Aiming towards evidence based interpretation of cardiac biomarkers in patients presenting with chest pain

The WESTCOR study

Hilde Lunde Tjora

Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
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UNIVERSITY OF BERGEN

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Scientific environment

The research was performed within the WESTCOR study group

Department of Clinical Science

University of Bergen

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In cooperation with:

Emergency Care Clinic, Haukeland University Hospital

Department of Heart Disease, Haukeland University Hospital

Department of Heart Disease, Stavanger University Hospital

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UNIVERSITY OF BERGEN
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Preface

Working as a specialist in pulmonary medicine, I often treated patients with severe COPD exacerbations. They had respiratory problems, but many had stable elevated troponin levels in several blood samples. This observation made me both curious and frustrated. I was curious about the mechanisms for elevated troponin levels and frustrated with lacking treatment options. Later on, as a cardiologist and during the work with this Ph.D. thesis, my understanding of troponins broadened. However, there still is a knowledge gap in what to do with patients with stable elevated troponin values.

Acknowledgements

When I asked to participate in the WESTCOR project, my main supervisor, associate professor Kristin Moberg Aakre approved, and I am deeply grateful for that. You have an encouraging and optimistic attitude, the door is always open, and no question is too small to be answered. Statistical questions, feedback on articles, and rehearsals for oral presentations exemplify what you have helped me with over the last years. Even when your summer holiday last year on Svalbard was partly interrupted by helping me answer difficult questions from reviewers on my third paper, you replied to the questions with the same enthusiasm as always.

My co-supervisors have been very helpful during this project. Professor Rune Bjørneklett, when I asked for your help to be a part of the WESTCOR project, you immediately said yes and later as my co-supervisor, you have given me precious advice on different aspects of the process of doing a Ph.D. thesis. Several times we discussed different topics when we met at the coffee machine or when we passed each other outside the offices at the Emergency Care Clinic. Professor Torbjørn Omland, thank you for your precise and critical questions regarding my work, making me reevaluate and improve it. Associate professor Kjell Vikenes, as the head of the Heart Department, the formal meetings in the WESTCOR group have been beneficial for me in several ways. The invaluable discussions we have had in your office with dark chocolate at hand, and also the slight pressure for reporting on the progression, have been important.

Thank you, Professor Paul Collinson, for your valuable and insightful discussions. They have improved my scientific work and my knowledge of the English language.

Thank also to the rest of the members of the WESTCOR group. Ole-Thomas Steiro for fruitful discussions and to Jørund Langørgen. for helping us out with adjudicating when we could not agree. Jørund Langørgens` clinical skills and comments on articles have been highly appreciated. I am grateful for the valuable feedback from Vernon V.S. Bonarjee, Øyvind Skadberg and Øistein R. Mjelva at Stavanger University Hospital.

I wish to thank the head of the Emergency Care Clinic, Anne Taule for facilitating for my scholarship, and my colleagues in the Emergency Care Clinic for a pleasant work environment.

A special thanks to the staff in the Emergency Department and respective wards for including and following up patient in the WESTCOR project.

Finally, I must thank my family for all their support. My parents taught me to be hard-working and independent, and for that, I am grateful. Erling, my best friend and husband, you mean the world to me. The support you have given me during this project is invaluable. Living with you and our three beloved daughters, Idunn, Tale, and Elisabeth, matters the most.

Abbreviations

ACC: American College of Cardiology

ACCF: American College of Cardiology Foundation

ACS: Acute coronary syndrome

ADP: Accelerated diagnostic protocol

AHA: American Heart Association

AUROC: Area under the Receiver Operating Characteristic

CABG: Coronary artery bypass graft

CAD: Coronary artery disease

CARE: Characteristics, Age, Risk factors, ECG

CCTA: Cardiac computed tomographic angiography

CK: Creatine kinase

CK-MB: Creatine kinase-myocardial band

cTn: Cardiac troponin

CV_A: Coefficient of variation

CVD: Cardiovascular disease

CV_I: Individual coefficient of variation

ECG: Electrocardiogram

ED: Emergency department

EDACS: Emergency Department Assessment of Chest Pain Score

ESC: European Society of Cardiology

GRACE: Global Registry of Acute Coronary Events

GWAS: Genome-wide association studies

HEART: **H**istory, **E**lectrocardiogram, **A**ge, **R**isk factors, and **T**roponin

High-STEACS: High-**S**ensitivity **T**roponin in the **E**valuation of patients with **A**cute **C**oronary **S**yndrome

hs-cTnI$_{(Abbot)}$: High-sensitivity cardiac troponin I (Abbott Diagnostics)

hs-cTnI$_{(Sgx)}$: High-sensitivity cardiac troponin I (Singulex Clarity System)

hs-cTnT: High-sensitivity cardiac troponin T (Roche Diagnostic)

ISFC: International Society and Federation of Cardiology

LDL: Low-density lipoprotein

LOB: Limit of blank

LOD: Limit of detection

LOQ: Limit of quantitation

MACE: Major adverse cardiovascular events

MI: Myocardial infarction

MONICA: MONItoring of trends and determinants in CArdiovascular disease

NCCP: Non-cardiac chest pain

NPR: Norwegian Patient Register

NPV: Negative predictive value

NSTE-ACS: Non-ST-elevation acute coronary syndrome

NSTEMI: Non-ST-elevation myocardial infarction

POC: Point of care

PPV: Positive predictive value

PCI: Percutaneous coronary intervention

RCV: Reference change value

SD: Standard deviation

STARD: **STA**ndards for the **R**eporting of **D**iagnostic accuracy studies

TIMI: Thrombolysis In Myocardial Infarction

T-MACS: Troponin-only Manchester Acute Coronary Syndromes

UAP: Unstable angina pectoris

UDMI: Universal Definition of Myocardial Infarction

URL: Upper reference limit

WESTCOR-D: WESTCOR Derivation cohort

WESTCOR-SUS: WESTCOR Validation Cohort from Stavanger University Hospital

WESTCOR-V: WESTCOR Internal Validation cohort

WHF: World Heart Federation

WHO: World Health Organization

List of Publications

Paper 1

Tjora HL, Steiro OT, Langørgen J, Bjørneklett R, Nygård OK, Renstrøm R, Skadberg Ø, Bonarjee VVS, Lindahl B, Collinson P, Omland T, Vikenes K, Aakre KM. Aiming toWards Evidence baSed inTerpretation of Cardiac biOmarkers in patients pResenting with chest pain - the WESTCOR study: Study design. Scand Cardiovasc J. 2019 Oct;53(5):280-285.

Paper 2

Tjora HL, Steiro OT, Langørgen J, Bjørneklett R, Nygård OK, Skadberg Ø, Bonarjee VVS, Collinson P, Omland T, Vikenes K, Aakre KM. Cardiac troponin assays with improved analytical quality - a trade-off between enhanced diagnostic performance and reduced long-term prognostic value. J Am Heart Assoc. 2020 Dec;9(23):e017465.

Paper 3

Steiro OT, Tjora HL, Langørgen J, Bjørneklett R, Nygård OK, Skadberg Ø, Bonarjee VVS, Lindahl B, Omland T, Vikenes K, Aakre KM. Clinical risk scores identify more patients at risk for cardiovascular events within 30 days as compared to standard ACS risk criteria. The WESTCOR study. Eur Heart J Acute Cardiovasc Care. 2021 May 11;10(3):287-301.

Paper 4

Tjora Hilde L, Ole-Thomas Steiro, Jørund Langørgen, Rune Bjørneklett, Øyvind Skadberg, Vernon V S Bonarjee, Øistein R Mjelva, Paul Collinson, Torbjørn Omland, Kjell Vikenes, Kristin M Aakre. Diagnostic performance of novel troponin algorithms for the rule-out of non-ST-elevation acute coronary syndrome. Clin Chem. 2022 Feb 1;68(2):291-302.

Abstract

Background: Chest pain patients admitted to the emergency department (ED) with possible acute coronary syndrome (ACS) encompasses about 10% of the ED population, only a relatively small proportion have ACS.

Method: The WESTCOR study is a prospective observational study, including patients with ACS symptoms. Different approaches for predicting major adverse cardiovascular events (MACE) are investigated and compared to European Society of Cardiology (ESC) recommendations: 1) novel 0/1 hour algorithms using a high sensitivity troponin I assay from Singulex (hs-cTnI$_{Sgx}$) with measurable results in >99% of healthy persons. 2) 0/3 hour ESC (2015) and the High-**S**ensitivity **T**roponin in the **E**valuation of patients with **A**cute **C**oronary **S**yndrome (high-STEACS) algorithms combined with the ACS criteria from ESC or eleven different risk scores: 3) novel hs-cTnT and hs-cTnI rule-out algorithms designed with low baseline/low (1-3) hour delta values.

Results: 1) The hs-cTnI$_{(Sgx)}$ baseline value for rule-out of non-ST elevation myocardial infarction (NSTEMI) had significant higher Area under the Receiver Operating Characteristic (AUROC) (0.95 vs. 0.91 for hs-cTnT$_{ESC}$, P<0.001 and 0.93 for hs-cTnI$_{(Abbott)}$, P=0.004, Delong test). The 0/1-hour hs-cTnI$_{(Sgx)}$ algorithms allocated 92% of patients to rule-in/rule-out, while comparator algorithms only ≤ 78%. The 0/1-hour hs-cTnI$_{(Sgx)}$ rule-out algorithm did not provide prognostic information for combined all-cause mortality and future nonfatal MI. 2) Combining 0/3- hour troponin algorithms from ESC or high-STEACS with the ACS criteria, ruled out 3.8-4.9% of patients who experienced MI, all-cause mortality, and revascularization within 30 days. A HEART score ≤3 reduced the event rate to 2.2-2.7%. 3) The clinical sensitivity for the hs-TnT 0/1-hour rule-out algorithm for predicting combined NSTEMI or unstable angina pectoris (UAP) during index hospitalization was 95% vs. 63% for the ESC algorithm (P<0.001)). The rule-out rate for the nove algorithm was significantly reduced, 21.0% vs 82% (P<0.001). The novel algorithm for hs-cTnI$_{(Abbot)}$ had slightly lower sensitivity 87% but better specificity 45%.

Conclusion: This work suggests several novel approaches for improving the diagnostic workup of chest pain patients in the ED.

Abstract in Norwegian

Bakgrunn: Ca 10% av alle pasientar i akuttmottak blir innlagt med brystsmerter og spørsmål om akutt koronarsyndrom (AKS), men under ¼ av pasientane har AKS.
Metode:WESTCOR studien er ein prospektiv observasjonsstudie som inkluderer pasientar i akuttmottak med mistanke om AKS. Ein har brukt ulike vinklingar for å identifisera kardielle endepunkt, og samanliknar endepunkta med anbefalingar frå European Society of Cardiology (ESC): 1) Utvikla 0/1 times algoritmar for eit nytt høg sensitivt troponin I assay frå Singulex (hs-cTnI$_{Sgx}$), 2) Samanlikning av ESC 0/3 timars algoritmar (2015) og High-**S**ensitive **T**roponin i **E**valuering av patientar med **A**kutt **K**oronar **S**yndrom (high-STEACS) algoritmar kombinert med AKS kriteria frå ESC eller elleve ulike risikoskårar, 3) Utvikling av nye hs-cTnT og hs-cTnI algoritmar som nyttar lave slutningsgrenser både for nullprøven og deltaprøvane (1 og 3 timar).
Resultat: 1) Samanlikna med andre troponinmetodar, hadde nullprøven frå hs-cTnI$_{(Sgx)}$ signifikant høgare areal under kurven (AUC) (0.95 versus 0.91 hs-cTnT$_{ESC}$, P<0.001 og 0.93 for hs-cTnI$_{ESC(Abbott)}$, P=0.004, Delong test) for å utelukka non-ST elevasjons myokardial infarkt (NSTEMI). 0/1 timars hs-cTnI$_{(Sgx)}$ algoritmane plasserte 92% av pasientane i enten låg eller høgrisikogruppa for NSTEMI, medan hs-cTnT$_{ESC}$ og hs-cTnI$_{ESC(Abbott)}$ plasserte ≤78%. 0/1 times hs-cTnI$_{(Sgx)}$ algoritmen gav ingen langtids prognostisk information (total mortalitet og MI). 2) Dei to 0/3 timar algoritmane kombinert med AKS kriteria plasserte 3,8-4,9% av pasientane som fekk det kombinerte endepunkt MI, total mortalitet, og revaskularisering innan 30 dagar i lågrisikogruppa. Kombinasjon med HEART score ≤3 reduserte endepunkta i lågrisikogruppa til 2.2-2.7%. 3) Den nye hs-TnT 0/1 times algoritmen hadde ein sensitivitet for det kombinerte endepunktet NSTEMI eller ustabil angina pectoris (UAP) under opphald på 95% versus 63% for ESC algoritmen (P<0.001) Evna til å senda ut pasientar med den nye hs-TnT 0/1 algorithm vart signifikant redusert, 21% versus 82 % (P<0.001). Algoritmen frå hs-cTnI$_{(Abbott)}$ hadde sensitiv på 87% og spesifisitet 45%.
Konklusjon: Dette arbeidet viser nye måtar å vurdera og forbetra diagnostikken av brystsmerterpasientar i akuttmottak.

Contents

1. Introduction

1.1 Background

Atherosclerosis is a complex chronic inflammatory disease that can arise in arteries of any size (1-3). The understanding of the mechanisms underlying its development has been evolving for many millennia. The first known description of angina is from a papyrus roll, the Ebers Papyrus, dated 1550 BCE, and examinations of mummies from the ancient era have revealed the presence of atherosclerosis (4, 5). In the most thorough study to date, the HORUS study, 34% of mummies had arteriosclerosis, and age was a significant factor for development of coronary artery disease (CAD) (5).

In the 15th to 16th centuries, Leonardo Da Vinci tried to correlate symptoms of angina with changes in the arteries of old men (6). During the 19th and 20th centuries, understanding of atherosclerosis developed rapidly, and by the mid-19th century, two theories addressed the pathophysiological process leading to development of atherosclerotic plaques. Virchow proposed that inflammation initiated the atherosclerotic process, whereas von Rokitansky suggested that the cause was a degenerative disease related to passive lipid deposition (6). The latter theory was the leading conceptualization until the end of the 20th century, when inflammation re-emerged as a suspected cause of atherosclerosis.

1.2 Current hypothesis for development of artheriosclerosis

In 1999, Ross's "response to injury" hypothesis proposed that atherosclerosis was an inflammatory disease (7). The pathogenesis is believed to be dominated by oxidative stress and endothelial dysfunction that leads to deposition of atheromatous plaques in the arterial wall. Several factors, such as an unhealthy diet, obesity, hypertension, high lipid levels, and age, influence the magnitude of atherosclerotic lesions (2), and especially oxidation of low-density lipoprotein (LDL) cholesterol particles is believed to play an important role in the development of atherosclerotic plaques (8, 9). Family history also has been regarded as an important risk factor for decades, and in recent

years, genome-wide association studies (GWAS) have confirmed this hypothesis, with more than 200 CAD-associated loci reported. For many but not all of these candidates, an inferred genetic mechanism for atherosclerotic development is known (10).

Treatment options have developed based on this improved knowledge of pathophysiological mechanisms. Lowering cholesterol is one of the main preventive treatment options for atherosclerosis, and the development of statins targeting HMG-CoA reductase and inhibiting in vivo cholesterol production has been crucial (11). More recently the pleiotropic effects of statins have been discovered, including an anti-inflammatory effect (12). GWAS studies have also revealed other mechanisms for lipid lowering e.g. the PCSK9 inhibitor; reducing LDL concentrations by increasing LDL uptake in the liver, and Ezetemib; reducing cholesterol uptake from the gastrointestinal tractus. It is expected that the evolving knowledge from GWAS studies can be used in future risk prediction, risk factor managing and treatment (10, 13). Anti-inflammatory treatment without lowering cholesterol is also effective, as has been highlighted in both the CANTOS and COLCOT studies (14, 15). In the CANTOS study, post-infarction patients treated with the monoclonal antibody canakinumab targeting interleukin-1β had a significantly lower incidence of future major adverse cardiac events (MACE), defined as nonfatal myocardial infarction (MI), nonfatal stroke, or cardiovascular death (14). In the COLCOT study, the use of colchicine after MI significantly reduced the frequency of MACE, defined as death from cardiovascular causes, resuscitated cardiac arrest, MI, stroke, or urgent hospitalization for angina leading to coronary revascularization (15).

1.3 Cardiovascular disease

The World Health Organization (WHO) defines cardiovascular disease (CVD) as coronary heart disease, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease, deep vein thrombosis, and pulmonary embolism (16). The first three are related to arteriosclerosis, and in principle, all organ systems

may be affected, including the kidney, brain, gut, and heart. CVD is the most common cause of death worldwide, accounting for 32% of deaths annually (16), and in Europe, the middle-income countries have the highest burden of CVD (17). CVD usually evolves over a long time span, with minor or moderate artery plaque development, and can become symptomatic on a spectrum from mild symptoms to sudden death (18).

1.3.1 Coronary artery disease

CAD encompasses acute and chronic coronary syndrome, and symptoms are related to deposition of atherosclerotic plaques in the coronary arteries (19, 20). Coronary plaques can be stable or unstable. Unstable plaques have a large necrotic core and a thin fibrous cap, with ongoing inflammation and microcalcification in the core (21). A rupture of an unstable plaque is typically followed by acute coronary syndrome (ACS) (19) whereas stable coronary plaques typically have a thick fibrous cap and macroscopic calcification (21-23). In addition, a plaque rupture can be silent or occur in chronic coronary syndrome (20, 24, 25). Several studies have found that patients with stable plaques and fixed stenoses may experience mismatch between oxygen supply and demand that may cause angina or a type 2 infarction (19, 20).

1.3.2 Acute coronary syndrome

ACS is one of the most feared cardiovascular conditions and affects a substantial number of younger and middle-aged people. During the last decades, its prevalence has decreased in high-income countries, but increasing prevalence is currently seen in low- and middle-income countries (26). In Norway, ACS is still associated with a substantial morbidity and mortality burden; CVD accounted for 23.5% of annual deaths in 2020, 7.4% of them from ACS (27). ACS is divided into three diagnoses based in part on pathophysiological manifestations and in part on the clinical presentation. ACS consists of ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI), and unstable angina pectoris (UAP) (19).

1.3.3 Myocardial infarction and unstable angina

MI is currently defined as the presence of myocardial ischemia detected by abnormal myocardial biomarkers and clinical evidence of acute myocardial ischemia (19), whilst UAP patients have myocardial ischemia and stable myocardial biomarker concentrations (19, 28) . The biomarker criterion for MI includes a significant rise and/or fall of cardiac troponins (cTn) with at least one value above the 99the percentile of the assay. The clinical evidence could be symptoms, ECG changes, imaging, angiography or autopsy findings. The universal definition of myocardial infarction (UDMI) subdivide MI into 5 categories (19) based on differences in pathophysiology, clinic and prognosis, see (**Table 1**).

Table 1 encompasses definition of types 1–5 MI

Myocardial Infarction	Definition
Type 1 MI	Atherothrombotic CAD with plaque rupture/erosion (19).
Type 2 MI	Imbalance in which oxygen demand is higher than supply, it is unrelated to acute coronary atherothrombosis. Conditions leading to a type 2 infarction include physiological stressors such as hypoxia, hypotension, or tachyarrhythmia. Other examples are severe anemia, fixed coronary atherosclerosis, coronary embolism, and coronary spasms (19).
Type 3 MI	Cardiac death. Symptoms are suggestive of myocardial ischemia, with presumed new ischemic ECG changes or ventricular fibrillation. Death occurs before blood samples can be obtained or before an increase in biomarkers (19).
Type 4 MI	MI after percutaneous coronary intervention (PCI). Type 4a is defined as PCI-related MI ≤48 hours after the index procedure. High-sensitivity cardiac troponin (hs-cTn) levels are 5 times the 99th percentile combined with either ischemic ECG changes, imaging evidence, or angiographic findings of disrupted cardiac artery flow. Type 4b is defined as stent thrombosis documented by angiography or autopsy. Type 4c is defined as restenosis related to PCI (19)
Type 5 MI	MI after coronary artery bypass graft (CABG), hs-cTn values are >10 times the 99th percentile upper reference limit (URL) combined with either ECG changes, image evidence, or disrupted cardiac artery flow (19).

A type 1 MI is characterized by either a plaque rupture/erosion with an occlusive or non-occlusive thrombus, and integrating the ECG findings are essential for treatment strategies (19). STEMI patients typically have an acute total or subtotal coronary occlusion. If this is not treated urgently, it will likely cause transmural myocardial necrosis (29). STEMI patients usually have severe clinical symptoms such as intense chest or radiating pain, often accompanied by nausea and perspiration (29). To be diagnosed with STEMI, the patients need to have ST-segment elevation in at least two contiguous leads on the ECG or new bundle branch blocks with ischemic repolarization patterns. STEMI patients should receive percutaneous coronary intervention or, if this is not feasible within 120 minutes, fibrinolytic therapy.

Patients with non-ST elevation myocardial infarction (NSTEMI) may have normal ECG, but in a major proportion of NSTEMI cases, there will be ST-depressions, T wave changes, and sometimes transient ST elevations. NSTEMI patients usually experience a plaque rupture with a non-occlusive coronary thrombus. This lesion will cause chest pain and a variety of accompanying clinical symptoms like nausea and perspiration. There is ongoing cardiomyocyte necrosis, and the condition is verified by increased and dynamic troponin concentrations (28) , detected using a serial sampling protocol. NSTEMIs are usually treated with revascularization procedures, but this procedure is less urgent compared with STEMI and usually undertaken within 24 hours for patients with at least one high risk criteron. This time frame is under debate, and clinically unstable patients should have prompt revascularization (28).

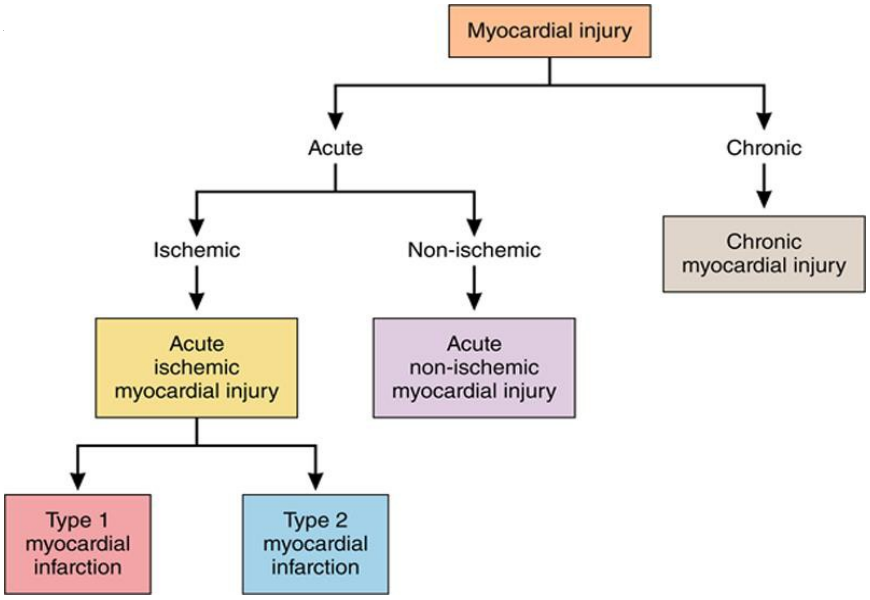
There are no specific ECG changes to diagnose UAP (19, 28) . UAP is characterized by a subtotal coronary occlusion causing myocardial ischemia at rest or with minimal activity, but without detection of acute cardiomyocyte injury/necrosis as defined using todays' troponin assays. The UAP diagnosis is based on clinical symptoms or a combination of clinical symptoms and different imaging modalities (including coronary angiography) confirming myocardial ischemia (28, 30). UAP patients are usually treated quite similar as NSTEMI patients, although the percentage receiving revascularization is lower. These patients have lower long-term risk of MACE compared with NSTEMI patients, but still higher risk than the general population, and the follow-up and treatment are under debate (30-32). After acute treatment, all ACS

patients should receive medical treatment focusing on future risk reduction and symptom relief.

1.4 Myocardial injury

In the fourth UDMI, published in 2018 introduce a new entity; myocardial injury. This is defined as elevated troponin values with at least one value above the 99th percentile (19). Myocardial injury may be acute ischemic (MI, as described above), acute non-ischemic, and chronic myocardial injury (19, 34) (**Figure 1**). The incidences of these different entities depend on the clinical setting where the cTn has been measured (33).

Figure 1. *Overview of the different types of myocardial injury. Figure by DeFilippis et al. Assessment and treatment of patients with type 2 myocardial infarction and acute nonischemic myocardial injury. Circulation 2019, Vol 140 (20) 1661-78, published under Open Access and reprinted under the terms of the of the Creative Commons CC*



1.4.1 Acute non-ischemic myocardial injury

Acute non-ischemic myocyte injury is defined as a rise and fall in cTn without simultaneous myocardial ischemia and should not be diagnosed as MI. It can be caused by direct trauma to the heart, for instance by contusion or operations, or by valvular heart disease, myocarditis, pulmonary embolism, Takotsubo cardiomyopathy, acute heart failure, sepsis, or rapid atrial pacing, and may even be seen during acute neurological events (19, 35). The clinical implications of acute myocardial injury is unclear. In a large observational cohort study from Sweden, patients with acute non-ischemic myocardial injury and chronic myocardial injury had similarly high risk of death during a 4-year follow-up, at 47% versus 48%, respectively. In the same study, patients diagnosed with type 1 MI had a 24% risk for death during follow-up. These findings highlight that acute non-ischemic myocardial injury can be as severe as chronic myocardial injury, see 1.4.2. No guidelines cover follow-up or treatment in this group (36).

A special cause of acute myocardial injury is strenuous exercise (37). Myocardial injury occurs in more than 80% of recreational athletes after endurance competitions (38). In some studies, it has been postulated that injury during exercise is physiological and reversible, but the mechanisms are not clear and this assumption mainly arises from the known favorable effects of physical activity on long-term cardiac outcomes (39). In other studies, such as that of older participants in the Nijmegen marches, exercise-induced cTnI elevation predicted higher mortality and cardiovascular events compared with participants who had normal cTnI after the exercise (40). Thus, the clinical interpretation of transient cTn increase during exercise remains unresolved (41).

1.4.2 Chronic myocardial injury

Stable elevated troponin concentrations (>99th percentile of the applicable troponin assay) is defined as chronic myocardial injury. Cardiac causes are severe CAD, heart failure, valvular heart disease, and infiltrative diseases such as amyloidosis and sarcoidosis (35). Non-cardiac causes include renal failure and pulmonary hypertension (35, 41). Current data indicate increased risk for future adverse cardiovascular events

and death among this group (33, 36, 42), but follow-up and treatment strategies are unclear.

