L	99.5	80.8			
		TnT_0 >206 ng/L and glucose >11 mmol/L	100	100			
		TnT_0 >52 ng/L	92.5	46.8			

* Validation cohort

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SUPPLEMEMENTAL DATA

Diagnostic definitions

Myocardial infarction was defined according to the third universal definition of myocardial infarction.[1]

Detection of a rise and/or fall of cardiac biomarker values (preferably cardiac troponins cTn) with at least one value above the 99th percentile upper reference limit (URL) and with at least one of the following:

- Symptoms of ischemia
- Development of pathologic Q waves in the electrocardiogram (ECG)
- New or presumed new significant ST-segment-T wave (ST-T) changes or new left bundle branch block (LBBB).
- Identification of an intracoronary thrombus by angiography or autopsy
- Imaging evidence of new loss of viable myocardium or a new regional wall motion abnormality

Unstable angina pectoris — UAP: Defined as symptoms suggestive of an ACS without elevation in biomarkers with or without ECG changes indicative of ischemia [2].

Stable angina was defined as typical angina symptoms lasting >1 month without an increase in magnitude, duration or frequency of the pain and a known history of coronary artery disease [3].

Pericarditis was diagnosed if at least two of four diagnostic criteria were present, as defined in several studies: typical pleuritic chest pain, detection of a pericardial rub on auscultation, typical ECG changes, new or increased amount of pericardial effusion on echocardiography [4]. *Myocarditis* was diagnosed according to the position statement of ESC from 2013 [5].

Takotsubo cardiomyopathy was diagnosed with the modified criteria suggested by The Mayo Clinic in 2008 [6].

Heart failure was defined according to the ESC diagnostic criteria of 2016 [7] .

Atrial fibrillation, atrial flutter and other supraventricular arrhythmias were diagnosed by ECG findings and the lack of symptoms and biochemical results supporting another disease.

Aortic stenosis and other valve diseases where diagnosed in accordance with echocardiographic results and a history supporting the valve disease as cause of the symptoms [8].

Myalgia was defined as chest pain provoked by palpation in lack of cardiac disease.

GERD was based on gastroscopic findings, also in the lack of cardiac disease.

Cholecystitis were defined by the Tokyo Guidelines of 2006 while other abdominal diseases where defined according to operative, endoscopic or radiological findings [9].

Pneumonia acquired typical symptoms and a chest X-ray supporting the disease, while the diagnosis of both pulmonary embolism and pneumothorax was based on radiologic results and the lack of concurrent cardiac disease.

COPD was defined in accordance with the criteria by Stephens MB from 2008 [10], while chest pain without any specific clinical, radiologic or biochemical findings where defined as non-specific chest pain.

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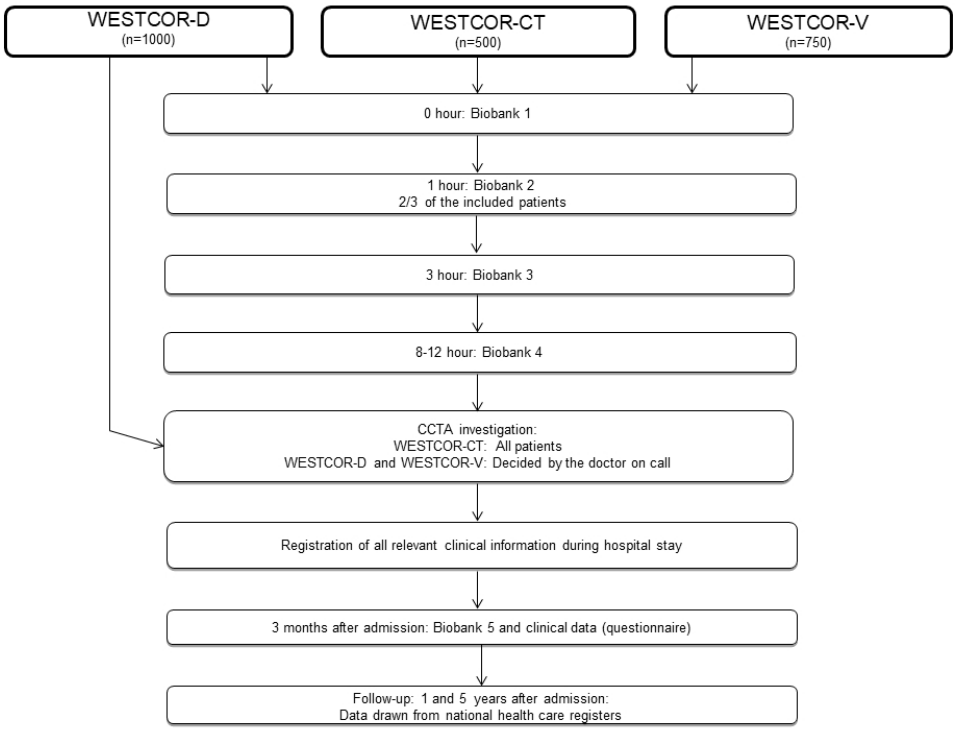

Figure 1: Flow chart outlining the study

254x190mm (96 x 96 DPI)