1.5 Cardiac troponin

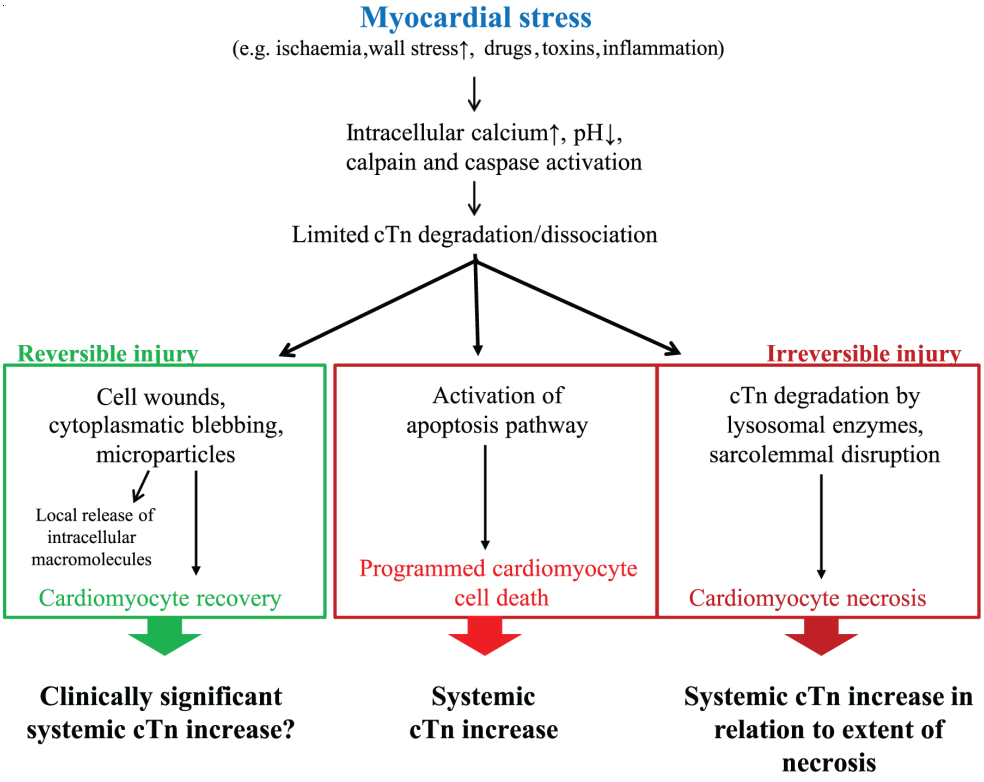
1.5.1 Function of troponin molecules

Troponin is a protein in the contractile apparatus of myocytes in both heart and skeletal muscle. Discovered in 1965, it plays an important role in muscle contraction. The sarcomere is the contractile unit, consisting of myosin (thick filament) and actin (thin filament). Both tropomyosin and troponin are attached to the actin filament. With calcium influx into the cardiomyocyte, troponin will remove tropomyosin from actin and expose actin's myosin-binding sites. An interaction between actin and myosin by a sliding movement leads to a muscle contraction (43). The three subunits of troponin are T, I, and C. The troponin T and troponin I isoforms are specific to cardiac myocytes (43), (cTnT and cTnI, respectively).

1.5.2 Release of cardiac troponin

During clinically steady-state cTnT is released in a diurnal rhythm, both in healthy and chronically diseased individuals. There is a peak in the morning, a gradual decrease during the daytime and increasing concentration throughout the night (44). This phenomenon is not observed for cTnI to the same extent. Baseline cTn concentrations correlate to myocardial mass (higher concentrations are seen in men) and increase with cardiovascular risk factors and older age (45, 46). cTn is released into the blood due to acute or chronic myocardial stress (47, 48). It is hypothesized that this myocardial injury can be reversible or irreversible, depending on the magnitude of stress factors (through time and intensity) the cardiomyocytes are exposed to (35), see **Figure 2**.

***Figure 2**. Different mechanisms for reversible and irreversible myocardial injury may lead to cTn release. Figure by Mair et al. How is cardiac troponin released from injured myocardium, Eur Heart J Acute Cardiovascular care, 2018, vol 7(6), 553-556, reprinted by permission of ESC (35).*



1.5.3 Cardiac troponin release in reversible myocardial injury

With reversible injury, cTn is released into the blood without cardiomyocytes undergoing necrosis or apoptosis. This release can be caused by transient ischemia, wall stretch, drugs, toxins, and inflammation (35) and typically is related to a high metabolic risk factor burden as is seen in, e.g., obesity, hypertension, and diabetes. Several mechanisms have been suggested, including cell injury from stretching or the formation of membranous blebs (35, 48). In a clinical study, the concentration of cTn increased significantly after 30 seconds with ischemia induced by inflating a balloon in the left anterior descending artery. The cTn increase was detected 15–30 minutes

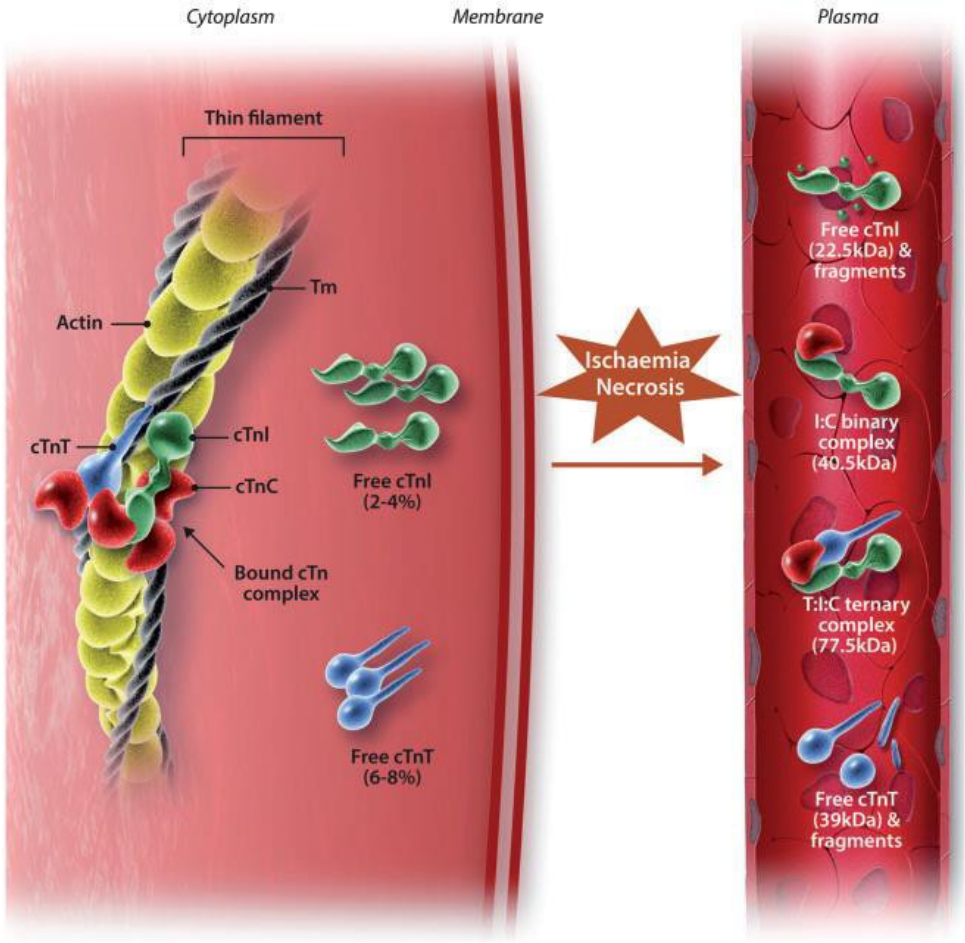
after the procedure, and cTn increased for 4 hours. These results suggested that cTn was released because of reversible ischemia, not necrosis (49).

1.5.4 Cardiac troponin release in irreversible myocardial injury

Irreversible myocardial injury is seen during MI, and the major mechanism is the necrosis of the cardiomyocyte, followed by release of cTn into the blood in a ternary or binary complex form or as free cTn fragments (43) (**Figure 3**). For patients given reperfusion therapy, there will be a significant cTn increase, with a peak at 10–20 hours, but without reperfusion treatment, the peak will be at 24–50 hours (48). The cTnT release is biphasic, with two peaks, while for cTnI, a single peak is usually seen (48).

Apoptosis of the cardiomyocyte is also a mechanism of release, and in an animal study, brief ischemia induced by clamping of the left anterior coronary artery was followed by apoptosis of cardiomyocytes (50). Apoptosis also was described in a study of patients with reversible left ventricular dysfunction after increased preload, and may have a role in post-infarction remodeling (51).

Figure 3. *Release of cTnI from the cardiomyocyte with ischemia and necrosis. Figure by Park et al, Cardiac troponins: from myocardial infarction to chronic disease, Cardiovasc Research, 2017,vol 113(14) 1708-1718, published under Open Access and reprinted under the terms of the Creative Commons CC BY license (43).*



1.6 Cardiac biomarkers in perspective

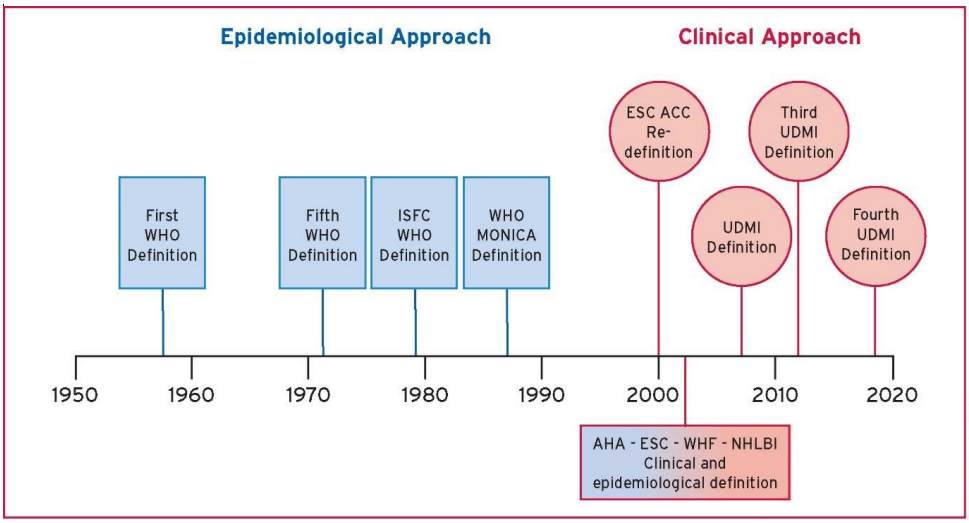
1.6.1 Biomarker development

MI causes tissue damage that leads to inflammation. In the beginning of the 20th century, observations of patients with heart attack revealed high white blood cell counts, and in 1933, a rise in erythrocyte sedimentation rate also was reported (47). From the 1950s, laboratory medicine developed rapidly, and knowledge about the biochemical enzymes related to muscle cells and the ability to measure them expanded (47). Aspartate transaminase was first, followed by lactate dehydrogenase, creatine kinase (CK), and creatine kinase-myocardial band (CK-MB). CK-MB is an isoenzyme of CK that is particularly abundant in myocardial tissue compared with other muscle tissues (skeletal and smooth muscle), so that it increases more than other isoforms of CK with cardiac muscle injury. This knowledge was used to distinguish different types of muscle injury (myocardial injury would have a higher CK-MB/CK ratio compared with injury to the skeletal muscle) and was an important advance in the ability to diagnose MI. During the 1980s, CK-MB could be measured with monoclonal antibodies, enhancing analytical specificity. The main drawback of the test was that it was not exclusively cardio specific and not clinically sensitive enough to detect minor MIs (47).

1.6.2 Biomarkers and development of the diagnostic definition of MI

During the 20th century, several definitions of MI emerged, and the ensuing confusion highlighted the need for a universal and common definition. In 1957, WHO published a report on atherosclerosis and ischemic heart disease that achieved this goal (52). With the increasing knowledge and understanding of MI, new definitions based on ECG changeswere published during the 1960s and 1970s, mostly intended for epidemiological purposes (**Figure 4**) (19).

Figure 4. *Historic development of the diagnostic definition of MI. Figure by Thygesen et al, Fourth universal definition of myocardial infarction (2018), European Heart Journal, 2019 vol 40 (3), 237-239, reprinted by permission of ESC (19).*



ACC = American College of Cardiology; AHA = American Heart Association; ESC = European Society of Cardiology; ISFC = International Society and Federation of Cardiology; MONICA = MONItoring of trends and determinants in CArdiovascular disease; NHLBI = National Heart, Lung, and Blood Institute; UDMI = Universal Definition of Myocardial Infarction; WHF = World Heart Federation; WHO = World Health Organization.

The development of cardiac biomarkers facilitated an improved diagnostic workup for MI, leading to a broader understanding of the pathology and playing an important role in the development of diagnostic definitions of MI. CK-MB was incorporated into the diagnostic criteria for MI in 1979 (53).

The discovery of troponins was a major step forward (47). In the 2000 European Society of Cardiology (ESC)/American College of Cardiology (ACC) consensus statement presented a universal definition of MI. This definition stated that any myocardial necrosis in the setting of myocardial ischemia should be labeled MI (54). Myocardial necrosis should be identified by measuring biomarkers, and cTnT or cTnI were preferred over CK-MB (47, 54). With the development of cTn assays, the definition of MI has changed several times over the two last decades, and the latest and fourth definition was published in 2018 (19) (**Table 2**).

Table 2. Diagnostic definitions of MI since 2000 and how they developed alongside troponin assays and accumulating clinical data describing the diagnostic and prognostic utility of the assays.

Year	Organization	MI definition	What's new	Biomarkers
2000	ESC/ACC consensus document (54)	Any myocardial necrosis in the clinical setting of myocardial ischemia should be defined as MI	-MI definition depends on combination of biochemical and clinical findings -Definition of NSTEMI and STEMI defined from ECG changes -MI related to PCI -MI related to CABG	cTnT and cTnI preferred over CK-MB CK-MB can be used if cTn assays are unavailable -One cTn value >99th percentile during the first 24 hours after the index event -Measure biomarkers at baseline, 6–9 hours, 12–24 hours after admittance No definition of the magnitude of biomarker increase when MI is related to PCI or CABG
2007	ESC/ACCF/AHA/ WHF universal definition of MI (55)	As above	MI defined in 5 subcategories Type 1 related to CAD with plaque rupture/erosion Type 2 related to increased demand or reduced supply Type 3 related to sudden death Type 4 related to PCI Type 5 related to CABG	-Rise and/or fall in cTn together with 1 value above the 99th percentile If cTn levels are above the 99th percentile, a 20% increase is indicative of additional myocardial necrosis (56) CK-MB can be used if cTn assays are unavailable -Repeated testing after 6–9 hours -MI related to PCI, cTn >3x 99th percentile URL -MI related to CABG, cTn >5x 99th percentile URL -Stable elevated cTn values should lead to search for other diagnoses
2012	ESC/ACCF/AHA /WHF third universal definition of MI (57)	As above	-Change in cTn levels for defining MI related to PCI and CABG	-Rise and/or fall in cTn together with 1 value above the 99th percentile 50% increase in cTn value if baseline value is ≤99th percentile URL 20% increase in cTn value if baseline value is above the 99th percentile URL (58)

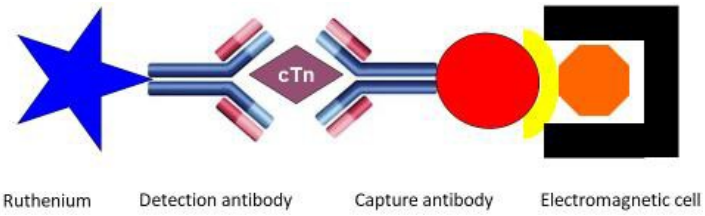
				CK-MB can be used if cTn assays not available Sex-dependent values may be recommended for hs-cTn assays - Repeated cTn testing after 3–6 hours MI related to PCI, cTn >5 x 99th percentile URL -MI related to CABG, cTn >10 x 99th percentile URL Several examples of conditions leading to myocardial injury with stable increased cTn levels
2018	ESC/ACCF/AHA /WHF fourth universal definition of myocardial infarction (20)	As above	-Myocardial injury included as a separate entity and divided into acute ischemic, acute non-ischemic, and chronic myocardial injury -Causes of type 2 MI specified -MINOCA defined -Takotsubo syndrome discussed	-The importance of hs-cTn assays is underlined - cTn kinetics, slower downslope than upslope; late presenters may not have significant changes in cTn over 1-2 hours but still have MI -Repeated testing after 3–6 hours recommended; benefits and pitfalls with earlier testing debated; identification of NSTEMI -Recommends use of sex-specific 99th percentile URL

1.7 Cardiac troponin assays

1.7.1 Development and characteristics of cardiac troponin assays

The cTn assays have been systematically improved since the late 1990s (59). cTnT and cTnI are measured using a so-called sandwich enzyme-linked immunosorbent assay method with at least two monoclonal antibodies (43, 60) (**Figure 5**).

Figure 5. Example of a sandwich immunoassay (cTnT from Roche Diagnostics). *Figure by Kristin Moberg Aakre, reprinted with permission.*



The assay consists of one capture antibody that will bind troponin to a well or another substrate. The simultaneous binding of a detection antibody will release a signal (typically excitation of some substance, such as ruthenium) detected by the analyzer (43). The strength of the signal is proportional to the concentration of cTn in the sample. The antibodies are targeted towards specific epitopes on cTnT or cTnI, and different manufacturers use different epitopes with different binding affinity to the molecule. For this reason, the assays can return different concentrations in the same samples and there is a need for assay-specific upper reference limits and cut-offs. Currently only one manufacturer offers a cTnT assay, but several assays are available for cTnI.

Since cTns became available, the assays have been continuously developed to increase analytical sensitivity and measure lower concentrations with higher precision (61).

1.7.2 Analytical characteristics

Limit of blank (LOB), limit of detection (LOD), and limit of quantitation (LOQ) are important measures that must be described for all cTn assays (62). These terms describe the smallest concentration of an analyte that can be reliably measured by an analytical procedure. LOB is the highest analyte concentration obtained when the duplicates of a sample containing no analyte are examined. It is calculated from measured duplicates of a blank sample, taking the mean result and the standard deviation (SD) of the mean: LOB = $\mu B + 1.645(SD_{mean})$. LOD is the lowest detectable concentration of the applicable analyte that may be distinguished from the LOB. It is defined as LOB + $1.645(SD_{low\ concentration\ sample})$. The LOQ is the smallest amount of an analyte that can be reliably and consistently detected and measured, usually similar to the concentration where a 20% analytical variation (coefficient of variation, or CV_A, defined as 100 x SD/mean) is obtained. It can be equal to LOD or a higher concentration (62).

1.7.3 The 99th percentile upper reference limit

Another important assay characteristic is the cTn 99th percentile upper reference limit (URL). To find this percentile, cTn is measured in a healthy population. Sandoval and Apple criticized previous definitions of the 99th percentile for different assays because of the lack of uniform procedures or guidelines for obtaining the value (63). Apple also criticized the variability of the 99th percentile with different assays and how to define a healthy population (59). Usually, a non-parametric method is used for estimating the 99th percentile; the values are plotted and the 1% highest results are removed, leaving the 99% lower results to define the 99th percentile, i.e., the upper normal values. A sample size of 300–400 individuals usually is required to define the 99th percentile, allowing for calculation of 90% or 95% confidence intervals, respectively. For cTns, sex-specific percentiles should be calculated because a sex-specific concentration difference is evident for all assays (64).

The International Federation of Clinical Chemistry and Laboratory Medicine provides updated information regarding these parameters (LOB, LOD, 99th percentile, and several more) for all cTn assays on a dedicated website (65).

1.7.4 Clinical implications of the high sensitiv cardiac troponin assays

By definition, high sensitivity cardiac troponin (hs-cTn) assays can measure a quantitative cTn concentration in 50% of a healthy reference population and show an analytical variation (CV_A) of ≤10% at the 99th percentile of the assay (66, 67). The first hs-cTn assay became commercially available in 2009. The improved sensitivity had two major clinical implications that were reflected in multiple seminal papers published within a few years.

First, two simultaneous publications (68, 69) in the *New England Journal of Medicine* used hs-cTn in a chest pain population in the emergency department (ED). Their major findings were that the high-sensitivity assays could reduce time to NSTEMI diagnosis because NSTEMI could be predicted by the admission sample in a large number of patients. This was the starting point for developing troponin-based rapid rule-out and rule-in algorithms for risk stratification of chest pain patients in the ED (59, 70-72). The field is still developing. Another seminal publication related to the utility of hs-cTn in the ED was the High-STEACS (High-**S**ensitivity **T**roponin in the **E**valuation of patients with **A**cute **C**oronary **S**yndrome) trial using the first hs-cTnI assay that became available in 2013 (73). The important but somewhat disappointing results of this trial showed that although use of a high-sensitivity vs. contemporary cTn assay increased the number of MI diagnoses, it did not reduce long-term mortality.

The second major clinical finding that emerged after introduction of hs-cTn assays was that stable increased cTn concentrations offer important prognostic information and can predict future heart failure and death in patients with stable coronary disease (74). This realization has propelled a large number of studies describing the prognostic value of cTn measurements in diverse cohorts (36, 73, 75, 76) and provided the basis for the diagnostic term chronic myocardial injury.

1.7.5 Algorithms for early identification of NSTEMI in the emergency department

Internationally, approximately 10% of all patients admitted to the ED will be investigated for ACS (77, 78). In Norway, one study showed that 13% of patients admitted to the ED had acute chest pain (79). Of these, 15%–20% likely would be diagnosed with MI (NSTEMI or STEMI) and 10% with UAP (29). In the first quarter of 2018, 9584 patients were admitted to the ED at Haukeland University hospital, of them, 950 (9.9%) had chest pain suspicious of NSTE-ACS. Based on this, the estimated admittance rate for chest pain patients in 2018 would be approximately 3800. The Norwegian Myocardial Infarction Registry reported 426 NSTEMIs at Haukeland University Hospital this year (80), which would (based on the estimates provided above) give an NSTEMI rate of 11% of patients admitted with chest pain. Chest pain patients are a major group in the ED, illustrated by this example, distinguish those with ACS rapidly and accurately is crucial. With the application of cTn algorithms, patients in the ED can be allocated to rule-out, observe, or rule-in for NSTEMI (28, 81), but no cTn algorithm excist for those with UAP.

In a 2018 review, the diagnostic and prognostic performance of hs-cTn algorithms and accelerated diagnostic protocols (ADPs) was evaluated (71) for the diagnostic endpoint MI. They also were evaluated for a prognostic endpoint of 30-day MACE, although MACE was defined differently among the studies. The review delineated three ways of handling chest pain patients with symptoms suspicious for ACS. All of them include measurement of hs-cTn as a marker of myocardial injury:

1. A cTn value corresponding to LOD or lower would signal rule-out, and all other patients would be ruled in. Patients allocated to rule-out should have 2-3 hours since onset of symptoms, negative ECG findings, and low clinical suspicion for ACS. According to the authors, this option was the most well adapted for safe rule-out of MI and safe discharge of patients from ED.
2. Serial cTn measurements should be obtained and different 0/1-, 0/2-, and 0/3-hour rule-out/rule-in algorithms would be used to allocate patients.

3. A combination of either a baseline hs-cTn or serial hs-cTn values with an ADP, such as additionally including pre-specified clinical risk scores for rule-out or rule-in of NSTEMI or ACS (71).

For the algorithms described under options 2 and 3, a high baseline concentration (typically 2-3 times the 99th percentile) allocates patients to rule-in. Non-allocated patients should be re-sampled after 1–3 hours (depending on the algorithm), and those who had a fairly low baseline concentration and showed a low delta (again taken together with ECG and clinical findings) should be allocated to rule-out, whereas those with larger delta values are allocated to rule-in. All other patients are allocated to observation and ultimately subjected to further investigations. The authors finally recommended ADP combined with serial hs-cTn measurements for evaluation of chest pain patients (71). With all of these strategies, there is a trade-off between safety and efficacy of the algorithms.

1.8 Variability in consecutive biomarker measurements

Delta values in consecutive measurements are an important part of the algorithms used for rule-out and rule-in of NSTEMI. Biomarker concentration delta values reflect either a clinically relevant change in an individual's condition or "natural" variations.

The "natural variations" seen in consecutive biomarker results during steady state are caused by analytical (CV_A) and intra-individual biological (CV_I) coefficient variation. CV_A is defined as the percentage variance seen when the same sample is measured several times in the same series: CV_A = 100 x SD/mean. CV_I is defined as the homoeostatic variation around a set point when the same biomarker is measured several times in a clinically stable individual; i.e., it is the physiological variation of a biomarker within an individual. The combined CV_A and CV_I (the total biomarker variability) is called the "reference change value" (RCV) (82). This measure may be calculated with different certainty levels (confidence intervals) and provide an estimate of the size of variation that may be seen in clinically stable individuals due to

physiological causes. The RCV cannot be used to diagnose disease, but knowledge about the magnitude of this unavoidable variation may be useful for suggesting diagnostic delta values for later validation in clinically diagnostic studies. The optimal delta values used for predicting or diagnosing disease should be determined using state-of-the-art design as described in the STARD (**STA**ndards for the **R**eporting of **D**iagnostic accuracy studies) checklist, designing a clinical diagnostic study, and calculating the diagnostic performance (sensitivity and specificity) for the different delta values (83).

1.9 Clinical risk scores

A clinical risk score can consist of clinical or biological data or a combination of both (84). Clinical risk scores are designed for risk assessment in patients admitted to the ED with chest pain suspicious for ACS, and for risk assessment in patients with verified ACS. The risk scores can be used to either predict risk for ACS patients without established disease or assess risk in patients with already known ACS (85). In recent years, numerous risk scores have been developed **(table 3).** EDACS (Emergency Department Assessment of Chest Pain Score) (86), T-MACS (Troponin –only Manchester Acute Coronary Syndromes) (87), and HEART score (88) are examples of risk scores developed for patients admitted to the ED with chest pain suspicious of ACS. This is in contrast to TIMI (Thrombolysis In Myocardial Infarction) (89) and GRACE (Global Registry of Acute Coronary Events) (90) which were developed for patients with established ACS.

Table 3. Components of 10 different risk scores and mHEART (taken from paper 3).

	History	Age	ECG	Risk factors	Troponin levels	Known CAD	Angina	SBP	Other	Low risk
HEART 2008	Typical=2p Atypical=1p	>65=2p >45=1p	ST-dep=2p Other=1p	≥3 or CAD=2p ≥1=1p	≥3x99th=2p ≥99th=1p					≤3p
mHEART 2017	Typical=2p Atypical=1p	>65=2p >45=1p	ST-dep=2p Other=1p	≥3 or CAD=2p ≥1=1p	≥99th=2p Measurable=1p					≤3p
CARE 2018	Typical=2p Atypical=1p	>65=2p >45=1p	ST-dep=2p Other=1p	≥3 or CAD=2p ≥1=1p						≤1p
TIMI 2000		>65=1p	ST-changes >0.5mm=1p	≥3 =1p	>99th=1p	1p	Severe =1p		Aspirin used within 7 days=1p	≤1p
GRACE 2003/2004		0-100p	ST-changes >0.5mm=17p		>99th=14p			0-40p	Pulse=0-34p Creatinine=0-28p Cardiac arrest=30p Killip class=0-44p	≤108p ≤89p
EDACS 2014	Diaphoresis=3p Radiation‡ =5p Resp. pain=-4p Reproduced by palpation=-6p	2-20p		≥3 or CAD*=4p					Male gender=6p	≤15p
sEDACS 2016	Radiation‡ =1p	0-6p		≥3 or CAD=1p					Male gender=1p	≤3p
T-MACS** 2017	Diaphoresis=d Radiation§=r Vomiting=v		Ischemia=i		By degree of elevation=t		Crescendo=c	<100=h		≤0.02
sT-MACS 2018	Diaphoresis=1p Radiation§=1p Vomiting=1p		Ischemia=1p		TnT>9 ng/L=1p		Crescendo=1p	<100=1p		≤0p
Geleijnse-Sanchis 2005	>10 symptom points=1p	≥67=1p		DM† =2p		1p	Severe =1p			≤1p
Goldman 1996			Ischemia= high-risk				Crescendo=1p	<110=1p	Bilateral pulmonary rales=1p	≤1p

CAD indicates coronary artery disease; SBP, systolic blood pressure; HEART, History, ECG, Age, Risk factors, Troponin; mHEART, modified HEART score with troponin points given if hs-Tn is measurable; CARE, characteristics, age, risk factors, EGG; GRACE, Global Registry of Acute Coronary Events; T-MACS, troponin-only Manchester Acute Coronary Syndromes; TIMI, Thrombolysis In Myocardial Infarction; EDACS, Emergency Department Assessment of Chest Pain Score and MI, myocardial infarction.
*Age 18-50

**Percentage risk of ACS calculated using the following formula: p=1/(1+e^-(1.713i+0.847c+0.607r+1.417v+2.058d+1.208h+0.089t-4.766)) where hs-TnT is continuous and the other factors dichotomous.
†Demanding insulin
‡ To *any* shoulder/arm/jaw
§To *right* arm/shoulder

TIMI (Thrombolysis In Myocardial Infarction) (89), GRACE (Global Registry of Acute Coronary Events) (90), and HEART score (88) are examples of widely used risk scores to be discussed in more detail (91, 92).

The TIMI score was developed in randomized trials in patients with known NSTE-ACS, and the aim was to predict 14-day outcomes, defined as all-cause mortality, new or recurrent MI, or severe recurrent ischemia requiring urgent revascularization (89). The TIMI score range is 0–7, and each factor is worth one point (Age ≥65, ≥3 cardiac risk factors, Acetylsalicylic acid within the seven last days, Prior coronary stenosis ≥50%, ≥2 angina rest episode in <24 hour, ST-segment deviation and Elevated cardiac markers). A score of 0-1 is considered low risk, and 2+ points is non-low risk (89, 91). The TIMI score was designed for patients with already known ACS, so its usefulness for patients admitted with chest pain suspicious for ACS in the ED has been questioned. In a 2010 meta-analysis, 1.8% of chest pain patients with a TIMI score of 0 would experience a cardiac event within 30 days. In that study, there was a strong linear relation between TIMI score and cumulative incidence of cardiac events ($P<0.001$). The authors concluded that TIMI score could not be used alone for rule-out of ACS (93).

The GRACE score was developed in the GRACE study, a large prospective multinational observational study including patients with ACS, undertaken over 10 years in 14 countries (90). The GRACE score consists of eight risk factors: age, systolic blood pressure, heart rate, creatinine concentration, Killip class, cardiac arrest at admission, ST segment deviation, and elevated cardiac biomarkers. Cut-offs are low risk at ≤108 points, intermediate risk at 109–140 points, and high risk at >140 points (94). The GRACE score was first developed to assess the risk of in-hospital mortality (95) but later was used to predict the combined endpoint of death or MI after 6 months (96). GRACE score is recommended to use in combination with the ESC algorithms for evaluation of chest pain patients in the ED (28, 97). In several articles, the treatment-risk paradox is discussed, meaning that patients with NSTE-ACS and a low

risk get more guideline-recommended therapy than the those with high risk who would benefit the most (98, 99). Applying the GRACE score for a proper risk assessment is used as an example of how to address this problem (28).

The HEART score was designed to identify ACS in patients admitted to the ED with possible ACS. HEART score contains five categories, and it is an acronym for History, ECG, Age, Risk factors and Troponin. Each category is given 0 (low risk), 1 (moderate risk) or 2 (high risk) points, and a patient can have a score from 0 to 10 (88). It was developed in an observational study from 2008 with only 122 patients admitted with chest pain and a median follow-up of 423 days. MACE was defined as MI, PCI, CABG, death, and a combined endpoint of MI, PCI, CABG, and death (88). An overall HEART score of 3 or lower indicates low risk, and in the development study, patients with scores ≤3 had a 2.5% risk for MACE. There was a linear trend between the HEART score and the endpoints (P for trend <0.001) (88).

The HEART score has been validated in several later studies. One meta-analysis from 2018 covered nine studies and data for 11,217 patients admitted to the ED with chest pain. The authors concluded that for patients with a HEART score of 0–3, the pooled sensitivity for predicting MACE was 96.7%, missing 3.3% of ACS cases. MACE was defined as a composite of prevalent or incident MI, PCI, CABG, and all-cause death. The authors debated if this risk was low enough for safe implementation of the HEART score and recommended clinicians consider circumstances that could influence the diagnostic probability locally (100).

1.10 Gaps in knowledge

During the planning of this study, several gaps in knowledge were identified and discussed. An important subject was whether cTn assays with improved analytical sensitivity could substantially improve ED logistics by better NSTEMI prediction in early presenters or more efficient pathways. Another important topic was the lack of robust predictive tools for UAP patients in the ED and whether combining cTn-based

NSTEMI algorithms with clinical risk scores would benefit this group. Finally, how to predict UAP raises the research question if it is possible to design novel cTn algorithms targeting UAP and still have acceptable performance for rule-out of patients without NSTE-ACS.

2. Aims of the thesis

2.1 General aims

The main aim of this thesis is to validate and develop cTn algorithms that can be used safely and efficiently in diagnostic pathways for patients admitted to the ED with acute chest pain suspicious for NSTE-ACS.

2.1.1 Specific aims

Paper 1: Describe the study design and explain the framework of the WESTCOR study and expectations regarding scientific findings.

Paper 2: Develop novel rule-in/rule-out algorithms for a novel hs-cTn assay from Singulex Clarity System. Estimate the precision and efficiency of the novel algorithms for identification of NSTEMI in all patients regardless of time from symptom onset and compare these to other hs-cTn protocols. Investigate the long-term prognostic properties (using a composite endpoint of all-cause mortality and nonfatal MI) of the novel and comparator algorithms.

Paper 3: Investigate the diagnostic performance and efficiency of the 0/3-hour hs-cTn algorithms from ESC and High-STEACS alone and in combination with either the ESC clinical ACS criteria or one of eleven different standardized clinical risk scores. The primary endpoint was a composite of all-cause mortality, nonfatal MI, or unplanned revascularization within 30 days. The secondary endpoint was NSTEMI during the index hospitalization.

Paper 4: Explore the diagnostic performance and efficiency of novel hs-cTn algorithms combining very low baseline and delta values for the primary endpoint to rule out NSTE-ACS patients (NSTEMI and UAP during the index hospitalization). The secondary endpoint was a combined MI, all-cause death within 30 days, and revascularization within 24 hours.

3. Materials and Methods

3.1 Thesis overview

Article	I	II	III	IV
Design	Cross-sectional prospective observational study			
Data source	NA	-WESTCOR-D cohort -Norwegian Patient Register (NPR) and Norwegian Cause of Death Registry	-WESTCOR-D cohort -NPR and Norwegian Cause of Death Registry	-WESTCOR-D and WESTCOR-V cohort -NPR and Norwegian Cause of Death Registry
Time of data collection	NA	2015–2017	2015–2017	2015–2019
Study population	Patients admitted to the ED with chest pain suspicious for ACS			
Numbers included in analyses	985	971	932	1504
Numbers of cTn assays included	NA	3 hs-cTnT, hs-cTnI (2)	2 hs-cTnT, hs-cTnI	2 hs-cTnT, hs-cTnI
Main laboratory method	NA	Roche Diagnostics Abbott Diagnostics Singulex Clarity System	Roche Diagnostics Abbott Diagnostics	Roche Diagnostics Abbott Diagnostics
Time points for biomarker collection used	NA	Index hospitalization (0, 1 hours)	Index hospitalization (0, 3 hours)	Index hospitalization (0, 1, 3 hours)
Outcomes	Study design	-Diagnostic endpoint: NSTEMI during index hospitalization -Prognostic endpoint: a composite of all-cause mortality and subsequent nonfatal MI	-Primary composite endpoint: 30-day nonfatal MI, all-cause mortality, and unplanned revascularization -Secondary endpoint: NSTEMI during index hospitalization	-Primary endpoint: NSTE-ACS during index hospitalization -Secondary endpoint: death, AMI within 30 days, and urgent revascularization within 24 hours
Follow up time	Study design	Until an endpoint occurred or a median follow-up of 723 days after inclusion (ranging from 4 to 900 days)	30 days	30 days
Main statistical analyses	Study design	Kruskal-Wallis, Chi square, and Fisher's exact tests; receiver operating characteristic (AUROC) analysis, DeLong test; Kaplan–Meier curves; Cox proportional hazard regression analysis	Chi square, Fisher's exact, and Mann-Whitney U tests, Cox-regression AUROC analysis, DeLong test. McNamar test; binominal logistic regression (to combine variables for AUROC analysis)	Kruskal-Wallis and Mann-Whitney U tests, Chi square and Fisher's exact tests; AUROC analysis, DeLong test; McNamar test; Kaplan–Meier curves
Conclusion	Back-ground and expect-ations	-cTn assays with improved analytical sensitivity may show improved ability for early identification of NSTEMI -Long-term cardiovascular risk should be considered in patients with slightly elevated cTn who are ruled out for NSTEMI	-cTn-based algorithms intended to identify NSTEMI should be combined with a clinical risk score to improve sensitivity and NPV for identification of patients with high risk of MI, death, or need for invasive treatment	-Low concentration/low-delta cTn algorithms improved the sensitivity for NSTE-ACS, and still rule out a substantial number of non-cardiac chest pain (NCCP) patients -The algorithms' diagnostic performance was influenced by the analytical performance of the assays

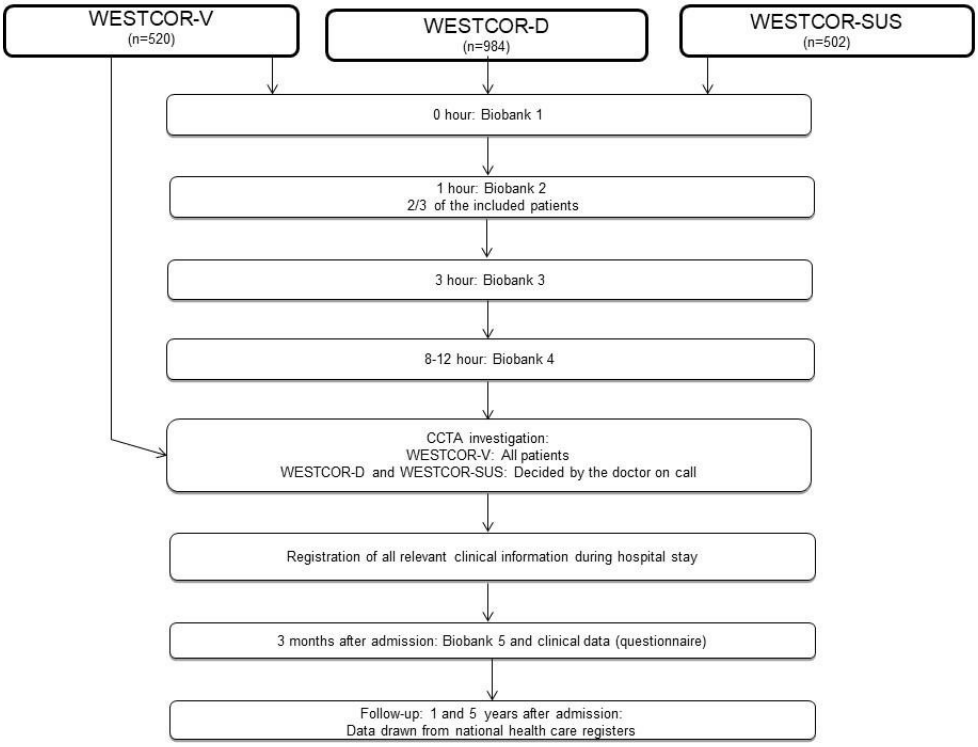
3.2 Study design

The WESTCOR (Aiming Towards Evidence Based Interpretation of Cardiac Biomarkers in Patients Presenting With Chest Pain) study (Clinical Trials number NCT02620202 is a regional two-center, prospective observational study. The study and biobank were approved by the Regional Committees for Medical and Health Research Ethics (2014/1365 REK West and 2014/1905 REK West). The study encompasses the university hospitals within the Western Norway Regional Health Authority. Patients were included at both Haukeland and Stavanger university hospitals. The inclusion period lasted from September 2015 until March 2020. As part of the study protocol, included patients were divided into a derivation and two validation cohorts (101) (**Figure 6**). All patients in the internal validation cohort (WESTCOR-V) were to undergo cardiac computed tomographic angiography (CCTA) unless it was contraindicated, and in the remaining cohorts, CCTA was done on clinical indication. Three months after the index hospitalization, patients were asked to fill out a questionnaire including the Seattle Angina Score, Rose Dyspnoea Score, RAND-12 (a truncated version of Short Form Health Survey-36), and Hospital Anxiety and Depression Scale and to take a blood test. Patients could be followed for at least 10 years through different national health care registers such as the Norwegian Patient Registry (NPR), Norwegian Cause of Death Registry, and Norwegian Prescription Database.

This PhD thesis includes only data from patients at Haukeland University Hospital who were included in the local derivation and internal validation cohorts. At Haukeland University Hospital, 984 patients were included in the derivation cohort (WESTCOR-D) and 520 patients in the internal validation cohort (WESTCOR-V).

Figure 6. *Flowchart outlining the study. Figure by Tjora et al, slightly modified.. Aiming toWards Evidence baSed inTerpretation of Cardiac biOmarkers in patients pResenting with chest pain-the WESTCOR study: study design (2019) vol 53(5), 280-285, reprinted by permission of Taylor & Francis (101).*

WESTCOR-V: WESTCOR Internal Validation cohort, WESTCOR-D: WESTCOR Derivation cohort, WESTCOR-SUS: WESTCOR Validation Cohort from Stavanger University Hospital



3.3 Study enrollment and biobanking

Unselected patients admitted with chest pain suspicious for NSTE-ACS were eligible for inclusion. They had to be age ≥18 years, not have a short life expectancy, and be able to perform informed consent (**Table 4**).

Table 4. Inclusion and exclusion criteria.

INCLUSION CRITERIA
Patients admitted with chest pain suspicious for 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

The inclusion was done in the ED by doctors and nurses on call. Serum samples were drawn at arrival, after 3 hours, and at 8–12 hours as part of routine clinical care, and additional aliquots were stored in a biobank. Measurement of hs-cTnT was performed on fresh samples with results reported to the attending clinician. After an implementation period, an additional biobank sample was drawn one hour after admission, without results being reported to the attending clinicians.

3.4 Biochemical analyses

All samples were centrifuged after 30 minutes, and material for the biobank was aliquoted and frozen at -80°C. hs-cTnT (Roche Diagnostics) was analyzed continuously on fresh material. hs-cTnI was measured in biobank.

hs-cTnT (Roche Diagnostics) had a LOB 3 ng/L, LOD 5 ng/L, 99th percentile 14 ng/L, and measurement range 4–10,000 ng/L (65). CV_A was 10% at 4.5 ng/L. The analysis was done using nine different reagents and calibrator lots. The $hs\text{-}cTnI_{(Abbot)}$ assay has a LOB 0.9 ng/L, LOD 1.7 ng/L, and 99^{th} percentile of 26 ng/L (65). The measurement range was 2–50,000 ng/L and the 10% CV_A was 4.6 ng/L. The analysis was done using

reagent lot 71164V100 and calibrator lot 65294V100 for the derivation cohort, and reagent lot 11151UI00 and calibrator lot 09906 UI00 for the internal validation cohort. For paper 2, hs-cTnI also was measured using the Singulex Clarity System (hs-$cTnI_{(Sgx)}$). For the time being, this assay is not in commercial use due to bankruptcy and measurements in the remaining cohort is therefor not available. hs-$cTnI_{(Sgx)}$ has a LOB of 0.02 ng/L, LOD 0.08 ng/L, 99th percentile 8.67 ng/L, and 10% CV_A at 0.53 ng/L. All other clinical chemistry tests were performed using Cobas e602 or Cobas 8000 from Roche Diagnostics. The glomerular filtration rate was estimated using the Chronic Kidney Disease Epidemiology Collaboration formula (102) with an enzymatic isotope dilution mass spectrometry traceable creatinine assay (Cobas 8000 from Roche Diagnostics) and a CV_A less than 3% for concentrations >60 μmol/L.

3.5 Adjudication

The index diagnosis was adjudicated by two cardiologists based on the information from the laboratory tests, including serial cTnT values, ECG, imaging findings including coronary angiography, coronary computer tomography angiography (CCTA), and echocardiography. A third adjudicator resolved disagreements. Specific diagnostic criteria were predefined for 22 different medical conditions based on guidelines available during the study planning. MI was defined according to the third UDMI (57), meaning that myocardial necrosis should be present in a clinical setting consistent with acute myocardial ischemia. Regarding biomarkers, there should be a rise and/or fall of cTn with at least one value above the 99th percentile combined with symptoms of ischemia, ECG changes, imaging evidence of loss of viable myocardium, or intracoronary thrombus. A significant change in cTn levels was defined as an increase in cTn >50% if the baseline concentration was below URL or 20% increase if the baseline value was above the URL (58). UAP was defined as symptoms suggestive of ACS without elevation in biomarkers, with or without ECG changes indicative of ischemia (97). NSTE-ACS was defined as NSTEMI and UAP combined.

3.6 Endpoints

Paper 2 included both diagnostic and long-term prognostic endpoints. The diagnostic endpoint was NSTEMI during the index hospitalization. The prognostic endpoint was a composite of all-cause mortality and subsequent nonfatal MI (all MIs after the index NSTEMI).

The third article was a post-hoc analysis, with a primary endpoint of 30-day composite nonfatal MI (type 1 or 2), all-cause mortality, and unplanned revascularization, including intention to treat. The secondary endpoint was NSTEMI during the index hospitalization.

In the fourth paper, the primary endpoint was a diagnosis of NSTEMI or UAP during the index hospitalization. The secondary endpoints were a composite of MI or all-cause mortality during the first 30 days after hospitalization or urgent (within 24 h after admission) revascularization.

3.7 Development of novel algorithms

Two papers present novel algorithms for rule-out and rule-in developed by the research group. In paper 2, the novel rule-out algorithms were designed to have a sensitivity for NSTEMI of ≥99% and the highest possible specificity. This was based upon an earlier study surveying cardiologist views on acceptable miss rates suggested that less than 1% of NSTEMIs should be falsely ruled out, so that the sensitivity for NSTEMI using rule-out algorithms should be ≥99%, meaning a maximum of 1 in 100 MIs could be missed (103). The rule-in algorithms should have a diagnostic specificity for NSTEMI of ≥95% (ruling in <5% non-NSTEMI, arbitrary chosen) and the highest possible sensitivity (104). During the practical development of the of the novel hs-cTnI$_{(Sgx)}$ algorithms in paper 2 (104) we calculated the percentage of NSTEMI ruled out if different admission concentrations and delta values were applied as cut-offs (**Table 5**). The first concentration would typically be the LOD or rounded to the nearest whole number. Then this concentration would be tested together with different delta values.

The most favorable algorithm would be the one yielding the lowest number of NSTEMIs ruled out and the highest number of non-coronary chest pain patients ruled out (101).

Table 5. Template for developing novel algorithms.

Concentrations and delta values tested	**NSTEMI**	**UAP**	**Non-ACS cardiac disease**	**NCCP**	**Other diseases**	**Total**
Baseline: LOD Δ value +1–5 ng/L						
Baseline: LOD + 1 ng/L Δ value +1–5 ng/L						
Baseline: LOD + 2 ng/L Δ value +1–5 ng/L						
Baseline: LOD + ***n*** ng/L Δ value +1–5 ng/L						

In paper 4, the novel rule-out algorithms had baseline values corresponding to the LOD and delta values that were based on RCVs (109). We hypothesized that these novel rule-out algorithms would have a sensitivity ≥95% (arbitrary chosen) for the primary endpoint of NSTEMI or UAP and ≥99% sensitivity for the secondary endpoint.

3.8 Comparatory troponin algorithms

In paper 2, the novel NSTEMI admission rule-out algorithm and 0/1-hour rule-out/rule-in algorithms were compared with already established algorithms like the ESC 2015 0/1-hour rule-out/rule-in of NSTE-ACS guideline (97) (**Table 6**). The novel hs-cTnI$_{(Sgx)}$ algorithms were also compared with other publications (Body et al (106) and Neumann et al (107)). In paper 3, the 2015 ESC 0/3-hour rule-out/rule-in algorithms (97) and the algorithms derived from the High-STEACS study (108) were examined. In paper 4, the novel rule-out algorithms were compared with the 0/1-hour rule-out algorithms in the ESC 2020 guidelines for NSTE-ACS (28).

Table 6. Overview of the different algorithms developed and evaluated in the three papers. Very low: concentration used for rule-out at admission; low: concentration used for rule-out in a two-sample algorithm; and high: concentration used to rule-in at admission. The rule-out and rule-in deltas are for a two-sample algorithm. All units are ng/L unless otherwise specified.

PAPER 2					
	Very low	**Low**	**Rule-out Δ**	**High**	**Rule-in Δ**
Novel 0/1-hour algorithms					
hs-cTnI (Singulex Clarity) (104)	<2	<8.67	<3	≥30	≥5
hs-cTnI (Singulex Clarity) (104)	<2	<10	<3	≥70	≥5
Comparator 0/1-hour algorithms					
hs-cTnI (Singulex Clarity) (107)	<1	<2	<1	≥25	≥6
hs-cTnI (Singulex Clarity) (106)	<1.5	NA	NA	NA	NA
hs-cTnT (Roche Diagnostics) (97)	<5	<12	<3	≥52	≥5
hs-cTnI (Abbott Diagnostics) (97)	<2	<5	<2	≥52	≥6

PAPER 3					
	Very low	**Low**	**Rule-out Δ**	**High**	**Rule-in Δ**
0/3-hour algorithms					
hs-cTnT (Roche Diagnostics) (97)	≤14	≤14	≤ 50%	>14	>50%
hs-cTnI (Abbott Diagnostics) (97)	≤26	≤26	≤ 50%	>26	>50%
hs-cTnT (Roche Diagnostics)(108)	<5	≤14	<3	>14	≥3
hs-TnI (Abbott Diagnostics) (108)	<5	≤16 (F) ≤34 (M)	<3	>16 (F) >34 (M)	≥3

PAPER 4					
	Very low	**Low**	**Rule-out Δ**	**High**	**Rule-in Δ**
Novel 0/1-hour algorithms					
hs-cTnT (Roche Diagnostics) (109)	NA	<5	<1	NA	NA
hs-cTnI (Abbott Diagnostics) (109)	NA	<2	<1	NA	NA
Novel 0/3-hour algorithms					
hs-cTnT (Roche Diagnostics) (109)	NA	<5	<1	NA	NA
hs-cTnI (Abbott Diagnostics) (109)	NA	<2	<1	NA	NA
Comparator 0/1-hour algorithms					
hs-cTnT (Roche Diagnostics) (28)	NA	<12	<3	NA	NA
hs-cTnI (Abbott Diagnostics) (28)	NA	<5	<2	NA	NA

3.9 Statistical considerations

3.9.1 Sample size and power calculations

Sample size calculations were based on the assumptions of alpha = 0.05 and 1- beta (power) of 80%. It was thought to be clinically meaningful to have a difference between two different algorithms or protocols of 5% for sensitivity, 5% for specificity, and 0.03 for the AUROC (110). The power calculations showed that 355 patients should be included to uncover a 5% difference in sensitivity or specificity (McNemar's test). For 80% power to detect a difference in AUROC of 0.03 (e.g., from 0.92 to 0.95), a total of 828 patients needed to be included (Delong test; rank correlation between tests was set to 0.9, ratio between negative and positive cases was set to 8), 92 patients with the condition (NSTEMI or NSTE-ACS as applicable) and 736 without the condition. Regarding prognosis, we estimated that 40% (111) of the total cohort would have an increased cTn concentration and a concomitant risk of the endpoint of >10%, compared with a low cTn population that would have a <5% risk of an endpoint. This resulted in a need to include 850 patients (log-rank survival analysis).

3.9.2 Statistical analysis

Baseline characteristics were analyzed using ordinary descriptive statistics, including non-parametric (Kruskal-Wallis and Mann-Whitney U test) statistical tests for continuous variables and Chi-square or Fisher's exact test for categorical variables, as appropriate (see **Point 3.1**). The baseline characteristics were reported as median with interquartile range (skewed data) for continuous data or mean with (±2 SD) (normally distributed data), and percentages for categorical data. For evaluation of the algorithms, calculation of sensitivity, specificity, NPV, and PPV was undertaken in all papers. Calculation of the AUROC was performed for all algorithms. Significant differences in AUROC were evaluated using the Delong test, and efficiency was defined as percentage of patients ruled out plus percentage of patients ruled in. Difference in sensitivity or specificity of algorithms was assessed using McNemar's test. Survival analysis was done with Kaplan–Meier curves, and risk prediction was estimated using unadjusted and adjusted Cox proportional hazards regression analysis (104). The

adjusted analysis corrected for age, sex, current or previous smoking, estimated glomerular filtration rate above vs. below 60 mg/min per 1.73 m^2, diabetes mellitus, hypertension, hyperlipidemia, and previous MI. For evaluation of the AUROC of the combination of cTn-based algorithms (categorical variable) and risk scores (continuous variable) , a combined variable using binominal logistic regression was created (105). Hypothesis testing was two-tailed, and P values <0.05 were considered statistically significant. Analysis was performed using IBM SPSS Statistics version 24/26 for Windows (IBM Statistics, Chicago, IL, USA) and MedCalc version 17.6 for Windows (MedCalc Software bvba, Ostend, Belgium).

4. Summary of main results

Paper 1 (101), describes the background and rationale for the study, the study design, and the expected outcome.

In Paper 2 (104), a baseline rule-out algorithm and 0/1-hour rule-out/rule-in algorithms based on a novel hs-cTnI assay from Singulex Clarity System (hs-$TnI_{(Sgx)}$) were designed for identification of NSTEMI during the index hospitalization as a primary endpoint. The secondary endpoint was a composite of all-cause mortality and subsequent nonfatal MI during a follow-up median of 723 days (range, 4–900 days). The novel algorithms were compared to previously published algorithms and performed better than the comparator algorithms. When the admission sample concentration was used as a continuous variable for NSTEMI versus not-NSTEMI, the Sgx assay had the best performance (AUROC was 0.95 vs. 0.91 for hs-cTnT, P<0.001 and 0.93 for hs-$cTnI_{(Abbott)}$, P=0.004 Delong test), mainly because of improved specificity for rule-out. The novel 0/1-hour $cTnI_{(Sgx)}$ algorithms were more efficient and allocated more patients to rule-in or rule-out (92%) compared with established 0/1-hour algorithms (≤78%). A survival analysis for the prognostic endpoint was undertaken, showing that for the novel $TnI_{(Sgx)}$ algorithm, the prevalence of endpoints was not significantly lower in the rule-out group compared with the observation or rule-in group. The reverse was seen for the comparator algorithms.

In paper 3 (105), two different sets of 0/3-hour hs-cTn based algorithms (ESC and High-STEACS) were evaluated in combination with either the ACS risk criteria as recommended in the ESC guidelines, or one of eleven clinical risk scores (HEART, mHEART, CARE, GRACE, T-MACS, sT-MACS, TIMI, EDACS, sEDACS, Goldman, and Geleijnse–Sanchis). The primary endpoint was nonfatal MI (type 1 and 2), all-cause mortality, and unplanned revascularization within 30 days. The secondary endpoint was NSTEMI during the index hospitalization. A total of 21% experienced a primary endpoint. The event rate was 3.8%–4.9% (2 NSTEMI, otherwise unplanned revascularization) in patients ruled out for NSTEMI when the ESC 0/3-hour algorithms were combined with ACS criteria. The combination of the High-STEACS algorithm

and the ACS criteria resulted in an event rate of 3.8%–4.3% (1 NSTEMI, the others unplanned revascularization). Replacing ACS criteria with a HEART score ≤3 reduced the event rate in the rule-out group to 2.5%–2.7% (ESC 0/3 hours) and 2.2%–2.5% (High-STEACS), and only unplanned revascularizations were ruled out. A total of 13% had a secondary endpoint. Using the troponin algorithms alone ruled out 2–7 NSTEMI (0.2%–0.9% of all patients ruled out). Combining the algorithms with ACS criteria or HEART ≤3 decreased the rule-out rate for NSTEMIs to 1 or 2 (<0.2%).

In paper 4 (109), 0/1-hour and 0/3-hour hs-cTnT/hs-cTnI rule-out algorithms combining low baseline (LOD) and low delta (based on the RCV derived from the assay's analytical and biological variation) were compared to the ESC 2020 0/1-hour algorithms for a primary endpoint (NSTEMI and UAP during the index hospitalization) and a secondary endpoint (MI, all-cause mortality (within 30 days) and urgent (24 hour) revascularization). The main findings were that the novel algorithms had higher clinical sensitivity for identification of the combination of NSTEMI and UAP than the comparator algorithms; e.g., the hs-TnT 0/1 had a clinical sensitivity of 95.4% versus 62.8% for the ESC 0/1-hour algorithm (P<0.001). The rule-out rate for UAP patients was much lower compared with prior published algorithms. The increase in clinical sensitivity came at a cost of lower clinical specificity of 21.0% for the 0/1-hour algorithm vs. 81.7% for the ESC algorithm (P<0.001), similar but less clear findings were seen for cTnI. The novel algorithms had no advantage over the ESC algorithms for the secondary endpoint.

4.1 Study population

There were 984 patients in the derivation cohort and 520 in the internal validation cohort. Biobank admission samples were available from 1504 patients. A 1-hour sample was available for (n=479) in the derivation cohort and (n=505) in the internal validation cohort.

4.1.1 Derivation cohort

The median age of the patients was 63 years, and 61% were male. NSTEMI was diagnosed in 13% (n=129), unstable angina in 11% (n=111), other diseases in 16% (n=155), and NCCP in 60% (n=589). Patients with ACS were older, had more risk factors for CVD, and used more medications than patients without ACS. Median time from symptom onset to first blood test was 8.1 hours. A total of 201 patients (20%) had onset of symptoms <3 hours before the admission sample was collected. CCTA was performed in 31%, and 8% had angiography without further treatment. About 16% were treated with either PCI or CABG. Revascularization was performed in 96/129 (74%) of patients with NSTEMI and 55/111 (50%) of patients with UAP. Overall, 3% of the patients had an outpatient coronary CT scan, and one patient (0.1% of total) had a significant stenosis. hs-cTnI$_{(Sgx)}$ was measurable in 99.9% of admission samples, hs-cTnT in 70%, and hs-cTnI$_{(Abbott)}$ in 84%.

4.1.2 Internal validation cohort

In the internal validation cohort, patients were slightly younger and had less comorbidity compared with the derivation cohort. The median age of patients was 60 years, and 60% were male. NSTEMI was diagnosed in 9% (n=45), unstable angina in 17% (n=90), other diseases in 11% (n= 59), and NCCP in 63% (n=326). As in the derivation cohort, the ACS patients were older, had more risk factors for CVD, and used more medications than patients without ACS. Median time from symptom onset to first blood test was 12.9 hours, and 93 patients (18%) had onset of symptoms <3 hours before first sampling. CCTA was performed in 51% of all patients, and 11% had angiography without further treatment. About 18% were treated with either PCI or CABG. Revascularization was performed in 34/45 (76%) of NSTEMI patients and 49/90 (54%) of patients with UAP. A total of 25 patients (5%) had an outpatient coronary CT scan, of whom two diagnosed as having NCCP (0.3% of total) had a significant stenosis.

There were measurable hs-cTnT concentrations in 71% of the patients in the internal validation cohort, numbers that were quite similar to those of the derivation cohort. A

lower total of 57% of the patients had a hs-cTnI$_{(Abbott)}$ measurable concentration above LOD. The hs-cTnI$_{(Sgx)}$ assay was not analyzed in the internal validation cohort.

4.2 Results Paper 1

Paper 1 describes the background and rationale for the study, study design, and expected outcome. The patient characteristics from the derivation cohort are also reported (see point 4.1.1).

4.3 Results Paper 2

The data analysis for this paper was done in the derivation cohort only. A baseline rule-out cut-off was investigated in 971 patients, and a 0/1-hour rule-out/rule-in algorithm was developed based on data from the 465 patients who had a 1-hour sample. When the baseline concentrations of the different cTn assays were compared for diagnosing NSTEMI using a AUROC analysis, the hs-cTnI$_{(Sgx)}$ assay came out as most favorable (AUROC 0.95 vs. 0.91 for hs-cTnT, P<0.001 and 0.93 for hs-cTnI$_{(Abbott)}$, P=0.004 Delong test)

4.3.1 Diagnostic performance and efficacy of the algorithms

When the whole derivation cohort was evaluated (regardless of time from symptom onset), the novel admission hs-cTnI$_{(Sgx)}$ algorithm (baseline value <2 ng/L) had a sensitivity >99%, fulfilling the pre-specified criteria. The hs-cTnI$_{(Sgx)}$ algorithm suggested by Neumann (107) had 100% sensitivity, and the hs-cTnI$_{(Sg)}$ algorithm developed by Body et al had >99% sensitivity (106). The ESC algorithms were evaluated after exclusion of patients with <3 hours since symptom onset. Doing this, the cTnT$_{ESC}$ algorithm had a similar sensitivity (98.9%), missing one patient, while the cTnI$_{(Abbot)}$ (97) algorithm inappropriately ruled out two NSTEMIs, resulting in a sensitivity of 97.9% (**Table 7**).

Table 7. Overview of baseline hs-cTn concentrations for the NSTEMI patients who were ruled out by one or several single (admission) sample rule-out algorithms. Patients with chest pain <3 hours were excluded from the analysis of the ESC algorithms.

Single sample rule-out algorithm	**Patient 1**	**Patient 2**	**Number of ruled-out NSTEMI**
hs-cTnT$_{ESC}$ <5 ng/L	cTnT$_{ESC}$ 4 ng/L	cTnT$_{ESC}$ 11 ng/L	1
hs-cTnI$_{(Abbott)ESC}$ <2 ng/L	cTnI$_{(Abbott)}$ 1.5 ng/L	cTnI$_{(Abbott)}$ 2 ng/L	2
hs-cTnI$_{(Sgx)}$ <2 ng/L	cTnI$_{(Sgx)}$ 1.3 ng/L	cTnI$_{(Sgx)}$ 9.3 ng/L	1
hs-cTnI$_{(Sgx)Neumann}$ <1 ng/L	cTnI$_{(Sgx)}$ 1.3 ng/L	cTnI$_{(Sgx)}$ 9.3 ng/L	0
hs-cTnI$_{(Sgx)Body}$ <1.5 ng/L	cTnI$_{(Sgx)}$ 1.3 ng/L	cTnI$_{(Sgx)}$ 9.3 ng/L	1

None of the investigated 0/1-hour algorithms ruled out any NSTEMI patients.

In our cohort, the novel hs-cTnI$_{(Sgx)}$ algorithm were highly efficient compared with the other algorithms, ruling out 37% of the patients, while the rule-out rate was 9%–24% for the comparator algorithms (Neumann (107) and Body (106), respectively). The 0/1-hour rule-out and rule-in algorithms allocated 92% of the patients to either rule-in or rule-out, while the numbers for the comparator algorithms were ≤ 78%.

4.3.2 Long-term prognostic value

There were 82 endpoint events in the group evaluated based on the admission sample, and 32 in the group evaluated based on the 0/1-hour cohort. Median follow-up was 723 days. Cox regression analysis showed that patients who were allocated to rule-out based on an admission sample experienced significantly fewer endpoints than patients in the observation/rule-in group. The exception was the hs-cTnI$_{(Sgx)}$ admission cut-off from Neumann et al, because this algorithm ruled out only 9% of the patients included in the WESTCOR cohort. Prognostic potential was also found for the previously published 0/1-hour algorithms from ESC, Body, and Neumann (97, 106, 107) as opposed to the novel algorithm allowing for rule-out even at hs-cTnI$_{(Sgx)}$ concentrations above the 99th percentile of the assay. The long-term event rate for patients ruled out

by the novel hs-cTnI$_{(Sgx)}$ 0/1-hour algorithm was as high as 5.3%, which was not significantly different from the event rate in the observation or rule-in group for this assay.

4.4 Results Paper 3

This paper reported data from 932 patients with suspected NSTE-ACS in the WESTCOR derivation cohort . The primary endpoint (30-day MI, all-cause mortality, and unplanned revascularization) was reached by 21%, and 13% had NSTEMI during the index hospitalization (secondary endpoint). Among patients allocated to low risk who were ruled out by the ESC 0/3-hour algorithms, 7.8%-9.2% would experience a primary endpoint (**Table 8**), compared with 7.3%-8.6%–with use of the High-STEACS algorithm. Combining the 0/3-hour algorithms with the clinical ACS criteria suggested by ESC reduced the number of primary endpoint events to 3.9%–4.9% (ESC 0/3-hour algorithm) and 3.8%–4.3% (High-STEACS algorithm). Most events were unplanned revascularizations (≤0.5% patients with MI were ruled out, none of the deaths were associated with rule-out). Replacing the ACS criteria with risk scores yielded variable results, but use of the HEART score would have led to increased NPV and sensitivity while maintaining efficacy. The combination of the 0/3-hour algorithms and HEART score ≤3 would lead to reductions in the rule-out of primary endpoints to 2.5%–2.7% (ESC 0/3 hour) and 2.2%–2.5% (High-STEACS). Again, almost all endpoints that were ruled out were unplanned revascularizations (no deaths were associated with rule-out, and one MI was ruled out for ESC hs-cTnI$_{(Abbott)}$).

Regarding the secondary endpoint, the ESC 0/3-hour hs-cTnT would have missed three index NSTEMIs and the High-STEACS algorithm would have missed two. The hs-cTnI ESC 0/3-hour algorithm missed seven NSTEMIs, and High-STEACS missed three (**Table 8**). The combination of hs-cTn algorithms and ACS criteria would have substantially reduced the number of ruled-out NSTEMI patients because this combination with hs-cTnT missed no patient, whereas the combination with hs-

cTnI$_{(Abbott)}$ missed two for the ESC and one patient for the High-STEACS combination. The combination with HEART score missed no index NSTEMIs except for one patient missed with the ESC hs-cTnI$_{(Abbott)}$ algorithm.

Table 8. Numbers of ruled-out patients with primary or secondary endpoint events when cTn algorithms were applied alone and in combination with the ACS criteria or the HEART score.

Algorithm	Very low	Low	Rule-out Δ	Risk criteria	Primary endpoint (%)	Secondary endpoint (%)	Rule-out rate (%)
ESC hs-cTnT	≤14	≤14	≤50%	NA	47 (7.8)	3 (0.5)	595 (63.8)
ESC hs-cTnI$_{(Abbott)}$	≤26	≤26	≤50%	NA	65 (9.2)	7 (0.9)	706 (75.8)
High-STEACS hs-cTnT	<5	≤14	<3	NA	42 (7.3)	2 (0.2)	574 (61.6)
High-STEACS hs-cTnI$_{(Abbott)}$	<5	≤16 (F) ≤34 (M)	<3	NA	57 (8.6)	3 (0.4)	661 (70.9)
ESC hs-cTnT	≤14	≤14	≤50%	ACS	15 (3.9)	0	376 (40.3)
ESC hs-cTnI$_{(Abbott)}$	≤26	≤26	≤50%		19 (4.9)	2 (0.5)	386 (41.4)
High-STEACS hs-cTnT	<5	≤14	<3	ACS	14 (3.8)	0	367 (39.4)
High-STEACS hs-cTnI$_{(Abbott)}$	<5	≤16 (F) ≤34 (M)	<3		16 (4.3)	1 (0.2)	376 (40.3)
ESC hs-cTnT	≤14	≤14	≤50%	HEART≤3	9 (2.5)	0	366 (39.3)
ESC hs-cTnI$_{(Abbott)}$	≤26	≤26	≤50%		10 (2.7)	1 (0.3)	374 (40.1)
High-STEACS hs-cTnT	<5	≤14	<3	HEART≤3	8 (2.2)	0	356 (38.2)
High-STEACS hs-cTnI$_{(Abbott)}$	<5	≤16 (F) ≤34 (M)	<3		9 (2.5)	0	364 (39.1)

4.5 Results Paper 4

In the derivation cohort, 24.4% of the patients had NSTE-ACS, compared with 25.8% in the validation cohort. The NSTEMI rate was lower in the validation cohort at 8.7% vs. 13.2% in the derivation cohort. The baseline concentration of hs-cTnT (analyzed

on fresh samples using multiple different reagent and calibrator lots) was similar for the diagnostic groups in the two cohorts. The hs-cTnI$_{(Abbott)}$ concentrations were analyzed in batches, so that one reagent and calibrator lot was used for each cohort, resulting in significantly lower baseline concentrations in the validation compared with the derivation cohort (P<0.001) for all diagnosis groups except NSTEMI. In both cohorts, UAP patients had significantly higher baseline concentrations (P<0.001) and larger hs-cTn delta values compared with the patients with NCCP (P<0.01), except for the 3-hour delta value for hs-cTnI$_{(Abbott)}$ (P=0.19).

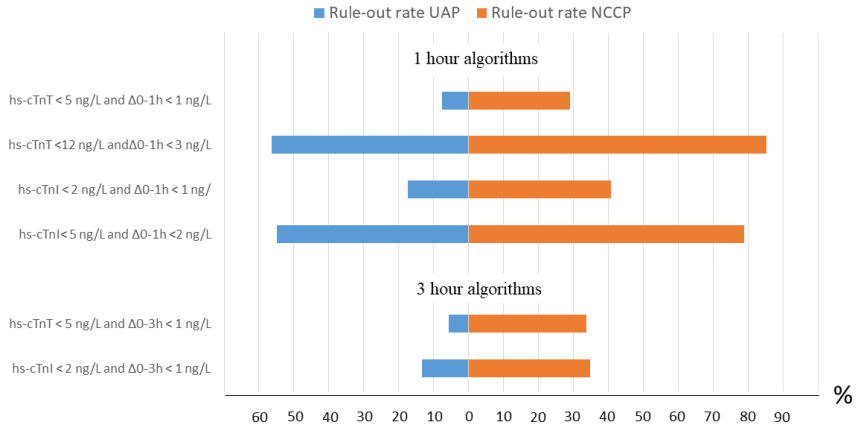
The novel low baseline/low delta hs-TnT 0/1-hour and 0/3-hour rule-out algorithms had superior clinical sensitivity for the primary endpoint (NSTEMI or UAP during the index hospitalization). In the validation cohort, sensitivity was 95.4% for 0/1-hour and 97.5% for 0/3-hour versus 62.8% for the ESC 0/1-hour algorithm (P<0.001). However, the specificity for the novel hs-TnT 0/1-hour and 0/3-hour rule-out algorithms was much lower at 21.0% for 0/1-hour and 30.6% for 0/3-hour versus 81.7% for the ESC algorithm (P<0.001). There was a 4.2% reduction in the rule-out rate compared with the hs-cTnT ESC algorithm. In the validation cohort, the novel 0/1-hour and 0/3-hour hs-cTnI$_{(Abbott)}$ algorithms did not fulfill the 95% sensitivity criterion, with sensitivities of 86.9% and 87.6%, respectively, but were still much better compared with the 63.9% for the hs-cTnI$_{(Abbott)}$ 0/1-hour ESC algorithm (P<0.001). The specificity was low at 45.1% for 0/1-hour and 38.6% for 0/3-hour versus 78.5% for the ESC algorithm. The rule-out rate was reduced by a factor of 1.8.

The novel algorithms did not perform better than the ESC algorithms regarding the secondary endpoint (MI, all-cause mortality within 30 days, or revascularization within 24 hours). The sensitivity for the novel algorithms was 100% (both cohorts), but despite 100% sensitivity of the ESC algorithms in the derivation cohort, they missed a few urgent revascularizations, returning sensitivities of 94% (ESC hs-cTnT) and 96% (ESC hs-cTnI$_{(Abbott)}$) in the validation cohort. The specificity of the novel algorithms was much lower than specificity of the ESC algorithms, and the novel 0/1-hour and 0/3-

hour rule-out algorithms had values of 19%–41%, versus 66%–77% for the ESC 0/1-hour algorithms.

In a subgroup analysis of UAP and NCCP patients, the 0/3-hour rule-out algorithm showed the best performance (**Figure 7)**. Using the low baseline/low delta hs-cTnT algorithm, only 6% of the UAP patients were ruled out (total cohort) with a concomitant rule-out rate of 34% in NCCP patients. For the hs-cTnI$_{(Abbott)}$ algorithm, corresponding numbers were 13% for UAP and 35 for NCCP.

***Figure** 7. Rule-out rates in patients with UAP and NCCP. Figure by Tjora et al. Diagnostic Performance of Novel Troponin Algorithms for the Rule-Out of Non-ST-Elevation Acute Coronary Syndrome, Clinical Chemistry, 2022,vol 68 (2) 291-302, published under Open Access and reprinted under the terms of the of the Creative Commons CC BY license.*



5. Discussion

5.1 Methodological considerations

5.1.1 Study Design

The WESTCOR study is a prospective observational study. However, prospectively colleceted data have also been used for cross-sectional analyses.

Advantages of the cross-sectional analyses include measuring exposure (hs-cTn measurements or clinical symptoms) and disease (e.g., NSTEMI or NSTE-ACS) in the same time frame (0–3 hours after admission), calculating prevalence, measuring multiple outcomes, and calculating diagnostic performance according to the STARD instrument (see below) (112).

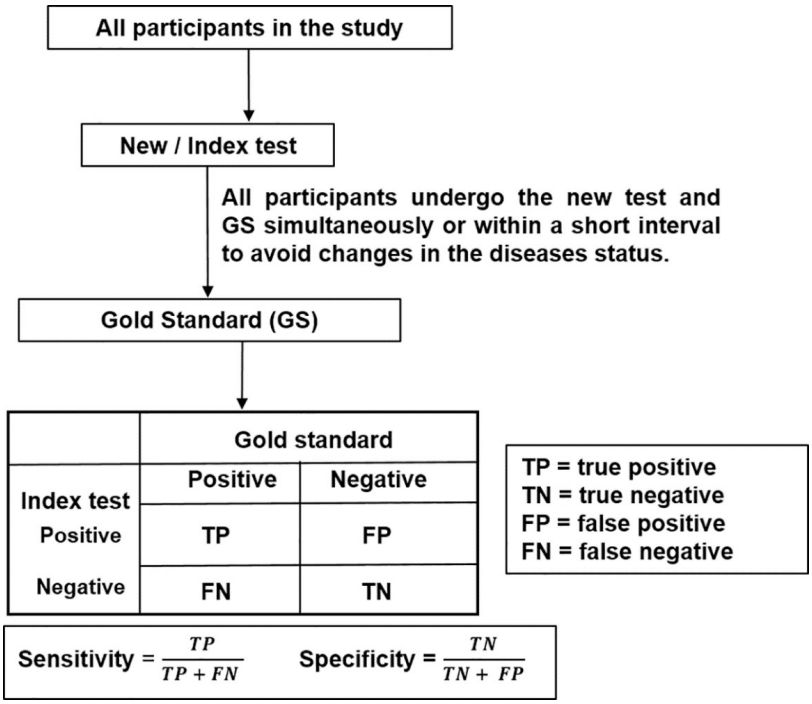
The prospective observational part of the study offered an opportunity to observe patients over a long time period after the exposure (admission to hospital because of chest pain). In general, a prospective design can facilitate investigation of associations between multiple exposures and outcomes, also calculation of incidence and relative risk can be made (113). A disadvantage of a prospective observational study design is that a long observation time from exposure to outcome is sometimes needed which is time-consuming. In addition, the observation period should be minimized to avoid introducing uncertainty about whether the outcome indeed is related to the exposure, so this study design is not best suited to studying diseases with long latency (113).

The most important disadvantage of observational studies (both cross sectional and prospective) is that this design does not allow for differentiation between cause and effect (112), and only associations and not causality can be established. Bias and confounding are major potential weaknesses in such studies, which can influence the results. Also, cohorts must be of sufficient size to allow for creation of groups to compare or for substudies (113).

5.1.2 The STARD instrument

The STARD instrument was developed to increase the quality of diagnostic performance studies (83, 114) and provides a detailed description of how diagnostic studies should be performed. According to this methodology, diagnostic studies should compare new (index) test accuracy in predicting the target diagnosis with the performance of a gold standard test (**Figure 8**). The gold standard test should have a sensitivity and specificity near 100%, so that it will discriminate in the best possible way between those with and without disease in the study population (115). Accuracy is defined as calculation of sensitivity, specificity, NPV, and PPV.

***Figure* 8**. *Outline of a diagnostic study design. Figure by Umemneku et al. Diagnostic test evaluation methodology: A systematic review of methods employed to evaluate diagnostic testes in the abcence of gold standard-An update, Plos One, 2019,vol 14 (10) 1-25, published under Open Access and reprinted under the terms of the of the Creative Commons CC BY license (115).*



5.1.3 The gold standard test

If no particular gold standard test is available, as is the case for MI, a clinical diagnosis may be applied as the gold standard. In such cases, the clinical diagnosis must be based on a uniform definition that is accepted throughout the field, such as the UDMI. Furthermore, it is crucial to have a valid and objective procedure for adjudication and to apply updated state-of-the-art diagnostic criteria consistently. In the WESTCOR study, two cardiologists adjudicated the diagnosis as defined in the third UDMI (available during the study planning), and the index test (e.g., different biomarkers, biomarker combinations, and clinical scoring systems was applied on the study population and compared with outcomes using the gold standard test (115). The design with shared decision-making for adjudication of diagnosis has several benefits, but there are also potential pitfalls. Pooling data can lead to more reliable information, and an open discussion is valuable for recalibration of the diagnosis (116), but it is important to be attentive to group dynamics when decisions are made. A group can strive for conformity and start to engage in groupthink, becoming less critical or less willing to consider a different diagnosis. Another problem can arise if the group is too diverse in clinical perspective or if members feel mistrust or competition with each other (116). In the WESTCOR study there was 5% disagreement of the diagnoses in the derivation cohort and 1.3% in the validation cohort, but after thorough discussions, this was reduced to 0.3%. This adjudication process is in line with the current state-of-the-art and applied in all major studies within this field (117, 118).

5.1.4 The index test

Another important prerequisite in the STARD instrument is that the gold standard test (i.e., clinical diagnosis) should not depend on the index test. This is a challenge in all studies where the index and gold standard test rely on e.g., the same biomarker for verification. However, the ability of an admission hs-cTn sample or a 0/1-hour hs-cTn algorithm to predict MI may be compared with a gold standard (i.e., UDMI diagnosis), because the gold standard definition takes into consideration a large number of

additional clinical and imaging data and several hs-cTn measurements, while the index test is designed for purely predicting probability of MI.

The index test can be prone to bias in other ways, including in recruitment, data collection, and analysis (119, 120), as further discussed below.

5.2 Bias

Bias could be defined as a systematic error that affects the magnitude of an observed difference in the outcomes of two groups that are compared. In a study, bias can arise during the planning, implementation, and publication of results, among other ways, and lead to false conclusions about the effects of the parameter being studied (121, 122).

5.2.1 Selection bias

Selection bias is a general term used to describe a group of biases and effects that arise in a sample that is systematically different from the population it is intended to represent (123). In the WESTCOR study, patients were selected in the ED, and we have to be aware of selection bias from this locus. Bias can have been introduced if the patients who refused to participate were differed in some ways from patients who agreed, so that the true chest pain population was not examined. In the planning of the study, we decided to have consecutive sampling in the ED. This proved to be difficult to perform during the study, mainly because the doctor or nurses on call decided who was eligible for inclusion, and in the sometimes overcrowded ED, study inclusion was not prioritized. From 2015–2019, the inclusion rate went down, suggesting that the extra labor burden from performing inclusion was too high and led to inclusion "burn-out" in the ED staff. This factor could offer one explanation for why the percentage of NSTEMI in the derivation cohort was higher (13%) compared with the later included internal validation cohort (9%), if staff found patients with less severe symptoms easier to include. Even so, the NSTE-ACS rate was the same in both cohorts, at approximately 25%. When compared with other studies, our study population seems to have been similar regarding age and number of patients with NSTE-ACS, frequency of other diseases, and rates of risk factors (124, 125). Focusing on sensitivity and specificity

(parameters independent of the prevalence of the disease studied) is important when cohorts are different; accordingly, we emphasize these indices more than the prevalence-dependent predictive values. The rule-out algorithms were given higher consideration than the rule-in algorithms because incorrectly rule-out of patients with NSTEMI would have major implications. However, since the internal validation cohort included a smaller proportion of NSTEMI patients, it is probably unlikely that there was any overestimation of the performance of rule-out algorithms.

Another source of selection bias that is applicable for our study is loss to follow-up or non-registered events. Because the follow-up data were collected from national registries, we view this potential for bias as minimal. The NPR is conducted under Norwegian legislation and registers all health-related contacts in Norway. Accordingly, only the infrequent situation of a patient's moving or being hospitalized outside of the country would result in this kind of bias.

5.2.2 Information bias

Information bias is caused by the studied variables not being measured, collected, or interpreted correctly (122). In our study, NSTEMI was diagnosed by the use of routine hs-cTnT measurements, we did not apply the hs-cTnI for adjudication. The few patients who would have been diagnosed with NSTEMI if the hs-cTnI assay had been used instead, could have affected the sensitivity and specificity of the hs-cTnI algorithms. It would have been more correct to have done the adjudication with both assays, and the lack of "double" cTn adjudication should be considered a weakness of the study.

Our long-term follow-up data are based on diagnoses from the NPR, so if the data in this register are not accurate, then the findings in the long-term follow-up could be flawed. We did not undertake systematic or independent adjudication of the diagnoses provided by the NPR, this another limitation of our study. Howewer, a recent study from Tromsø validated the MI diagnoses set in the NPR registry, and showed that the diagnoses were highly correct (126).

5.3 Confounding

Confounding can occur when a variable is associated with both the exposure and the outcome being investigated, but not on the same causal pathway between the two (122). To overcome this problem in the WESTCOR study, we performed a multivariable Cox regression analysis in which we corrected for factors described as potential confounders: age, sex, smoking, glomerular filtration rate, diabetes mellitus, hypertension, hyperlipidemia, and previous MI (104). Thus, some of the variables that were significantly related to the endpoint in the univariable analysis would not be significant in the multivariable analysis. It is important to be aware that residual confounding can also be a problem (112) if an unknown and thus unadjusted parameter influences the outcome. For adjusting the analysis in this study, we chose independent variables based on clinical and scientific knowledge, but other strategies that could have been used include automatic stepwise forward or backward selection, measures of explanatory variables (in univariate analysis), or use of a model based on the quality of the statistical model (e.g., Akaike Information Criteria).

5.4 Choosing the endpoints

We chose slightly different endpoints in the different papers, focusing on the index event and in papers 3 and 4 also including one endpoint looking at 30-day MI, death, and unplanned or urgent revascularization. We found these endpoints to be of high importance because they measure whether an algorithm can discriminate between patients with high versus low risk of acute CAD also when disease is not discovered immediately during the index event. In paper 2, the prognostic endpoint was a composite of all-cause mortality and subsequent nonfatal MI (meaning all MIs after the index NSTEMI), but contrary to papers 3 and 4, patients were followed until an endpoint occurred or until a median follow up time of 723 days. The knowledge of how these algorithms perform for an intermediate-term event (30 days) is missing, which may be considered a weakness of that paper. The inclusion of all-cause mortality as an

endpoint may be debated. If the cause of death is not related to CVD, then this endpoint can underestimate the efficacy of, for example, rule-out algorithms because they cannot be expected to predict death from non-cardiac causes. Another point is the age of the patients at study inclusion. We had no upper age limit for inclusion, and older patients of course have a shorter life expectancy on average. This factor is still unlikely to have had a major effect on the data because the median age in our study was similar to that in other studies within this field (73, 127).

5.5 Measuring diagnostic performance

There is debate about whether sensitivity or NPV should be used as the safety measure for rule-out algorithms for MI (128, 129). NPV depends on disease prevalence, and with a low prevalence, it will increase, whereas it decreases with a high prevalence (given the same clinical sensitivity) (130). Accordingly, diagnostic clinical sensitivity ≥99% might be a better measure (71, 104). For ruling in MI, most studies require a high PPV ≥70%–80% to prevent allocation of too many patients without NSTEMI to the acute cardiology ward and angiography (28, 71, 131). PPV also depends on prevalence, so that specificity for NSTEMI ≥95% (ruling in <5% of non-NSTEMI patients) with maximized sensitivity for NSTEMI can be a better safety measure (104). The power calculations were targeted to yield a study power of 80% with alpha of 0.05 to detect differences between algorithms. The expectation of finding a 5% difference in sensitivity between rule-out algorithms turned out not to be realistic because the currently recommended rule-out algorithms for NSTEMI have a sensitivity ≥95%. However, this was less clear during the planning of the study (before 2012). Similarly, the specificity for the rule-in algorithms was similar to that of other published algorithms, most of which have documented a specificity close to 95%. The differences between algorithms would therefore be evident in the specificity of rule-out algorithms (higher rule-out rate of non-NSTEMI patients) and specificity of rule-in algorithms

(lower rule in-rate for non-NSTEMI patients), and examples of significant differences between algorithms related to these parameters were demonstrated in the three articles.

5.6 Internal and external validity

A study with high internal validity has a good correlation between the exposure (different algorithms investigated) and endpoint (e.g. diagnosis of NSTEMI or NSTE-ACS) measured (132). As described above, for the WESTCOR study, we tried to eliminate or at least acknowledge sources of bias as far as possible and to select endpoints that were representative for the research question. Finally, in paper 4, we evaluated the novel algorithms from the derivation cohort in an internal validation cohort, increasing the confidence of the data overall and identifying potential uncertainties that should be acknowledged, such as how the analytical quality of the assay could influence the diagnostic performance of the algorithms (see point 5.8.4. below).

The external validity of our study refers to the generalizability of our findings, so that the data are applicable in other cohorts and in health care systems where chest pain patients are investigated using different logistics systems from ours (132). Our strategy to ensure external validity has been to focus on sensitivity and specificity (which are not dependent on disease prevalence) and to use broad inclusion criteria without limitations regarding age, renal function, and time frame since symptom onset. The last factor may be the most important because the algorithms have been criticized for poorer performance in early and late presenters, which we observed, as well. Finally, all patients had a final adjudicated diagnosis, an important step because exclusion of patients for whom NSTEMI may not be certainly determined or excluded could positively bias algorithm performance.

In paper 4 we include UAP in the primary endpoint. The diagnostic definition of UAP is not consistant between studies and it should be notet that we have a broad definition including patients with hs-cTnT concentrations above the 99th percentile amongst those diagnosed with UAP. Our prevalence of UAP seemingly is higher that many other studies, especially in the validation cohort. The impose uncertainty on our findings

related to UAP, and these needs validation in other cohorts. A universal consensus regarding the definition of UAP seems highly warranted.

5.7 Ethical considerations

The study and biobank were approved by the Regional Committees for Medical and Health Research Ethics (2014/1365 REK Vest and 2014/1905 REK vest). The study was conducted in accordance with the Declaration of Helsinki, and all patients provided written informed consent. A few patients withdrew consent and were removed from the study. The patients had to undergo some extra blood sampling, and otherwise there were no risks related to study participation, and inclusion did not interfere with treatment or follow-up.

5.8 Discussion of main findings

5.8.1 Introduction

In this thesis, the main goal has been to improve the diagnostic pathways for patients with chest pain suspicious for ACS who are admitted to the ED. To improve the work-up, new algorithms were designed with the purpose of obtaining an ideal balance between sensitivity and specificity ensuring safe follow-up while preserving efficacy. Another way to improve diagnostics has been to explore the combination of different scoring criteria and estimate long-term cardiovascular risk. The research question has been investigated from different angles, focusing on any clinical effect of improved analytical sensitivity of hs-cTn assays, the role of clinical risk scores, and whether hs-cTn assays may be useful for identifying UAP patients.

5.8.2 Pros and cons for rule-out/rule-in algorithms using high sensitivity cardiac troponin assays with improved analytical sensitivity

In paper 2 (104), a hs-cTnI assay from Singulex Clarity System was used to design novel algorithms. This assay (not currently in commercial use) has a higher analytical sensitivity than other hs-cTn assays available during the study, showing measurable concentrations (above the LOD) in 99.9% of a healthy population versus 25%–90% for other hs-cTn assays (59, 133). Two earlier studies (106, 107) indicated that a higher analytical sensitivity would improve the diagnostic work-up for patients, so this feature seemed important to further elaborate. Another study has also explored the hs- $cTnI_{(Sgx)}$ assay in patients with chronic CAD, showing its superiority in diagnosing obstructive CAD compared with hs-cTnT (134). Our data support the interpretation that the increased analytical sensitivity of the hs-$cTnI_{(Sgx)}$ translated into several important observations documented in our study.

First, the algorithms developed using this assay provided rule-out (both admission and 0/1 hour) with similar sensitivity but higher specificity compared with other algorithms using hs-cTnT or hs-$cTnI_{(Abbott)}$. This increase resulted in a higher rule-out rate for the novel algorithms. Body et al (106) reported a similar sensitivity a higher rule-out rate compared with a hs-cTnT algorithm. For the rule-in algorithm, both higher sensitivity and specificity were detected, resulting in a higher rule-in rate for patients with NSTEMI and lower rule-in rate for patients without NSTEMI in contrast to comparator algorithms. Together, these improvements led to a higher overall efficiency for the novel algorithms (104, 135). Neumann et al (107) compared a novel 0/1-hour algorithm using hs-$cTnI_{(Sgx)}$ with a corresponding ESC algorithm from hs-$cTnI_{(Abbott)}$ and found improved diagnostic performance and efficacy for the novel algorithm, in line with our data.

Although these findings are in accord with what we would expect from an assay with higher analytical sensitivity and with the existing literature, they also could be cohort dependent and should therefore be seen as hypothesis generating. The algorithms developed in the cohorts investigated by Body and Neumann (106, 107), respectively, differed from ours, and the diagnostic performance and rule-out rate for the single-

sample test and 0/1-hour algorithm gave very different results when these algorithms were applied in our cohort. The reason for these differences could be related to the different health care systems, leading to clinical inequalities among the three cohorts. The demand for a very high clinical sensitivity allowing for only 1% of NSTEMIs to be false rule-outs leads to cut-offs derived based on the very few NSTEMI patients with the lowest baseline and delta values (those on the lower "tail" of hs-cTn concentrations). The low number of data determing the cut offs would be expected to increase uncertainty and give the appearance of large differences in suggested cut offs if these NSTEMI patients by coincidence differ only slightly. Another important issue is that reagent and calibrator lot variations can influence the diagnostic performance of the cut-offs. The measured hs-cTn concentrations on admission were higher in the WESTCOR cohort compared with the Neumann cohort (no data on admission concentrations were available from the cohort reported by Body et al) (**Table 9**). The higher baseline concentrations observed in the WESTCOR study correspond to the lower rule-out rates calculated when the cut-offs suggested by Neumann (107) were applied in our cohort, supporting this explanation. Most patients with acute chest pain have low cTn concentrations, so that reagent and calibrator lot shifts can have larger effects on the efficacy of algorithms using a low concentration (rule-out algorithms). Furthermore, these analytical challenges are known to be more prominent at lower concentrations. Thus, it is less surprising that the rate of ruled-in patients in our study (104) was quite similar to that of the Neumann study (107) when the rule-in algorithm (as rule-in algorithms use higher concentrations as cut offs) from the Neumann study was applied in the WESTCOR cohort.

Table 9. Admission sample concentrations, rule-out rate, and rule-in rate reported for the hs-cTnI$_{(Sgx)}$ assay, for similar algorithms across different cohorts.

Cohort	**Admission hs-cTnI (ng/L)**	**Rule-out rate (%)**			**Rule-in rate (%)**
		cTnI <1 ng/L	cTnI <1.5 ng/L	cTnI <2 ng/L	TnI ≥25.0 ng/L or Δ0-1 ≥6 ng/L
WESTCOR	2.8 (1.5–7.7)	9	24	37	16
NEUMANN	2.1 (0.9–1.2)	27	No data	47*	18
BODY	No data	No data	40 and 39ǂ	No data	No data

* Numbers are reported for the following algorithm: TnI <2.0 and Δ0-1 <1 ng/L.
ǂ Derivation and validation cohort

The major divergence between our results and the studies of Body and Neumann (106, 107) highlights the importance of investigating the assay and algorithms in different chest pain cohorts, using different reagent and calibrator lots for the assay and that all novel algorithms need extensive internal and external validation in large study cohorts. The hs-cTnI$_{(Sgx)}$ assay is no longer commercially available, and further validation is not possible. Our data can still serve as an example of how algorithms using high-sensitivity assays can be developed and used.

Another important finding was that the hs-cTnI$_{(Sgx)}$ assay seemingly could detect a clinically relevant increase in cTn concentration earlier compared with other assays, suggesting the potential to reduce the time to myocardial injury detection without any reduction in clinical diagnostic sensitivity. If this finding could be confirmed, all patients could be evaluated using the admission sample, regardless of time since symptom onset. This ability would be a clear advantage because the present recommendations state that only patients who have been symptomatic for 3 hours or more should be evaluated based on an admission sample alone (28) . Up to 40% of patients are early presenters, so this limitation on the utility of the current algorithms significantly affects ED logistics. Body (106) reported a similar observation.

The last important finding was that patients ruled out by the novel 0/1-hour hs-cTnI$_{(Sgx)}$ algorithm experienced more future MIs or deaths than patients ruled out by the hs-cTnI$_{(Abbott)}$ and hs-cTnT 0/1-hour algorithms from ESC, indicating that the hs-cTnI$_{(Sgx)}$ algorithm could not be used for prognostication. The most likely explanation is that the

hs-cTnI$_{(Sgx)}$ 0/1-hour algorithm used a baseline cut-off value above the 99th percentile, so that more patients with subclinical or even overt chronic myocardial injury were ruled out. It is well documented that these patients have higher long-term risk of cardiovascular events (33, 36, 104).

Based on this finding, algorithms that are extremely efficient at targeting NSTEMI may be less useful for future risk classification (135), and such classification might better be done using a dedicated scoring system for long-term risk evaluation.

5.8.3 Combining risk scores with cardiac troponin algorithms

Paper 3 investigated the utility of risk scores when chest pain patients are evaluated in the ED. In that paper, the combination of a clinical risk score such as HEART score ≤3 and hs-cTn 0/3 hour algorithms was evaluated and proved superior for detecting the primary endpoint (MI, death, or urgent revascularization within 30 days) compared with the combination of standard ACS criteria/hs-cTn algorithms suggested by ESC (105).

Our paper showed that risk scores like T-MACS, mHEART, and HEART score performed better than the ACS criteria from ESC and that the HEART score performed particularly well (105). In contrast, the latest ESC guidelines for NSTE-ACS recommend the GRACE score (not HEART) to be used as an investigation tool in the ED (28). Compared with earlier guidelines, this recommendation has gone from 1B to 2B, meaning there is currently less evidence supporting the use of this particular risk score for validation of the NSTE-ACS (28, 136). In a 2021 meta-analysis of data for 40,262 chest pain patients from 33 studies, TIMI, GRACE, and HEART scores were compared indirectly among one another regarding predictive value for risk stratification for MACE (92). The pooled sensitivities and specificities were respectively 0.95 and 0.36 for TIMI, 0.96 and 0.50 for HEART, and 0.78 and 0.56 for GRACE resulting in TIMI and HEART being superior to GRACE for predicting MACE (92), in line with our findings.

Few studies have evaluated a combination of ESC algorithms and clinical risk scores. Recently a modified HEART (mHEART) score has been developed *(137, 138),* that uses hs-cTn concentrations in a similar way as the ESC rule-out algorithms. In the mHEART score, the cTn levels need to be below the 99th percentile in more than one sample combined with a score of ≤3 for the remaining four categories to be considered low risk (137, 138). In two meta-analyses undertaken for HEART score and mHEART score, sensitivity for MACE was quite similar, 96% versus 97%, the incidence of MACE was, however, lower in the study using mHEART score (utilizing a lower cTn cut off resembling the ESC algorithms) 1.6% versus 0.8% *((100, 139)*, similar to our findings when combing HEART and cTn algorithms. The study from McCord et al. (137) concluded that a combination of hs-cTnT below the 99th percentile combined with an mHEART score ≤3 could identify a low-risk group in the ED who could be discharged without further testing (137). A study from Sanchis et al. included chest pain patients with a baseline hs-cTnT value below the 99th percentile, examining one-year outcomes. Undetectable hs-cTnT values were not helpful for predicting outcomes. Adding the hs-cTnT value as a continuous variable above LOD, however, improved the discrimination ability for a one-year outcome of MACE in both early and late presenters (C-statistics: 0.754, p=0.007: and 0.847, p=0.001), adding the HEART score improved reclassification in early presenters (140). This study supports the hypothesis that in patients being admitted to the ED with chest pain and hs-cTnT levels below the 99th percentile, adding a risk score can be helpful for further assessment of early presenters (140).

In paper 3 we used the 0/3 hour algorithm from 2015. In the latest ESC NSTE-ACS guidelines from 2020, 0/1-hour rule-out/rule-in algorithms are recommended, for a negative predictive value (NPV) of >99% and positive predictive value (PPV) of 70%–75% (28). In a meta-analysis from 2022, the new ESC 0/1-hour and previous 0/3-hour algorithms were evaluated. The 0/1-hour algorithm had a pooled sensitivity of 99.1% and an NPV of 99.8% for rule-out of MI. The pooled specificity for rule-in was 97%, with a PPV of 94%. The 0/1-hour algorithms performed better than the 0/3-hour algorithm (81). The previously recommended 0/3-hour algorithm had a lower pooled sensitivity of 93.7% and NPV of 98.7% for rule-out of MI, and the pooled specificity

was only 93.2% (PPV 64.4%). This is similar as seen in our data, the 0/3 hour algorithms from hs-cTnT and hs-cTnI$_{(Abbott)}$ (Paper 3) performed poorer compared to the corresponding 0/1 hour algorithms (Paper 2).

Further studies in this field are warranted especially because some NSTE-ACS patients have UAP, and even though they have increased risk for an adverse outcome (30, 32), they may not be identified using cTn algorithms alone (140). A clinical risk score or clinical gestalt is necessary to identify these patients, and it seems feasible to include the tool with the highest diagnostic precision into structured ED investigation pathways.

5.8.4 Using high sensitivity cardiac troponin algorithms to identify UAP

The ESC algorithms for rule-in/rule-out of NSTE-ACS are designed for rule-in/rule-out of NSTEMI. UAP patients can not be identify by these algorithms since they by definition have stable hs-cTn values. After the development and implementation of the hs-cTn assays, more patients were diagnosed with NSTEMI and fewer with UAP (141), and there was an expectation that the UAP diagnosis would vanish (142). This has not happened, and the reported incidence of UAP has varied from 2.8% (High-STEACS) (31) to 8.9% (APACHE) to 15% in a SWEDEHEART registry study (32). In our study, the UAP incidence was 11% in the derivation cohort and 17% in the internal validation cohort (109). One reason for the variable incidence is related to the defininition of UAP because this diagnosis is not clearly delineated in the UDMI. Some studies, such as High-STEACS, diagnosed UAP only in patients with ischemic symptoms and hs-cTn levels below the 99th percentile (31). Other studies have defined UAP also to include stable elevation of cTn values ≥99th percentile as a result of chronic myocardial injury (30, 32, 141). Several studies have measured hs-cTn levels in UAP and explored the association with outcomes (30, 32, 143). In general, UAP patients will have increased risk of death related to the cTn level, but the severity of CAD is not dependent on an elevated hs-cTn level (30, 144). One study showed that 29% of UAP patients with hs-cTn <LOD had PCI within a month (30), and in another using angiography on UAP

patients with hs-cTn levels below the 99th percentile, multivariable regression analysis showed that male sex (and not hs-cTn concentrations) was the only significant predictor of CAD (P=0.002) (144). Another recent publication from Lambrakis et al. indicates that identifying NSTEMI alone is insufficient. This study was the first randomized clinical trial evaluating the 0/1 hour algorithms. Patients were randomized to either a 0/1-hour algorithm using hs-cTnT or a 0/3-hour algorithm which only reported hs-cTnT concentrations down to 29 ng/L simulating a "conventional" cTnT assay, defined as the masked group. When the patients were evaluated for a 12 months endpoint, those with low hs-cTnT concentrations (< 29 ng/L) who were included in the 0/1-hour protocol had an increased hazard ratio for MI or all-cause death (P=0.030) compared to the masked 0/3-hour group (145). The authors discussed if the study targeted a group of patients with chronic myocardial injury with high risk for a future coronary event, mostly not MI. This group would then not benefit from an 0/1 –hour protocol. Also, the authors debated if rapid testing can miss subclinical CAD (145).

In our paper, we hypothesized that this ischemia would cause slightly higher baseline concentrations and larger variation in hs-cTn delta values in UAP patients compared to what is seen in presumable healthy individuals. The findings confirmed the hypothesis that the baseline and delta values were significantly higher in the UAP group compared with the NCCP patients (P<0.001) in both the validation and derivation cohorts (109). The novel algorithms were superior for allocating both NSTEMI and UAP patients to rule-in, but they were less specific, so that the rule-out rate declined 2–4 fold compared with the ESC algorithms that target identifying NSTEMI. The 0/3-hour hs-cTnT algorithm had the best performance, ruling out only 6% of the UAP patients and at the same time 34% of the NCCP patients. This performance could be attributable to easier detection of multiple minor cTn releases from cardiomyocytes in UAP patients if the observation period is slightly expanded. These algorithms did not perform better than the ESC algorithms for allocating NSTEMI patients or for the secondary endpoint of MI or death at 30 days or urgent revascularization within 24 hours.

Our study also show an example of how analytical performance of an assay may effect clinical outcomes. In the hs-cTnI assay, there was a calibrator shift in the internal

validation cohort, affecting the analytical sensitivity of the assay. In the derivation cohort, hs-cTnI was measured in 975 patients, and 84.4% had a value ≥1.7 ng/L (LOD). In the internal validation cohort, cTnI was measured in 519 patients, and only 57.2% had a concentration at LOD or higher. This outcome again affected the diagnostic clinical sensitivity of the novel hs-cTnI 0/1-hour and 0/3-hour algorithms, applying a cut-off concentration close to the LOD (2 ng/L). It is likely that this result was related to a calibrator shift as hs-cTnI was analyzed in batches using one reagent and calibrator lot for each cohort. This approach gave significantly lower hs-cTnI levels in the internal validation cohort compared with the derivation cohort, and the lower concentrations seemingly affected the sensitivity and specificity of the algorithms. In contrast, hs-cTnT was measured using multiple calibrator and reagent lots, yielding measurable results in approximately 70% of the patients in both cohorts and no difference in analytical performance between cohorts. This contrast highlights that using algorithms with low baseline and low delta values creates susceptibility to analytical quality of an assay, and that low long term analytical variation at the applicable concentrations is necessary to achive robust results.

To sum up, this paper shows that it is possible to design algorithms that can rule in UAP patients, and it will be important to explore even more sensitive hs-cTn assays when they are on the market. The challenges with diagnosing UAP and the need to explore hs-cTn assays has recently been discussed (146). Because of the lower efficacy of the algorithms and the described limitations related to analytical performance, all novel suggestions must be firmly validated in several independent cohorts.

6. Conclusion

A cardiac injury marker with higher analytical performance compared to current assays may improve ED efficiency by predicting NSTEMI in early presenters and allocating more patients to rule-out and rule-in compared to current recommendations. However, if high cut-offs are used for rule-out, patients ruled out for NSTEMI can have a subclinical myocardial injury and increased long-term risk. Accordingly, these patients must be identified using another risk stratification tool.

Troponin-based algorithms combined with clinical risk scores will identify a slightly higher proportion of patients with MI, death, or 30-day need for revascularization compared with using ACS low-risk criteria recommended by ESC or troponin-based algorithms alone. Our study evaluating 11 risk scores in combination with the ESC 0/3-hour and High-STEACS algorithms showed that the HEART score performed best regarding safety and rule-out rate.

The use of low baseline concentrations and delta values may improve clinical sensitivity for NSTE-ACS, as this option seems to better differentiate patients with UAP versus NCCP. The specificity is reduced, however, and compared with the ESC algorithms, the overall rule-out rate of patients investigated for NSTE-ACS was reduced by a factor of 2 to 4, which would result in a less efficient patient flow through the ED. Timing of samples, lot variations, and analytical variability may substantially influence diagnostic performance, and further improvement of analytical hs-cTn assays may still yield clinical benefit.

Both papers 2 and 4 demonstrate that it is important not to underestimate inter-cohort variability or analytical variation of assays when algorithm performance is determined, and robust data from multiple studies should be available before algorithms are implemented.

7. Future perspectives

Novel hs-cTn assays are under development, and for all novel assays rule-out and rule-in algorithms for NSTEMI must be developed and validated extensively.
Furthermore, it will be essential to explore if novel assays have improved abilities to predict or diagnose UAP compared to the assays being in commercial use now. If algorithms using low concentrations or low delta values are evaluated, developers should be aware of the recently described circiadian rhythm of hs-cTnT and investigate if this fluctuation can affect the interpretation of low baseline low delta hs-cTn algorithms (false rule-in patients) (44, 46). If such algorithms are successfully developed, a randomized controlled trial comparing standard care using the ESC 0/1-hour algorithms to the algorithms also identifying UAP would be beneficial, with follow-up for one year for MI, death, and revascularization.

Point-of-care (POC) assays that can provide a hs-cTn result within few minutes could be valuable tools in a hectic ED if such could be used for more rapid allocation of chest pain patients (147). POC assays need to be analytically and clinically robust to the same degree as the currently available hs-cTn assays, returning reliable and accurate results (147, 148). If the patient also needs other blood tests for diagnostic work up, the value of POC might not be as high in the ED, but this needs to be further investigated, preferable in a randomized clinical trial . POC may have greater value in outpatient clinics or in the ambulance for decision-making, if improved clinical diagnostic performance and efficiency may be scientifically demonstrated (148).

If the ESC 0/1-hour rule-out/rule-in algorithms are applied, a large group of patients will be ruled out. If imaging investigations (e.g. CCTA) is undertaken in this group non-significant stenosis or minor coronary arthery disease could be detected in proportions of patients. Future trials should investigate if subpopulations within the ruled out group could benefit from further investigations (e.g, imaging) and if proven beneficial clinical trials identifying the the optimal treatment should be undertaken as there is a need for structured follow-up in this group of patients.

8. References

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9. Papers 1- 4

Journal of the American Heart Association

ORIGINAL RESEARCH

Cardiac Troponin Assays With Improved Analytical Quality: A Trade-Off Between Enhanced Diagnostic Performance and Reduced Long-Term Prognostic Value

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BACKGROUND: Cardiac troponin (cTn) permits early rule-out/rule-in of patients admitted with possible non–ST-segment–elevation myocardial infarction. In this study, we developed an admission and a 0/1 hour rule-out/rule-in algorithm for a troponin assay with measurable results in >99% of healthy individuals. We then compared its diagnostic and long-term prognostic properties with other protocols.

METHODS AND RESULTS: Blood samples were collected at 0, 1, 3, and 8 to 12 hours from patients admitted with possible non–ST-segment–elevation myocardial infarction. cTnT (Roche Diagnostics), $cTnI_{(Abbott)}$ (Abbott Diagnostics), and $cTnI_{(sgx)}$ (Singulex Clarity System) were measured in 971 admission and 465 1-hour samples. An admission and a 0/1 hour rule-out/rule-in algorithm were developed for the $cTnI_{(sgx)}$ assay and its diagnostic properties were compared with $cTnT_{ESC}$ (European Society of Cardiology), $cTnI_{(Abbott)ESC}$, and 2 earlier $cTnI_{(sgx)}$ algorithms. The prognostic composite end point was all-cause mortality and future nonfatal myocardial infarction during a median follow-up of 723 days. non–ST-segment–elevation myocardial infarction prevalence was 13%. The novel $cTnI_{(sgx)}$ algorithms showed similar performance regardless of time from symptom onset, and area under the curve was significantly better than comparators. The $cTnI_{(sgx)0/1\ hour}$ algorithm classified 92% of patients to rule-in or rule-out compared with ≤78% of comparators. Patients allocated to rule-out by the prior published 0/1 hour algorithms had significantly fewer long-term events compared with the rule-in and observation groups. The novel $cTnI_{(sgx)0/1\ hour}$ algorithm used a higher troponin baseline concentration for rule-out and did not allow for prognostication.

CONCLUSIONS: Increasingly sensitive troponin assays may improve identification of non–ST-segment–elevation myocardial infarction but could rule-out patients with subclinical chronic myocardial injury. Separate protocols for diagnosis and risk prediction seem appropriate.

Key Words: chest pain ■ chronic myocardial injury ■ myocardial infarction ■ 0/1 hour algorithm

Clinical suspicion of non–ST-segment–elevation myocardial infarction (NSTEMI) is a frequent cause of hospital admission,[1] and cardiac troponin (cTn, T, or I) measurement is a cornerstone in evaluation of these patients.[2] Approximately 40% of patients are "early presenters,"[3,4] and accurate detection of low cTn

See Editorial by McCarthy and Januzzi

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CLINICAL PERSPECTIVE

What Is New

- Troponin assays with analytical sensitivity and precision beyond the current high sensitivity troponin assays are likely to show improved diagnostic performance for non–ST-segment–elevation myocardial infarction (NSTEMI).
- Diagnostic algorithms that are very precise for identification of NSTEMI will to a lesser extent identify patients with subclinical or overt chronic myocardial injury and consequently show lower long-term prognostic power compared with less precise algorithms.

What Are the Clinical Implications?

- Troponin assays with improved analytical sensitivity have a high ability for early identification of NSTEMI, making early presenters with low admission troponin concentrations eligible for rule-out.
- Development of efficient diagnostic follow-up schemes allocating >90% of patients presenting with chest pain to rule-in or rule-out for NSTEMI should improve the logistics in the emergency room.
- Long-term cardiovascular risk should be considered even in patients who are ruled out for NSTEMI.

Nonstandard Abbreviations and Acronyms

CMI	chronic myocardial injury
ESC	European Society of Cardiology
LOD	limit of detection
Sgx	Singulex Clarity System

concentrations for immediate rule-out of NSTEMI may therefore have clinical utility. The European Society of Cardiology (ESC) recommends that patients with a detectable baseline cTn concentration undergo serial testing. Based on the baseline and delta concentrations obtained, such cases can be classified as rule-out, observation, or rule-in for NSTEMI.[2] A few limitations apply when troponin-based algorithms are used for rule-out of NSTEMI, for example, the suboptimal analytical sensitivity (unavailability to provide measurable concentrations in all healthy individuals) and large analytical imprecision (reproducibility of sequential measurements) at low troponin concentrations, which could produce a false low delta value leading to an inappropriate rule-out. Improved analytical sensitivity and precision of these assays might facilitate algorithms with higher specificity allowing for admission rule-out in all individuals with low concentrations regardless of time from symptom onset and greater reliability of deltas allowing for rule-out in subjects with high normal or increased baseline concentrations.

Stable troponin concentrations above the 99th percentile is considered to indicate chronic myocardial injury (CMI) and are associated with poor long-term outcomes.[5–7] Studies have shown that risk rises continuously with troponin concentrations below the 99th percentile.[8] Some studies have identified this association even at concentrations lying between the limit of detection (LOD) and limit of blank of current assays.[9] Thus, identifying subclinical myocardial injury (ie, myocardial injury with stable troponin concentration <99th percentile) may be of clinical relevance because it could indicate increased cardiovascular risk in patients with acute chest pain.[10–13] Whether assays with improved analytical performance could allow for further improvement in long-term risk prediction is not known.

Two previous reports[14,15] described findings with a cTn assay (Singulex Clarity System) ($cTnI_{(sgx)}$) with improved analytical sensitivity, providing measurable results in >99% of healthy individuals (versus corresponding values of 72% for Roche Diagnostics and 85% for Abbott Diagnostics[16]). This assay also provided 10% analytical variation at concentrations below 1 ng/L.[16] Neither study, however, directly addressed whether this increased analytical quality translated into improved clinical utility. Although this assay is currently unavailable because the company stopped trading in 2019, the data derived from it are highly relevant to understand the possible benefits and drawbacks of improved analytical quality with troponin or other cardiac injury biomarker assays.

In this cross-sectional observational study, we hypothesized that compared with currently used or suggested cTnT and cTnI algorithms, $cTnI_{(sgx)}$ could offer better performance, with a greater rate of correct rule-out and rule-in of patients presenting with possible NSTEMI. For its development, the admission sample algorithm should include all patients regardless of time between sampling and symptom onset, and the 0/1 hour algorithm should allow rule-out in patients with increased baseline concentration, given low delta values. Because algorithms using high baseline troponin concentration can rule out patients with both subclinical myocardial injury and CMI, we also analyzed data from a prospective follow-up period to evaluate the relative long-term risk-prediction ability of these algorithms.

METHODS

Study Design

The data that support the current findings are available from the corresponding author upon reasonable

request. The WESTCOR (Aiming Towards Evidence Based Interpretation of Cardiac Biomarkers in Patients Presenting With Chest Pain) study (Clinical Trials number NCT02620202) is a two-center, cross-sectional, prospective observational study described in detail earlier.[17] The cross-sectional study design was used to investigate the accuracy of different algorithms. Patients were then prospectively followed to determine if the different algorithms could predict future cardiovascular outcomes.[18]

The current article reports data from the WESTCOR derivation cohort (WESTCOR-D) including 985 patients admitted to Haukeland University Hospital, Norway, with suspected non−ST-segment elevation acute coronary syndrome (ACS). The inclusion period lasted from September 2015 to February 2017. The study and biobank were approved by the Regional Committees for Medical and Health Research Ethics (2014/1365 REK West and 2014/1905 REK West).

Study Enrollment and Biobanking

Patients age >18 years admitted with chest pain or symptoms suggesting non−ST-segment elevation-ACS and who did not have a short life expectancy (eg, advanced cancer) and could provide informed consent were eligible for inclusion.[17] Patients had serum samples drawn on arrival to the emergency department and after 3 and 8 to 12 hours. Samples were centrifuged after 30 minutes, and material for the biobank was aliquoted and frozen. High-sensitivity cTnT was measured in fresh samples, and the results were reported to the attending clinician. After an initial period of fine-tuning of the study, an additional biobank sample was drawn 1 hour after admission, and the results were not reported to the attending clinician. This adjustment was planned a priori as a part of the study.[17] Biobank admission samples were available from 971 patients, and a 1-hour sample was available for 465 patients.

Biochemical Analyses

Routine and 1-hour samples were measured for cTnT (Roche Diagnostics) with limit of blank 3 ng/L, LOD 5 ng/L, 99th percentile 14 ng/L, and analytical within-series coefficient of variation 10% at 4.5 ng/L. For cTnI (biobanked samples), measured using the Abbott Diagnostics assay ($cTnI_{(Abbott)}$), these values were limit of blank 0.9 ng/L, LOD 1.7 ng/L, 99th percentile 26 ng/L, and 10% coefficient of variation 4.6 ng/L. For the cTnI measured using the Singulex Clarity System, these values were limit of blank 0.02 ng/L, LOD 0.08 ng/L, 99th percentile 8.67 ng/L, and 10% coefficient of variation 0.53 ng/L.[16] All other clinical chemistry tests were measured using Cobas e602 or Cobas 8000 from Roche Diagnostics. The glomerular filtration rate was estimated using the Chronic Kidney Disease Epidemiology Collaboration formula and an enzymatic isotope dilution mass spectrometry traceable creatinine assay (Roche Diagnostics).

Diagnosis

The diagnostic end point was NSTEMI during the index hospitalization. The adjudicating process has been described earlier,[17] but briefly, 2 independent cardiologists adjudicated the final diagnosis based on all available clinical, routine laboratory (including cTnT at admission and at 3 and 8–12 hours from admission), electrocardiogram, ultrasound, and imaging findings, including cardiac computed tomographic angiography and conventional angiography. A third adjudicator resolved disagreements. Specific diagnostic criteria were predefined for 22 different medical conditions based on guidelines that were available during planning of the study (see Data S1). NSTEMI was defined according to the third universal definition for myocardial infarction (MI), including a significant rise and fall of cTn with at least 1 value above the 99th percentile combined with symptoms of ischemia, electrocardiogram changes, and image evidence of loss of viable myocardium or intracoronary thrombus.[19] Delta values of 20% (baseline cTnT concentration >14 ng/L) or 50% (baseline cTnT concentration ≤14 ng/L) in serial cTnT measures were regarded as significant, as suggested by the ESC in 2012.[17,19] Since 2012, several studies have found a significantly lower 99th percentile concentration of cTn for women compared with men,[20–22] but knowledge of sex-specific cutoffs for women regarding diagnosing NSTEMI is partial because of a lack of data on pathophysiology.[23] Consequently, we chose to apply a common cutoff for all patients.

Follow-Up and Prognostic End Points

Follow-up data were collected through the Norwegian Patient Register and Norwegian Cause of Death Registry. The prognostic end point was a composite of all-cause mortality and subsequent nonfatal MI (all MIs after the index NSTEMI). Patients were followed until an end point occurred or until a median follow-up time of 723 days after inclusion (ranging from 4 to 900 days).

Comparator Algorithms

According to current recommendations, patients are eligible for early discharge or further investigations for ACS based on the troponin results.[2] The algorithms encompass an initial review of the admission sample in patients who present more than 3 hours after symptom onset. If the concentration is below

the LOD of the troponin assay, the patient is eligible for "rule-out" and may be discharged if the electrocardiogram and/or the clinical symptoms suggest a lower likelihood of ACS. The remaining patients undergo serial sampling at 1-hour intervals. Based on the baseline and delta values obtained, patient status is established as "rule-out," "observation," or "rule-in" for NSTEMI. Again, ruled-out patients may be discharged if the clinical suspicion of ACS is low, those who are ruled in may go directly to cardiac angiography and eventually invasive treatment, and those in the observation group undergo diagnostic follow-up. We compared the recommended ESC (cTnT and $cTnI_{(Abbott)}$) algorithms[2] and those suggested by Body et al[14] and Neumann et al[15] to the novel algorithms described in Tables 1 and 2.

Development of Novel Algorithms

The novel $cTnI_{(sgx)}$ rule-out algorithms were defined based on the following hierarchy of criteria: diagnostic sensitivity for NSTEMI ≥99.0%, as previously described,[24] and the maximum possible specificity. Sensitivity was preferred over negative predictive value as the criterion because sensitivity is independent of disease prevalence and applicable in chest pain cohorts with higher and lower prevalences compared with our cohort. Applicable concentrations for the rule-in algorithms were based on the following criteria: diagnostic specificity for NSTEMI ≥95% (<5% rule-in of patients with non-NSTEMI) and a simultaneously maximized sensitivity for NSTEMI. Specificity was considered preferable to positive predictive value because specificity is independent of prevalence.

We chose the preferred algorithms based on the number of ruled-out and ruled-in patients who would give a sensitivity and specificity corresponding to the prespecified criteria. "Direct rule-out" was defined as rule-out regardless of time since symptom onset. Diagnostic performance was calculated with and without early presenters, defined as patients with <3 hours since symptom onset.

Statistical Analysis

The baseline characteristics are reported as medians with interquartile ranges for continuous data and percentages for categorical data. The data were analyzed using the nonparametric Kruskal–Wallis test for continuous variables and the chi-square and Fisher's exact test for categorical variables, as appropriate. Statistical analyses included calculation of sensitivity, specificity, negative predictive value, positive predictive value, and likelihood ratios, receiver operating characteristic curve analysis, and calculation of area under the curve (AUC) for all algorithms. Significant differences in AUC were evaluated using the Delong test, and efficiency

Table 1. Single Sample Rule-Out of NSTEMI

	Sensitivity	Negative Predictive Value	Negative Likelihood Ratio	Specificity	Positive Predictive Value	Positive Likelihood Ratio	Area Under the Curve
Direct rule-out (N=971)							
$cTnT_{ESC}$ <5 ng/L	98.4 (94.4–99.8)	99.3 (97.4–99.8)	0.04 (0.01–0.18)	35.1 (31.8–38.4)	15.5 (17.7–19.3)	1.52 (1.44–1.60)	0.67 (0.64–0.70)
$cTnI_{(Abbott)ESC}$ <2 ng/L	97.6 (93.3–99.5)	98.7 (96.0–99.6)	0.09 (0.03–0.28)	26.0 (23.0–29.1)	16.6 (15.9–17.2)	1.32 (1.26–1.38)	0.62 (0.59–0.65)
$cTnI_{(sgx)Neumann}$ <1.0 ng/L	100 (97.1–100)	100 (NC)	0.0 (NC)	10.3 (8.3–12.6)	14.4 (14.1–14.7)	1.11 (1.09–1.14)	0.55 (0.52–0.58)
$cTnI_{(sgx)Body}$ <1.5 ng/L ng/L	99.2 (95.7–99.9)	99.6 (97.1–99.9)	0.03 (0.00–0.20)	28.0 (25.0–31.1)	17.2 (16.5–17.8)	1.38 (1.32–1.44)	0.64 (0.60–0.67)
$cTnI_{(sgx)}$ <2 ng/L*	99.2 (95.7–100.0)*	99.7 (98.1–100.0)*	0.02 (0–0.13)*	42.5 (39.2–46.0)*	20.6 (20.0–21.6)*	1.73 (1.63–1.83)*	0.71 (0.68–0.74)*
Admission sample rule-out and history ≥3 h (N=772)							
$cTnT_{ESC}$ <5 ng/L	98.9 (94.2–100.0)	99.5 (96.8–99.9)	0.03 (0–0.22)	34.5 (30.9–38.2)	18.4 (17.5–19.3)	1.51 (1.42–1.60)	0.67 (0.63–0.70)
$cTnI_{(Abbott)ESC}$ <2 ng/L	97.9 (92.5–99.7)	98.8 (95.2–99.7)	0.08 (0.02–0.34)	25.4 (22.2–28.9)	16.4 (15.7–17.1)	1.31 (1.24–1.38)	0.62 (0.58–0.65)
$cTnI_{(sgx)}$ <2 ng/L*	98.9 (94.2–100.0)*	99.6 (97.4–100.0)*	0.03 (0–0.18)*	42.0 (38.2–45.8)*	20.3 (19.2–21.4)*	1.70 (1.59–1.82)*	0.70 (0.67–0.74)*

Values are n (%) with 95% CIs. $cTnI_{(Abbott)}$ indicates cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$, cardiac troponin I (Singulex Clarity System); cTnT, cardiac troponin T; ESC, European Society of Cardiology; and NSTEMI, non–ST-segment elevation myocardial infarction.
*ESC and earlier published Singulex Clarity System (sgx) algorithms and the novel algorithm are referenced in the table. A minor nonsignificant change in the diagnostic performance, for example, sensitivity, is seen when early presenters are excluded because of a change in the nominator.

Table 2. Serial 0/1 Hour Algorithms for Rule-Out and Rule-In of NSTEMI

	Sensitivity	Negative Predictive Value	Negative Likelihood Ratio	Specificity	Positive Predictive Value	Positive Likelihood Ratio	Area Under the Curve
0/1 h rule-out (N=465)							
$cTnT_{ESC}$ <12 ng/L and $\Delta_{0\text{-}1}$ <3 ng/L	100.0 (94.1–100.0)	100 (NC)	0 (NC)	74.3 (69.7–78.5)	37.0 (33.2–40.9)	3.88 (3.29–4.58)	0.87 (0.84–0.90)
$cTnI_{(Abbott/ESC}$ <5 ng/L and $\Delta_{0\text{-}1}$ <2 ng/L	100.0 (94.1–100.0)	100 (NC)	0 (NC)	65.4 (60.5–70.0)	30.4 (27.6–33.3)	2.89 (2.52–3.30)	0.83 (0.79–0.86)
$cTnI_{(sgx/Neumann}$ <2 ng/L and $\Delta_{0\text{-}1}$ <1 ng/L	100 (94.1–100.0)	100	0	46.0 (41.1–51.0)	21.9 (20.4–23.4)	1.85 (1.69–2.03)	0.73 (0.69–0.77)
$cTnI_{(sgx)}$ <8.67 ng/L and $\Delta_{0\text{-}1}$ <3 ng/L	100 (94.1–100.0)	100 (NC)	0 (NC)	87.1 (83.5–90.2)	53.7 (47.4–59.9)	7.77 (6.03–10.01)	0.94 (0.91–0.96)
$cTnI_{(sgx)}$ <10 ng/L and $\Delta_{0\text{-}1}$ <3 ng/L*	100.0 (94.1–100.0)*	100 (NC)*	0 (NC)*	88.6 (85.1–91.5)*	57.0 (50.3–63.5)*	8.78 (6.69–11.53)*	0.94 (0.92–0.96)*
0/1 h rule-in (N=465)							
$cTnT_{ESC}$ ≥52 ng/L or $\Delta_{0\text{-}1}$ ≥5 ng/L	78.7 (66.3–88.1)	96.8 (94.9–98.0)	0.22 (0.14–0.36)	96.5 (94.3–98.1)	77.4 (66.8–85.4)	22.7 (13.4–38.6)	0.88 (0.84–0.90)
$cTnI_{(Abbott/ESC}$ ≥52 ng/L or $\Delta_{0\text{-}1}$ ≥6 ng/L	91.8 (81.9–97.3)	98.7 (97.1–99.4)	0.09 (0.04–0.20)	94.6 (91.9–96.6)	71.8 (62.7–79.4)	16.9 (11.2–25.5)	*0.93 (0.90–0.95)
$cTnI_{(sgx/Neumann}$ ≥25 ng/L or $\Delta_{0\text{-}1}$ ≥6 ng/L	90.2 (79.8–96.3)	98.5 (96.8–99.3)	0.10 (0.05–0.22)	95.3 (92.8–97.2)	74.3 (64.9–81.9)	19.2 (12.3–30.0)	0.93 (0.90–0.95)
$cTnI_{(sgx)}$ ≥70 ng/L or $\Delta_{0\text{-}1}$ ≥5 ng/L*	90.5 (80.4–96.4)*	98.7 (97.2–99.4)*	0.10 (0.05–0.21)*	97.0 (94.9–98.5)*	80.6 (70.3–87.9)*	30.5 (17.4–53.5)*	0.94 (0.91–0.96)*
$cTnI_{(sgx)}$ ≥30 ng/L or $\Delta_{0\text{-}1}$ ≥5 ng/L*	93.4 (84.1–98.2)*	99.1 (97.7–99.6)*	0.07 (0.03–0.18)*	96.3 (94.0–97.9)*	77.4 (67.5–85.0)*	25.2 (15.3–41.5)*	0.95 (0.92–0.97)*

Values are n (%) with 95% CIs. $cTnI_{(Abbott)}$ indicates cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$, cardiac Troponin I (Singulex Clarity System); cTnT, cardiac troponin T; ESC, European Society of Cardiology; NC, not calculated; and NSTEMI, non–ST-elevation myocardial infarction.

*ESC and earlier published Singulex Clarity System (sgx) algorithms and the novel algorithm are referenced in the table.

(defined as percentage of patients ruled out plus percentage of patients ruled in) was calculated for all algorithms. Kaplan–Meier curves were drawn for the composite end point stratified according to categories, and the number of events was calculated. Cox proportional hazards regression analysis was used to calculate the unadjusted hazard ratio for the composite end point, and adjusted analysis was undertaken using age, sex, current or previous smoking, chronic kidney disease, hypertension, hyperlipidemia, diabetes mellitus, and previous MI as covariates. Definitions of the different risk factors are given in Data S1. We used SPSS Statistics 24 and MedCalc for the statistical analyses.

RESULTS

Baseline Characteristics and Troponin Concentrations

The characteristics of patients according to diagnostic category are presented in Table S1 (total cohort, n=971) and Table S2 (0/1 hour cohort, n=465). The prevalence of NSTEMI was 13%, and the prevalence of unstable angina pectoris was 11%. Figure 1 shows the median (25 and 75 percentiles) troponin concentrations at admission and 1 hour for the three different assays. $cTnI_{(sgx)}$ was measurable in 99.9%, cTnT in 74%, and $cTnI_{(Abbott)}$ in 87% of samples obtained at admission.

Derivation of a Direct Rule-Out and a 0/1 Hour Rule-Out and Rule-In Algorithm

The number of NSTEMIs that would be ruled out at different admission sample $cTnI_{(Sgx)}$ concentrations was calculated (see Table S3). A direct rule-out algorithm using <2 ng/L as the cutoff showed a diagnostic sensitivity >99%, in accordance with the prespecified criteria (Table 1). The 0/1 hour algorithm was developed in a similar way by calculating the number of NSTEMIs ruled out at different admission and delta value concentrations combined (see Tables S4 and S5). The optimal rule-out algorithm was a baseline $cTnI_{(Sgx)}$ concentration <10 ng/L and a delta value of <3 ng/L (Table 2). This algorithm did not rule out any patients with NSTEMI and consequently had a sensitivity of 100%, with a corresponding specificity of 89%. Of note, this decision threshold is higher than the 99th percentile of the assay.

If 8.67 ng/L was used as the baseline concentration in the algorithm, the resulting sensitivity was 100% and specificity was 87%. Regarding rule-in, the optimal algorithm showed a specificity of 97% using a baseline concentration of ≥70 ng/L or a delta value of ≥5 ng/L (Table S5 and Table 2) as cutoffs. An alternative

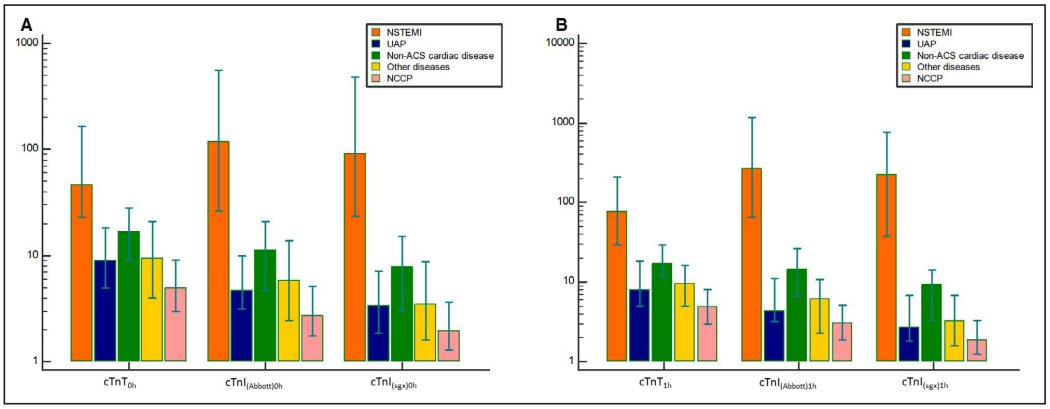

Figure 1. Cardiac troponin concentrations; median, (interquartile range) stratified according to diagnosis.
A, Cardiac troponin concentrations at admission. **B**, Cardiac troponin concentrations after 1 hour. ACS indicates acute coronary syndrome; $cTnI_{(Abbott)}$, cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$, cardiac troponin I (Singulex Clarity System); cTnT, cardiac troponin T; NCCP, non-cardiac chest pain; NSTEMI, non–ST-segment–elevation myocardial infarction; and UAP, unstable angina.

algorithm using a lower baseline concentration of ≥30 ng/L or a delta value of ≥5 ng/L showed similar (within 95% CIs) specificity and sensitivity.

Comparing Diagnostic Performance of Direct Rule-Out Algorithms

Baseline concentrations analyzed as a continuous variable showed a higher AUC for $cTnI_{(Sgx)}$ compared with the other assays (Figure 2; Delong test, $P \leq 0.004$). Direct rule-out by $cTnI_{(Sgx)}$ was the only direct rule-out algorithm that fulfilled the criterion of sensitivity >99% (Figure 3 and Table 1), and only 1 patient was falsely ruled out. A similar sensitivity was achieved for $cTnT_{ESC}$ when a time lag of 3 hours between testing and symptom onset was applied.

This patient with NSTEMI was inappropriately ruled out by all admission algorithms. She was a 65-year-old woman admitted with chest pain lasting more than 3 days. A few years earlier, she had been treated with percutaneous coronary intervention in all three coronary vessels because of unstable angina pectoris. Upon admission, her electrocardiogram showed nonspecific T changes, and she had a high clinical risk for ACS (eg, a HEART (History, Electrocardiogram, Age, Risk factors, Troponin) score of 7). At 72 hours after symptom onset, the baseline troponin samples were cTnT 4 ng/L, $cTnI_{(Abbott)}$ 1.5 ng/L, and $cTnI_{(sgx)}$ 1.3 ng/L, which increased significantly to cTnT 71 ng/L/, $cTnI_{(Abbott)}$ 80 ng/L, and $cTnI_{(sgx)}$ 56 ng/L after 3 hours. The coronary angiogram revealed a thrombus in a small vessel that was not available for percutaneous coronary intervention.

The $cTnI_{(Abbott)ESC}$ algorithm had the lowest sensitivity, missing a second patient, a 73-year-old woman with one previous MI. She was admitted to the hospital with a history of chest pain related to defecation and baseline blood samples taken 3.5 hours after symptom onset showing cTnT 11 ng/L, $cTnI_{(Abbott)}$ 2 ng/L, and $cTnI_{(sgx)}$ 9.25 ng/L. After 3 hours, values for $cTnI_{(Abbott)}$ increased to 5 ng/L, but cTnT and $cTnI_{(sgx)}$ showed stable values. At 18 hours after admission, the cTnT increased to 52 ng/L and the cTnI to 29 ng/L. Unfortunately, the coronary angiography failed because of difficult arterial access.

Comparing Diagnostic Performance Between 0/1 Hour Rule-Out and Rule-In Algorithms

None of the algorithms ruled out any patient with NSTEMI, so that the sensitivity was 100% (Figure 3 and Table 2). The novel $cTnI_{(sgx)}$ 0/1 hour rule-out algorithm had a higher rule-out rate of patients without NSTEMI (higher specificity) and an overall higher AUC (Delong test, $P<0.001$) than comparators.

Concerning the rule-in algorithms, results were quite similar for all algorithms: a few patients without NSTEMI were ruled in (false positive), for a specificity of 95% to 97%. However, the $cTnT_{ESC}$ 0/1 hour algorithm showed a lower AUC (Delong test, $P<0.05$) compared with the cTnI algorithms because of a slightly higher number of patients with NSTEMI allocated to the observation group (lower sensitivity). More than 90% of NSTEMIs were ruled in using the cTnI algorithms, whereas 78% were ruled in by $cTnT_{ESC}$ (Table S6).

Efficiency of the Algorithms

The novel $cTnI_{(sgx)}$ direct rule-out and 0/1 hour algorithms were more efficient than the comparators

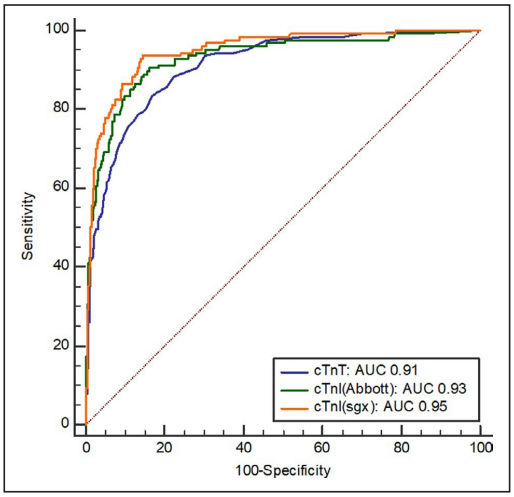

Figure 2. AUC-ROC for admission troponin concentrations as a continuous variable in patients with NSTEMI vs patients with non-NSTEMI.
AUC indicates area under the curve; $cTnI_{(Abbott)}$, cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$, cardiac troponin I (Singulex Clarity System); cTnT, cardiac troponin T; NSTEMI, non–ST-segment–elevation myocardial infarction; and ROC, receiver operating characteristic curve.

(Figure 3). The algorithm classified 37% of admitted patients as candidates for early discharge, compared with 31% with $cTnT_{ESC}$, 23% with $cTnI_{(Abbott)ESC}$, 24% with $cTnI_{(sgx)Body}$, and 9% with $cTnI_{(sgx)Neumann}$. When the early presenters were excluded for the ESC algorithms in accordance with the guideline, the direct rule-out rate dropped to 24% with $cTnT_{ESC}$ and 18% with $cTnI_{(Abbott)ESC}$ (Figure 3).

Concerning 0/1 hour serial sampling algorithms, the novel $cTnI_{(sgx)}$ rule-out algorithm would have suggested discharge for 77% of patients, compared with 64% with $cTnT_{ESC}$ and 57% with $cTnI_{(Abbott)ESC}$ (Table S4). Only 40% would be eligible for discharge if the $cTnI_{(sgx)\,Neumann}$ 0/1 hour algorithm were applied. Rule-in would be recommended for 13% to 17% (Table S5). Total efficiency values showed that the novel $cTnI_{(sgx)}$ 0/1 hour algorithms would allocate 92% (95% CI, 89%–94%) of the patients to either rule-out or rule-in. Corresponding numbers for the $cTnT_{ESC}$, $cTnI_{(Abbott)\,ESC}$, and $cTnI_{(Neumann)}$ 0/1 hour algorithms were 78% (95% CI, 74%–82%), 74% (95% CI, 70%–78%), and 56% (95% CI, 51%–60%), respectively.

Long-Term Prognostic Value

A total of 82 events occurred among the 971 patients included in the admission sample cohort. Table S7 shows the number of end points stratified according to the different algorithms. With the exception of $cTnI_{(sgx)\,Neumann}$ (which allocated only 9% of patients to rule-out), the direct rule-out algorithms showed a significant ability to predict long-term end points. The discrimination power of the rule-out algorithms was confirmed in a Cox regression analysis (Table 3), after adjustment for well-established risk factors.

The 465 patients included in the 0/1 hour cohort experienced 32 events. Patients who were ruled out by the novel $cTnI_{(sgx)}$ 0/1 hour algorithm had an event rate of 5.3% (Table S7), which was not significantly different from the event rate in the observation and

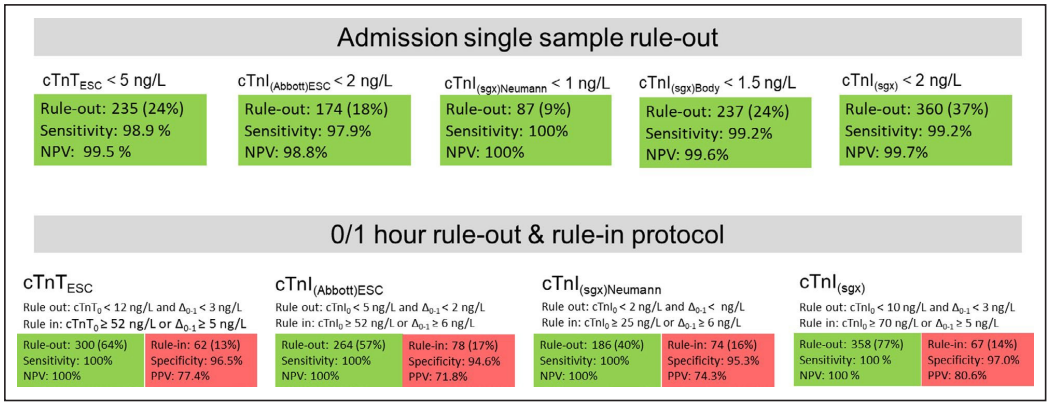

Figure 3. Main diagnostic performance measures and efficiency of the different algorithms.
Diagnostic performance of the admission ESC rule-out algorithm was calculated based on late presenters (n=772) because the ESC does not recommend direct rule-out until >3 hours after onset of symptoms. The cardiac troponin I from the Singulex Clarity System ($cTnI_{(sgx)}$) data are based on all participants (N=971). Efficiency was calculated as the percentage eligible for rule-out from the total cohort (all algorithms). $cTnI_{(Abbott)}$ indicates cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$, cardiac troponin I (Singulex Clarity System); cTnT, cardiac troponin T; ESC, European Society of Cardiology; and NPV, negative predictive value.

Table 3. Cox Regression Analysis

	Univariable		Multivariable*	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Direct rule-out (n=971)				
$cTnT_{ESC}$ <5 ng/L vs observation and rule-in	9.361 (3.423–25.583)	<0.001	3.421 (1.170–10.000)	0.025
$cTnI_{(Abbott)\ ESC}$ <2 ng/L vs observation and rule-in	8.179 (2.582–25.909)	<0.001	3.050 (0.917–10.146)	0.069
$cTnI_{(sgx)Neumann}$ <1 ng/L vs observation and rule in	8.296 (1.155–59.609)	0.035	2.365 (0.318–17.583)	0.400
$cTnI_{(sgx)}$ <2 ng/L vs observation and rule-in	7.769 (3.363–17.840)	<0.001	3.286 (1.359–7.946)	0.008
0/1 h rule-out (n=465)				
$cTnT_{ESC}$ <12 ng/L and Δ_{0-1} <3 ng/L vs observation and rule-in	4.959 (2.294–10.718)	<0.001	3.190 (1.345–7.562)	0.008
$cTnI_{(Abbott)\ ESC}$ <5 ng/L and Δ_{0-1} <2 ng vs observation and rule-in	3.456 (1.599–7.469)	0.002	2.227 (0.972–5.105)	0.058
$cTnI_{(sgx)Neumann}$ <2 ng/L and Δ_{0-1} <1 ng/L, vs observation and rule in	6.537 (1.991–21.461)	0.002	3.671 (1.046–12.885)	0.042
$cTnI_{(sgx)}$ <10 ng/L and Δ_{0-1} <3 ng/L, vs observation and rule-in	2.373 (1.172–4.805)	0.016	1.603 (0.754–3.406)	0.220

95% CI in brackets. Unadjusted and adjusted hazard ratios for the composite end point of future non-fatal myocardial infarction and all-cause mortality. Patients are dichotomized based on the admission sample algorithm, that is, rule-out (reference category) vs observation/rule-in. $cTnI_{(Abbott)}$ indicates cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$, cardiac troponin I (Singulex Clarity System); cTnT, cardiac troponin T; ESC, European Society of Cardiology; and HR, hazard ratio.

*Included in the multivariable model: algorithm as applicable, age, sex, current or previous smoking, estimated glomerular filtration rate above vs below 60 mL/min per 1.73 m^2, diabetes mellitus, hypertension, hyperlipidemia, and previous MI.

rule-in groups of 10% to 13% (Figure 4 and Table 3). In contrast, those allocated to rule-out by the ESC or the Neumann 0/1 hour algorithms had significantly lower event rates (1.6%–3.4%) compared with the corresponding observation and rule-in groups. Cox regression analysis results confirmed the discrimination power of rule-out by these algorithms (Table 3).

DISCUSSION

This study yielded three main findings. First a cardiac injury marker with improved analytical sensitivity and precision beyond the current high-sensitivity assays could improve logistics and categorizing of patients investigated for NSTEMI. Second, the cost of this screening could be higher rates of rule-out of patients with increased long-term risk because of subclinical myocardial injury or CMI. Third, our data are in agreement with the proposed ESC algorithms but only partly correspond to 2 earlier reports for the $cTnI_{(sgx)}$ assay. This pattern highlights a need for robust validation before rule-in and rule-out algorithms are implemented for any particular assay.

Our data suggest that the improved analytical sensitivity of the $cTnI_{(sxg)}$ assay translates into a better "signal-to-noise ratio" compared with the other high sensitivity assays and reduces the time window required for reliably detecting myocardial injury. Body et al[14] reported similar outcomes using a slightly lower cutoff than ours (1.5 ng/L). The very low direct rule-out cutoff suggested by Neumann et al[15] was 100% sensitive but proved clinically unsuitable in our cohort because of the low number of patients eligible for rule-out (9%). Using a $cTnI_{(sgx)}$ cutoff of <2 ng/L would lead to allocation of more patients to direct rule-out than the comparator algorithms suggested by ESC and Body et al.[4,14,25,26]

We used a 0/1 rule-out algorithm that depended more on delta values and allowed for rule-out at high baseline concentrations (>99th percentile of the assay), without compromising sensitivity. This approach resulted in a highly specific algorithm, ruling out large numbers of patients without NSTEMI. It is noteworthy that the novel $cTnI_{(sgx)}$ 0/1 hour algorithm could allocate more than 90% of patients to either rule-out or rule-in, with a similar or higher diagnostic accuracy compared with the other algorithms.

The second important finding in our study is that the ability to predict long-term MI and all-cause mortality seems to depend on the algorithm used for rule-out. The direct rule-out algorithms that used low troponin concentrations as cutoffs showed an excellent prognostic ability. This finding was robust for the $cTnI_{(sgx)}$ and $cTnT_{ESC}$ algorithms after adjusting for well-known risk factors and borderline significant for $cTnI_{(Abbott)ESC}$.[11,27,28] That the direct rule-out according to Neumann et al did not predict long-term prognosis may be explained by the low rule-out frequency of 9%.

Concerning the 0/1 hour algorithms, our data suggest that the $cTnI_{(sgx)}$ algorithm could not predict long-term risk. This algorithm had twice as many end points compared with the others. The higher baseline concentration used for rule-out included more patients with subclinical myocardial injury (high-normal troponin concentrations) and even CMI,[5] which could explain this observation because these patients have increased long-term risk.[11,27,28] For all algorithms, we observed a similar event rate in patients allocated to observation and to rule-in. The prevalence of NSTEMI and CMI was

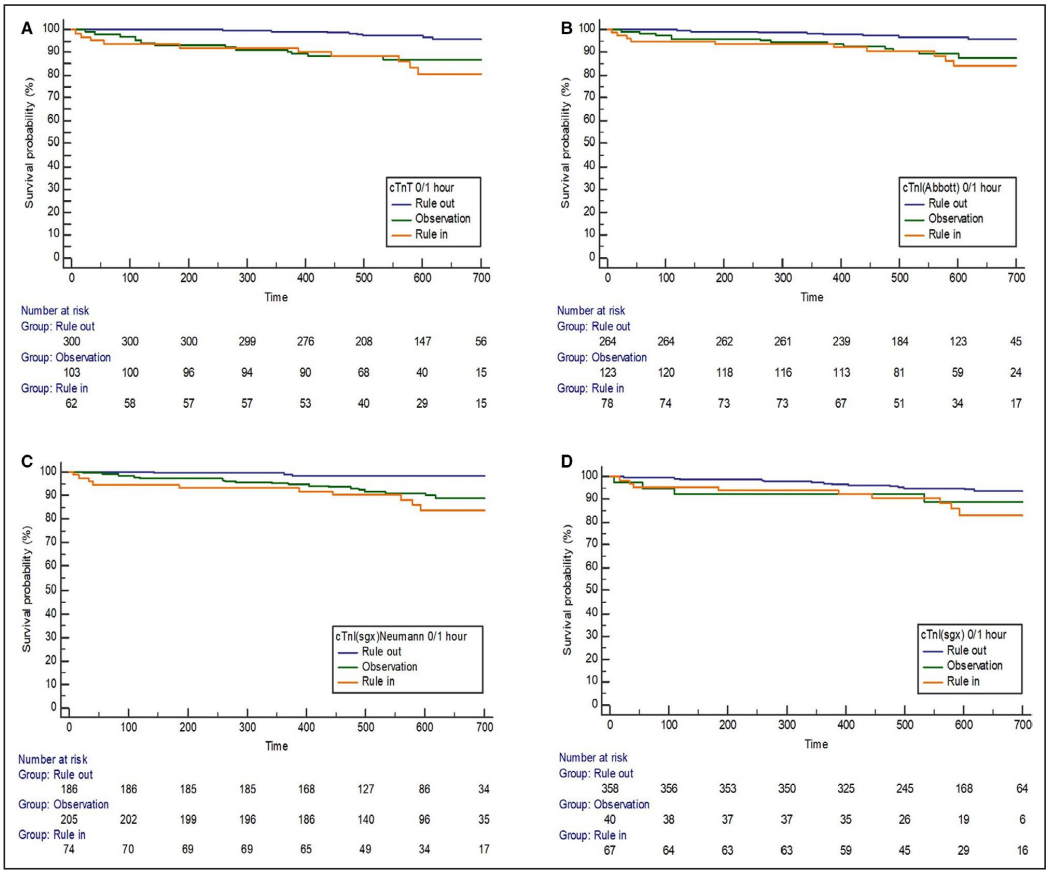

Figure 4. Kaplan–Meier curves for the prognostic composite end point of future myocardial infarction (MI) and all-cause mortality stratified according to the algorithms.
A, 0/1 hour $cTnT_{ESC}$. **B**, 0/1 hour $cTnI_{(Abbott)ESC}$. **C**, 0/1 hour $cTnT_{(sgx)Neumann}$. **D**, 0/1 hour $cTnI_{(sgx)}$. $cTnI_{(Abbott)}$ indicates cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$, cardiac troponin I (Singulex Clarity System); cTnT, cardiac troponin T; and ESC, European Society of Cardiology.

reciprocal in the two groups, with high NSTEMI frequency in the rule-in group and high CMI frequency in the observation group (Tables S6 and S7).[27,29] These findings highlight that NSTEMI and CMI are both serious conditions with increased long-term risk. Future studies should target identifying more accurate diagnostic and treatment options for patients with CMI.

The last important observation is the discrepancy between our findings and the data reported by Neumann et al.[15] The reason could be related to coincidence, as the cutoffs in both studies were based on the few patients with NSTEMI with low baseline concentration. Also, both studies were single center, and differences in health care systems could have affected the patient cohort that was recruited. The fact that Neumann et al also ruled out fewer patients for the $cTnI_{(ESC)}$ algorithm suggests that the cohorts likely were different. Furthermore, analytical issues such as reagent and calibrator lot variations are highly likely to influence the performance of cutoffs in the low range of an assay.[30] This assumption is strengthened by the observation that the diagnostic performance for the different rule-in algorithms showed better alignment, given that lot-to-lot differences are usually less prominent at higher cTn concentrations. Our data demonstrate the need for large sample sets, validation in several different patient cohorts, and knowledge about long-term analytical performance before rule-in and rule-out algorithms are implemented into practice.

An obvious strength of our study is the comparison of the $cTnI_{(sgx)}$ algorithm to well-validated algorithms

from ESC, including cTnT and another cTnI assay. Other strengths are a long observation time during the index hospitalization, during which the patients were observed for at least 8 hours, ensuring the validity of the adjudicated diagnosis. The study closely mirrored clinical practice by not excluding patients with end-stage renal disease or with more than a 12-hour history of symptoms suggestive of ACS. The last strength is a long follow-up period registering end points after the index NSTEMI/hospitalization, allowing for prognostication.

Study Limitations

The main limitation of the study is that the $cTnI_{(sgx)}$ assay currently is no longer available on the market because of bankruptcy. The baseline concentrations used in the rule-out and rule-in algorithms were chosen based on sensitivity, specificity, and efficiency for diagnosing NSTEMI, and more studies are necessary to confirm those concentrations that can indicate subclinical myocardial injury and increased long-term risk. The suggested algorithms should therefore be taken as an example of possibilities and limitations that might be expected from high-precision cardiac injury markers with measurable concentrations in almost all healthy participants. Other limitations are the relatively low number of patients used for development of the 0/1 hour algorithm, the single-center inclusion, the lack of a validation cohort, and the relatively low number of early presenters. As we have noted, our data should be seen as hypothesis-generating and as offering examples, and all new high-sensitivity biomarkers and algorithms need extensive validation in multiple cohorts before they can be ready for clinical use. Another limitation is that cTnT was used as part of the adjudication process. This use could have introduced a positive bias for the cTnT algorithms and underestimation of the performance of cTnI algorithms. Finally, this study involved a long inclusion period, which is a common problem in similar studies; however, the broad inclusion criteria should ensure a broad and representative inclusion. In addition, the NSTEMI rate and patient characteristics in this cohort are similar to those from comparable studies.[31,32]

CONCLUSIONS

In this study, we show that cardiac injury markers with improved analytical performance may improve emergency department efficiency by ruling out and ultimately categorizing more patients compared with current recommendations and algorithms. Our data indicate that patients with increased baseline troponin concentrations who might suffer from subclinical myocardial injury should not be deemed low risk even if they are ruled out for NSTEMI. Future studies should aim at simultaneous development of dedicated algorithms identifying both patients with NSTEMI and those with increased long-term risk. Our final observation is that the intercohort variability in algorithm performance should not be underestimated, and validation including several different cohorts and clinical settings is necessary for all suggested emergency department algorithms.

ARTICLE INFORMATION

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Disclosures

Aakre has served on one advisory board for Roche Diagnostics and received speaker's honoraria from Siemens Healthineers. 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. Skadberg has received lecture fees from Abbott Diagnostics. The remaining authors have no disclosures to report.

Supplementary Material

Data S1
Tables S1–S7
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Supplemental material paper 2

Diagnostic definitions

Myocardial infarction was defined according to the third universal definition of myocardial infarction [19].

Detection of a rise and/or fall of cardiac biomarker values (preferably cardiac 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

Prior Myocardial infarction **was defined by Q waves or QS complexed in the absence of QRS confounders in patients with ischaemic heart disease regardless of symptoms.**[19]

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 [33].

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[34] . *Myocarditis* was diagnosed according to the position statement of ESC from 2013 [35].

Takotsubo cardiomyopathy was diagnosed with the modified criteria suggested by The Mayo Clinic in 2008 [36].

Heart failure was defined according to the ESC diagnostic criteria of 2016 [37] .

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 [38].

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 [39].

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 [40], while chest pain without any specific clinical, radiologic or biochemical findings where defined as non-specific chest pain.

Definition of risk factors

Diabetes was defined by the use of insulin, oral antidiabetic or diet to lower the level of blood glucose.

Hypertension was based on the use of antihypertensive medication.

Hypercholesterolemia was defined by the use of statin or other lipid lowering drugs.

Chronic kidney disease was defined as eGFR below 60 ml/min/1.73m^2.

PAPER 2
SUPPLEMENTAL TABLES

Table S1. Baseline characteristics for the total cohort.

Baseline characteristics	**Total N=971**	**NSTEMI 127 (13.1)**	**UAP 111 (11.4)**	**Other diseases 153 (15.7)**	**NCCP 580 (59.7)**	**p-value**
Age, years	63 (52.0-74.0)	70 (57.0-79.0)	69 (62.0-77.0)	70 (58.0-80.0)	59.0 (49.0-70.0)	<0.001
Male	588 (60.6)	87 (68.5)	83 (74.8)	91 (59.5)	327 (56.4)	<0.001
Hours from symptom onset to first troponin sample	8.0 (3.4-46.2)	5.2 (2.8-25.4)	14.6 (5.5-81.6)	8.5 (3.5-47.7)	8.0 (3.3-45.7)	<0.001
Early presenters < 3 hours	N=199 (20.5)	N=34 (27)	N=17(15.3)	29 (18.9)	119 (20.5)	0.163
Percentage of patients observed > 8 hours	N=941 (96.8)	N=127 (100)	N=110(99.1)	141(92.2)	563 (97.1)	<0.001
Risk factors*						
Hypertension	403 (41.5)	62 (48.8)	60 (54.1)	64 (41.8)	217 (37.4)	0.003
Diabetes mellitus	120 (12.4)	22 (17.3)	28 (25.2)	16 (10.5)	54 (9.3)	<0.001
Current smoker	202 (20.8)	23 (18.1)	20 (18.0)	33 (21.6)	124 (21.4)	0.89
Previous smoking	410 (42.2)	69 (54.3)	59 (53.2)	59 (38.6)	219 (37.8)	0.001
History						
Previous MI	203 (20.9)	33 (26)	43 (38.7)	28 (24.3)	96 (16.6)	<0.001
Previous PCI	204 (21.0)	29 (22.8)	52 (46.8)	26 (17.0)	97 (16.7)	<0.001
Previous CABG	81 (8.3)	17 (13.4)	28 (25.2)	11 (7.2)	25 (4.3)	<0.001
Previous heart failure	46 (4.7)	8 (6.3)	6 (5.4)	14 (9.2)	18 (3.1)	0.013
Medication						
Statins/other lipidlowering	385 (39.6)	48 (37.8)	71 (64.0)	61 (39.9)	205 (35.3)	<0.001
Diuretics	176 (18.1)	23 (18.1)	30 (27.0)	38 (24.8)	85 (14.7)	0.002
ACE inhibitor/ARB	326 (33.6)	46 (36.2)	50 (45.0)	56 (36.6)	174 (30.0)	0.012
Beta-blocker	336 (34.6)	45 (35.4)	59 (53.2)	68 (44.4)	164 (28.3)	<0.001
Aspirin	340 (35.0)	54 (42.5)	70 (63.1)	106 (47)	169 (29.1)	<0.001
Oral Anticoagulant	118 (12.2)	12 (9.4)	13 (11.7)	39 (25.5)	54 (9.3)	<0.001
Antithrombotic agents	71 (7.3)	7 (5.5)	22 (19.8)	9 (5.9)	33 (5.7)	<0.001
Baseline measurements						
BMI, kg/m^2 (n=454)	26.4 (24.2-29.7)	25.9 (24.1-28.6)	25.8 (24.5-29.6)	27.2 (25.7-29.3)	26.3 (24.1-29.8)	0.337
HEART score	4.0 (3.0-5.0)	6.0 (5.0-7.0)	5.0 (4.0-6.0)	4.0 (2.5-6.0)	3.0 (2.0-5.0)	<0.001
Baseline biomarkers						
Glucose, mmol/L	5.8 (5.3-6.7)	6.5 (5.8-8.0)	5.9 (5.4-6.7)	6.1 (5.5-7.3)	5.6 (5.2-6.4)	<0.001

eGFR, ml/min/1.73m^2	85.2 (70.2-97.1)	79.6 (62.8-92.3)	77.7 (64.8-91.4)	74.3 (58.0-91.6)	88.4 (75.9-100.1)	<0.001
cTnT, ng/L	7.0 (3.0-18.0)	47 (23.0-168.0)	9 (5.0-18.0)	13 (5.5-24.0)	5 (3.0-9.0)	<0.001
cTnI, ng/L	4.0 (2.1-11.2)	117.7 (26.1-570.9)	4.7 (3.1-10.0)	8.0 (3.2-18.0)	2.7 (1.7-5.2)	<0.001
$cTnI_{(sgx)}$, ng/L	2.8 (1.5-7.7)	91.0 (23.1-487.7)	3.4 (1.9-7.3)	4.8 (2.1-12.2)	2.0 (1.3-3.6)	<0.001
ECG findings						
ST depression	33 (3.4)	17 (13.4)	3(2.7)	7 (4.6)	6 (1.0)	<0.001
ST elevation	15 (1.5)	2(1.6)	0	7 (4.6)	6 (1.0)	<0.001
T-wave inversion	30 (3.1)	10 (7.9)	6 (5.4)	4 (2.6)	10 (1.7)	<0.001

Values are n (%) or median (interquartile range).

ACE=angiotensin-converting enzyme; ARB=angiotensin receptor blocker; BMI=body mass index; cTnI=cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$=cardiac troponin I (Singulex Clarity system); cTnT=cardiac troponin T; CABG=coronary artery bypass graft; eGFR=estimated glomerular filtration rate; HEART score=acronym for History, ECG=electrocardiogram, A=age, R=risk factors, T=troponin; NCCP=non-cardiac chest pain; NSTEMI=non–ST-elevation myocardial infarction; MI=myocardial infarction; PCI=percutaneous coronary intervention; UAP=unstable angina pectoris.

*Hypercholesterolemia is defined as treatment with lipid-lowering drugs.

Table S2. Baseline characteristics for the 0/1 hour cohort (n=465).

	Total N=465	**NSTEMI 61 (13.1)**	**UAP 56 (12.0)**	**Other diseases 66 (14.2)**	**NCCP 282 (60.6)**	**p-value**
Age, years	61 (51.0-71.0)	67 (56.5.0-78.0)	68 (62.0-72.8)	70 (58.8-80.0)	57.0 (49.0-67.00)	<0.001
Male	278 (59.8)	43 (70.5)	47 (83.9)	33 (50.0)	278 (59.8)	<0.001
Hours from symptom onset to first troponin sample	8.8 (3.5-49.1)	5.2 (2.8-27.6)	25.4 (7.2-173.4)	5.8 (3.3-26.5)	8.8 (3.7-47.9)	<0.001
Risk factors*						
Hypertension	199 (43.1.5)	28 (45.9)	28 (50.0)	33 (51.6)	110 (39.1)	0.173
Diabetes mellitus	51 (11.0)	7 (11.5)	16 (28.6)	9 (13.6)	19 (6.7)	<0.001
Current smoker	110 (23.7)	8 (13.1)	13 (23.2)	15 (22.7)	74 (26.3)	0.61
Previous smoking	189 (40.7)	34 (55.7)	28 (50.0)	31 (47.0)	96 (34.2)	0.018
Previous PCI	83 (17.8)	12 (19.7)	25 (44.6)	8 (12.1)	38(13.5)	<0.001
Previous CABG	32 (6.9)	9 (14.8)	10 (17.9)	5 (7.6)	8 (2.8)	<0.001
Previous heart failure	14 (3.0)	2 (3.3)	3 (5.4)	4 (6.1)	5 (1.8)	0.170
Medication						
Statins/other lipidlowering	171 (36.8)	25 (41.0)	33 (58.9)	29 (43.9)	84 (29.8)	<0.001
Diuretics	81 (17.4)	9 (14.8)	15 (26.8)	15 (22.7)	42 (14.9)	0.100
ACE inhibitor/ARB	165 (35.5)	19 (31.1)	24 (42.9)	29 (43.9)	93 (33.0)	0.198
Beta-blocker	147 (31.6)	21 (34.4)	25 (44.6)	33 (50.0)	68 (24.1)	<0.001
Aspirin	154 (33.1)	28 (45.9)	37 (66.1)	22 (33.3)	67 (23.8)	<0.001
Oral Anticoagulant	41 (8.8)	4 (6.6)	3 (5.4)	13 (19.7)	21 (7.4)	<0.022
Antithrombotic agents	31 (6.7)	3 (5.0)	9 (16.1)	4 (6.2)	15 (5.4)	<0.057
Baseline measurements						
BMI, kg/m^2 (n=231)	26.4 (24.2-29.7)	25.9 (24.1-28.6)	25.8 (24.5-29.6)	26.3 (24.1-29.8)	27.2 (25.7-29.3)	0.337
HEART score	4.0 (2.0-5.0)	6.0 (5.0-7.0)	5.0 (4.0-6.0)	4.0 (3.0-5.0)	3.0 (2.0-4.0)	<0.001
Glucose, mmol/L	5.8 (5.3-6.6)	6.3 (5.7-7.6)	6.1 (5.4-7.6)	6.2 (5.7.5)	5.6 (5.2-6.2)	<0.001
eGFR, ml/min/1.73m^2	87.9 (72.4-98.6)	86.3 (71.6-97.0)	83.1 (69.9-94.8)	74.7 (58.0-93.9)	90.5 (76.1-101.2)	<0.001
cTnT, ng/L	7.0 (3.0-16.0)	49.0 (21.5-185.0)	8.5 (5.0-19.5)	12 (7.0-20.5)	5 (3.0-8.0)	<0.001

$cTnI_{(Abbott)}$, ng/L	3.8 (2.0-10.1)	144.5 (27.1-549.2)	3.9 (2.7-9.9)	8.1 (3.3-14.9)	2.7 (1.7-4.6)	<0.001
$cTnI_{(sgx)}$, ng/L	2.6 (1.4-6.8)	105.9 (28.4-501.7)	2.6(1.8-7.0)	4.6 (2.0-10.8)	1.8 (1.2-3.1)	<0.001
ECG findings						
ST depression	10 (2.2)	6 (9.8)	1 (1.8)	1 (1.5)	2 (0.7)	<0.001
ST elevation	3 (0.6)	2 (3.3)	0 (0.0)	1 (1.5)	0 (0.0)	<0.001
T-wave inversion	13 (2.8)	5 (8.2)	4 (7.1)	0	4 (1.4)	<0.001

Values are n (%) or median (interquartile range).

ACE=angiotensin-converting enzyme; ARB=angiotensin receptor blockers; BMI=body mass index; cTnI=cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$=cardiac troponin I (Singulex Clarity system); cTnT=cardiac troponin T; CABG=coronary artery bypass graft; eGFR=estimated glomerular filtration rate; HEART score=acronym for History, ECG=electrocardiogram, A=age, R=risk factors, T=troponin; NCCP=non-cardiac chest pain; NSTEMI=non–ST-elevation myocardial infarction; MI=myocardial infarction; PCI=percutaneous coronary intervention; UAP=unstable angina pectoris.

*Hypercholesterolemia is defined as treatment with lipid-lowering drugs.

Table S3. Number of patients ruled out using different protocols.

	NSTEMI	UAP	Non-ACS cardiac disease	Non-cardiac chest pain	Other diseases	Total
Rule-out, total cohort	**N=127**	**N=111**	**N=73**	**N=580**	**N=80**	**N=971**
$cTnT_{ESC}$ < 5 ng/L	2 (2)	20 (18)	6 (8)	247 (43)	23 (29)	298 (31)
$cTnI_{(Abbott)ESC}$ < 2 ng/L	3 (2)	11 (10)	3 (4)	192 (33)	13 (16)	222 (23)
$cTnI_{(sgx)Neumann}$ < 1.0 ng/L	0	5 (4.5)	0	76 (13.1)	6 (7.5)	87 (9.0)
$cTnI_{(sgx)Body}$ < 1.5 ng/L	1 (1)	15 (14)	2 (3.0)	203 (35)	16 (20)	237 (24)
$cTnI_{(sgx)}$ < 2 ng/L	1 (1)	31 (28)	6 (8)	293 (51)	29 (36)	360 (37)
Rule-out, total cohort (all early presenters were automatically ruled-in)	**N=127**	**N=111**	**N=73**	**N=580**	**N=80**	**N=971**
$cTnT_{ESC}$ < 5 ng/L	1 (1)	16 (14)	3 (4)	194 (33)	21 (26)	235 (24)
$cTnI_{(Abbott)ESC}$ < 2 ng/L	2 (1.5)	9 (8)	2 (3)	149 (26)	12 (15)	174 (18)
$cTnI_{(sgx)Neumann}$ < 1.0 ng/L	0	3 (3)	0	54 (12)	5 (6)	62 (6)
$cTnI_{(sgx)Body}$ < 1.5 ng/L	1 (1)	12 (11)	1 (1)	159 (27)	15 (19)	188 (19)
$cTnI_{(sgx)}$ < 2 ng/L	1 (1)	24 (22)	4 (5)	231 (40)	26 (33)	286 (29)
Rule-out, late presenters only (≥ 3 hours)	**N=93**	**N=94**	**N=55**	**N=461**	**N=69**	**N=772**
$cTnT_{ESC}$ < 5 ng/L	1 (1)	16 (17)	3 (6)	194 (42)	21 (30)	235 (30)
$cTnI_{(Abbott)ESC}$ < 2 ng/L	2 (2)	9 (10)	2 (4)	149 (32)	12 (17)	174 (23)
$cTnI_{(sgx)Neumann}$ < 1.0 ng/L	0	3 (3.2)	0	54 (12)	5 (7)	62 (8)
$cTnI_{(sgx)Body}$ < 1.5 ng/L	1 (1)	12 (13)	1 (2)	159 (35)	15 (22)	188 (24)
$cTnI_{(sgx)}$ < 2 ng/L	1 (1)	24 (26)	4 (7)	231 (50)	26 (38)	286 (37)

Percentages in brackets.

The upper panel shows the number of patients who would be ruled out if all patients (independent of time between symptom onset and testing) were included.

Middle panel shows number of rule-outs in the total cohort when all early presenters were directly transformed to serial sampling, and the last panel shows the number of rule-outs in late presenters only (used for calculation of diagnostic performance in late presenters; see Table 1, main text).

ACS=acute coronary syndrome; $cTnI_{(Abbott)}$=cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$=cardiac troponin I (Singulex Clarity system); cTnT=cardiac troponin T; ESC=European Society of Cardiology; NCCP=non-cardiac chest pain; NSTEMI=non–ST-elevation myocardial infarction; UAP=unstable angina pectoris.

Table S4. Number of patients ruled in using 0/1 hour protocols.

	NSTEMI	UAP	Non-ACS cardiac disease	Non-cardiac chest pain	Other diseases	Total
0/1 hour rule-out	**N=61**	**N=56**	**N=30**	**N=282**	**N=36**	**N=465**
Evaluation of cTn_{ESC} and Neumann algorithms:						
$cTnT_{ESC}$ < 12 ng/L and $\Delta_{0\text{-}1}$ < 3 ng/L	0	33 (59)	8 (31)	237 (84)	22 (61)	300 (64)
$cTnI_{(Abbott)ESC}$ < 5 ng/L and $\Delta_{0\text{-}1}$ < 2 ng/L	0	32 (57)	6 (20)	212 (75)	14 (39)	264 (57)
$cTnI_{(sgx)Neumann}$ < 2.0 and $\Delta_{0\text{-}1}$ < 1 ng/L	0	20 (36)	2 (7)	152 (54)	12 (33)	186 (40)
Evaluation of $cTnI_{(sgx)}$ baseline and delta values combined:						
$cTnI_{(sgx)}$ < 4.0 and $\Delta_{0\text{-}1}$ < 3 ng/L	0	35 (64)	8 (27)	230 (82)	19 (53)	293 (63)
$cTnI_{(sgx)}$ < 6.0 and $\Delta_{0\text{-}1}$ < 3 ng/L	0	41 (73)	11 (37)	256 (91)	25 (69)	333 (71)
$cTnI_{(sgx)}$ < 8.0 and $\Delta_{0\text{-}1}$ < 3 ng/L	0	44 (79)	14 (47)	264 (94)	28 (78)	350 (75)
$cTnI_{(sgx)}$ < 8.67 and $\Delta_{0\text{-}1}$ < 3 ng/L	0	44 (79)	15 (50)	264 (94)	29 (81)	352 (76)
$cTnI_{(sgx)}$ < 10.0 and $\Delta_{0\text{-}1}$ < 3 ng/L	**0**	**45 (80)**	**16 (53)**	**259 (95)**	**39 (87)**	**358 (77)**
$cTnI_{(sgx)}$ < 12.0 and $\Delta_{0\text{-}1}$ < 3 ng/L	1 (2)	46 (82)	19 (63)	268 (95)	31 (86)	365 (78)

Percentages in brackets.

ESC protocols for cTnT and $cTnI_{(Abbott)}$ and different protocols for $cTnI_{(sgx)}$.

ACS=acute coronary syndrome; $cTnI_{(Abbott)}$=cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$=cardiac troponin I (Singulex Clarity system); cTnT=cardiac troponin T; ESC=European Society of Cardiology; NCCP=non-cardiac chest pain; NSTEMI=non–ST-elevation myocardial infarction; UAP=unstable angina pectoris.

Table S5. Number of patients ruled in using 0/1 hour protocols.

	NSTEMI	UAP	Non-ACS cardiac disease	Non-cardiac chest pain	Other diseases	Total
0/1 hour rule-in	**N=61**	**N=56**	**N=30**	**N=282**	**N=36**	**N=465**
$cTnT_{ESC} \geq 52$ ng/L or $\Delta_{0\text{-}1} \geq 5$ ng/L	48 (79)	2 (4)	8 (27)	2 (1)	2 (6)	62 (13)
$cTnI_{(Abbott)ESC} \geq 52$ ng/L or $\Delta_{0\text{-}1} \geq 6$ ng/L	56 (92)	6 (11)	9 (30)	5 (2)	2 (6)	78 (17)
$cTnI_{(sgx)Neumann} \geq 25.0$ ng/L or $\Delta_{0\text{-}1} \geq 6$ ng/L	55 (90)	5 (9)	7 (23)	5 (2)	2 (5)	74 (16)
Evaluation of $cTnI_{(sgx)}$ baseline and delta values combined:						
$cTnI_{(sgx)} \geq 8.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L	61 (100)	12 (21)	16 (53)	20 (7)	8 (22)	117 (25)
$cTnI_{(sgx)} \geq 10.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L	61 (100)	12 (21)	16 (54)	20 (7)	8 (22)	117 (25)
$cTnI_{(sgx)} \geq 12.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L	60 (98)	10 (18)	11 (37)	16 (6)	5 (14)	102 (22)
$cTnI_{(sgx)} \geq 14.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L	58 (95)	10 (18)	10 (33)	13 (5)	4 (11)	95 (20)
$cTnI_{(sgx)} \geq 18.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L	58 (95)	9 (16)	7 (23)	10 (4)	3 (8)	86 (19)
$cTnI_{(sgx)} \geq 20.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L	58 (95)	8 (14)	7 (23)	10 (4)	3 (8)	86 (19)
$cTnI_{(sgx)} \geq 30.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L	58 (95)	6 (11)	7 (23)	8 (3)	2 (6)	81 (17)
$cTnI_{(sgx)} \geq 40.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L	57 (93)	6 (11)	7 (23)	7 (3)	2 (6)	79 (17)
$cTnI_{(sgx)} \geq 50.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L	57 (93)	6 (11)	7 (23)	7 (3)	2 (6)	79 (17)
$cTnI_{(sgx)} \geq 60.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L	57 (93)	6 (11)	7 (23)	7 (3)	2 (6)	79 (17)
$cTnI_{(sgx)} \geq 70.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L	57 (93)	6 (11)	7 (23)	7 (3)	2 (6)	78 (17)
$cTnI_{(sgx)} \geq 80.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L	57 (93)	4 (7)	6 (20)	4 (1)	2 (6)	73 (16)
$cTnI_{(sgx)} \geq 90.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L	57 (93)	4 (7)	6 (20)	4 (1)	2 (6)	73 (16)
$cTnI_{(sgx)} \geq 100.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L	57 (93)	3 (5)	6 (20)	4 (1)	2 (6)	72 (16)
$cTnI_{(sgx)} \geq 150.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L	57 (93)	2 (4)	6 (20)	4 (19	2 (6)	71 (15)
$cTnI_{(sgx)} \geq 10.0$ or $\Delta_{0\text{-}1} \geq 5$ ng/L	60 (98)	11 (20)	14 (47)	13 (5)	5 (14)	103 (22)
$cTnI_{(sgx)} \geq 20.0$ or $\Delta_{0\text{-}1} \geq 5$ ng/L	57 (93)	6 (11)	7 (23)	5 (2)	3 (8)	78 (17)
$cTnI_{(sgx)} \geq 30.0$ or $\Delta_{0\text{-}1} \geq 5$ ng/L	**57 (93)**	**3 (5)**	**7 (23)**	**3 (1)**	**2 (6)**	**72 (16)**
$cTnI_{(sgx)} \geq 40.0$ or $\Delta_{0\text{-}1} \geq 5$ ng/L	55 (90)	3 (5)	7 (23)	2 (1)	1 (3)	68 (15)
$cTnI_{(sgx)} \geq 50.0$ or $\Delta_{0\text{-}1} \geq 5$ ng/L	55 (90)	3 (5)	7 (23)	2 (1)	1 (3)	68 (15)
$cTnI_{(sgx)} \geq 60.0$ or $\Delta_{0\text{-}1} \geq 5$ ng/L	55 (90)	3 (5)	7 (23)	2 (1)	1 (3)	68 (15)
$cTnI_{(sgx)} \geq 70.0$ or $\Delta_{0\text{-}1} \geq 5$ ng/L	**55 (90)**	**3 (5)**	**6 (20)**	**2 (1)**	**1 (3)**	**67 (14)**

$cTnI_{(sgx)} \geq 80.0$ or $\Delta_{0\text{-}1} \geq 5$ ng/L	55 (90)	3 (5)	6 (20)	2 (1)	1 (3)	67 (14)
$cTnI_{(sgx)} \geq 90.0$ or $\Delta_{0\text{-}1} \geq 5$ ng/L	55 (90)	3 (5)	6 (20)	2 (1)	1 (3)	67 (14)
$cTnI_{(sgx)} \geq 100.0$ or $\Delta_{0\text{-}1} \geq$ 5 ng/L	55 (90)	3 (5)	6 (20)	2 (1)	1 (3)	67 (14)
$cTnI_{(sgx)} \geq 110.0$ or $\Delta_{0\text{-}1} \geq$ 5 ng/L	55 (90)	3 (5)	6 (20)	2 (1)	1 (3)	67 (14)
$cTnI_{(sgx)} \geq 120.0$ or $\Delta_{0\text{-}1} \geq$ 5 ng/L	55 (90)	3 (5)	6 (20)	2 (1)	1 (3)	67 (14)
$cTnI_{(sgx)} \geq 130.0$ or $\Delta_{0\text{-}1} \geq$ 5 ng/L	55 (90)	3 (5)	6 (20)	2 (1)	1 (3)	67 (14)
$cTnI_{(sgx)} \geq 140.0$ or $\Delta_{0\text{-}1} \geq$ 5 ng/L	55 (90)	3 (5)	6 (20)	2 (1)	1 (3)	67 (14)
$cTnI_{(sgx)} \geq 150.0$ or $\Delta_{0\text{-}1} \geq$ 5 ng/L	55 (90)	3 (5)	6 (20)	2 (1)	1 (3)	67 (14)
$cTnI_{(sgx)} \geq 250.0$ or $\Delta_{0\text{-}1} \geq$ 5 ng/L	55 (90)	3 (5)	6 (20)	2 (1)	1 (3)	67 (14)

Percentages in brackets.

ACS=acute coronary syndrome; $cTnI_{(Abbott)}$=cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$=cardiac troponin I (Singulex Clarity system); cTnT=cardiac troponin T; ESC=European Society of Cardiology; NCCP=non-cardiac chest pain; NSTEMI=non–ST-elevation myocardial infarction; UAP=unstable angina pectoris.

ESC protocols for TnT and $cTnI_{(Abbott)}$ and different protocols for $cTnI_{(sgx)}$.

Table S6. Allocation of NSTEMI patients.

Rule-out / rule-in protocol	Rule-out	Observation	Rule-in
$cTnT_{ESC}$ 0/1 hour	0	13 (21.3)	48 (78.7)
$cTnI_{(Abbott)ESC}$ 0/1 hour	0	5 (8.2)	56 (91.8)
$cTnI_{(sgx)Neumann}$ 0/1 hour	0	6 (9.8)	55 (90.2)
$cTnI_{(sgx)}$ 0/1 hour	**0**	**6 (9.8)**	**55 (90.2)**

Percentages in brackets.

The table shows the category to which the different 0/1 hour rule-out and rule-in protocols would allocate patients who were finally diagnosed with an index NSTEMI (n=61). $cTnI_{(Abbott)}$=cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$=cardiac troponin I (Singulex Clarity system); cTnT=cardiac troponin T; ESC=European Society of Cardiology; NSTEMI=non–ST-elevation myocardial infarction.

Table S7. Prevalence of events stratified according to protocol classification.

	None-fatal MI and all-cause mortality
Direct rule-out	N=82
Rule out	
cTnT < 5 ng/L	4 (1.4)
$cTnI_{(Abbott)}$ < 2 ng/L	3 (1.4)
$cTnI_{(sgx)Neumann}$ < 1 ng/L	1 (1.2)
$cTnI_{(sgx)}$ < 2 ng/L	**6 (1.7)**
Observation/rule in	
cTnT	78 (11.6)
$cTnI_{(Abbott)}$	79 (10.6)
$cTnI_{(sgx)Neumann}$	81 (9.2)
$cTnI_{(sgx)}$	**76 (12.4)**
0/1 hour protocol	N=32
Rule-out	
cTnT < 12 ng/L and Δ_{0-1} < 3 ng/L	9 (3.0)
$cTnI_{(Abbott)}$ < 5 ng/L and Δ_{0-1} < 2 ng/	9 (3.4)
$cTnI_{(sgx)Neumann}$ < 2 and Δ_{0-1} < 1 ng/L	3 (1.6)
$cTnI_{(sgx)}$ < 10 and Δ_{0-1} < 3 ng/L	**19 (5.3)**
Observation	
cTnT	13 (12.6)
$cTnI_{(Abbott)}$	13 (10.6)
$cTnI_{(sgx)Neumann}$	19 (9.3)
$cTnI_{(sgx)}$	**4 (10.0)**
Rule-in	
cTnT ≥52 ng/L or Δ_{0-1} ≥ 5 ng/L	10 (16.1)
$cTnI_{(Abbott)}$ ≥ 52 ng/L or Δ_{0-1} ≥ 6 ng/L	10 (12.8)
$cTnI_{(sgx)}$ ≥ 25.0 or Δ_{0-1} ≥ 6 ng/L	10 (13.5)
$cTnI_{(sgx)}$ ≥ 70.0 or Δ_{0-1} ≥ 5 ng/L	**9 (13.4)**

Percentages in brackets.

$cTnI_{(Abbott)}$=cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$=cardiac troponin I (Singulex Clarity system); cTnT=cardiac troponin T; MI=myocardial infarction.

ESC European Society of Cardiology

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ORIGINAL SCIENTIFIC PAPER

Clinical risk scores identify more patients at risk for cardiovascular events within 30 days as compared to standard ACS risk criteria: the WESTCOR study

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Aims Troponin-based algorithms are made to identify myocardial infarctions (MIs) but adding either standard acute coronary syndrome (ACS) risk criteria or a clinical risk score may identify more patients eligible for early discharge and patients in need of urgent revascularization.

Methods and results Post-hoc analysis of the WESTCOR study including 932 patients (mean 63 years, 61% male) with suspected NSTE-ACS. Serum samples were collected at 0, 3, and 8–12 h and high-sensitivity cTnT (Roche Diagnostics) and cTnI (Abbott Diagnostics) were analysed. The primary endpoint was MI, all-cause mortality, and unplanned revascularizations within 30 days. Secondary endpoint was non-ST-elevation myocardial infarction (NSTEMI) during index hospitalization. Two combinations were compared: troponin-based algorithms (ESC 0/3 h and the High-STEACS algorithm) and either ACS risk criteria recommended in the ESC guidelines, or one of eleven clinical risk scores, HEART, mHEART, CARE, GRACE, T-MACS, sT-MACS, TIMI, EDACS, sEDACS, Goldman, and Geleijnse–Sanchis. The prevalence of primary events was 21%. Patients ruled out for NSTEMI and regarded low risk of ACS according to ESC guidelines had 3.8–4.9% risk of an event, primarily unplanned revascularizations. Using HEART score instead of ACS risk criteria reduced the number of events to 2.2–2.7%, with maintained efficacy. The secondary endpoint was met by 13%. The troponin-based algorithms without evaluation of ACS risk missed three-index NSTEMIs with a negative predictive value (NPV) of 99.5% and 99.6%.

Conclusion Combining ESC 0/3 h or the High-STEACS algorithm with standardized clinical risk scores instead of ACS risk criteria halved the prevalence of rule-out patients in need of revascularization, with maintained efficacy.

Keywords Chest pain • High-sensitivity troponin assay • ESC 0/3 h algorithm • High-STEACS • Risk score • Revascularization

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Introduction

Chest pain is a frequent cause of admittance to the emergency department (ED).[1] Many patients have non-cardiac causes of pain that could be handled outside of hospitals, implying an unnecessary high burden on the healthcare system. Early rule-out of patients unlikely to have acute coronary syndrome (ACS) can ease the pressure on crowded EDs and reduce unnecessary examinations in low-risk patients.

The introduction of high-sensitive troponin assays and rapid rule-out or rule-in algorithms for non-ST-elevation myocardial infarction (NSTEMI) have led to swift and safe identification of these patients[2–6] and are recommended by the European Society of Cardiology (ESC).[7] Patients with unstable angina pectoris (UAP) may present similar history, clinical, and electrocardiographic (ECG) findings. Concentrations of cTn, however, are stable and often low, and troponin-based algorithms are hence less useful. Even though patients with UAP have lower mortality rates than patients with NSTEMI, the possible pitfalls of a troponin-centred evaluation might partly explain the slow implementation of troponin-based rule-out/rule-in algorithms as reported in the literature.[8] Even in Europe, where high-sensitivity troponin assays (cTn) have been available for more than 10 years, only 60% of laboratories use high-sensitivity assays, and only half use serial sampling of <_3 h.[9]

To avoid patients with UAP being discharged without correct diagnosis (pending further examinations), clinical gestalt may be sufficient in EDs with continuous presence of experienced physicians. A recent study showed that physicians correctly overruled the ESC 0/1 h algorithm in most patients who were in need of revascularization, preventing early discharge of patients with UAP.[10] However, the use of clinical gestalt is questioned, and other studies show fairly low diagnostic accuracy.[11,12] A reasonable supplement might, therefore, be use of standardized clinical risk scores, developed and validated to identify patients with high risk of coronary artery disease.[13] The main goal of this study was to replace the ACS risk criteria recommended in ESC guidelines with standardized clinical risk scores in a double rule-out algorithm and measure the optimal combined diagnostic performance for ACS. We assessed the ESC 0/3 h rule-out algorithms, the High-STEACS algorithms and 11 different clinical risk scores' ability to identify patients in need of immediate follow-up for ACS after hospitalization due to chest pain [composite endpoint of non-fatal myocardial infarction (MI), all-cause mortality or unplanned revascularization]. Furthermore, we evaluated the same diagnostic tools for a secondary endpoint defined as NSTEMI during index hospitalization.

Methods

Study design and population

The Aiming Towards Evidence-Based Interpretation of Cardiac Biomarkers in Patients Presenting with Chest Pain (WESTCOR) is a cross-sectional and prospective observational study conducted at two University hospitals in Norway (Clinical Trial NCT02620202).[14] The current article contain data from the WESTCOR derivation cohort (WESTCOR-D) including 984 patients form Haukeland University Hospital. The study was conducted according to the Declaration of Helsinki and approved by the regional ethics committee (REC number 2014/1365).

Patients >18 years who were admitted to the ED from September 2015 to February 2017 with suspected NSTE-ACS were eligible for inclusion, irrespective of symptom onset. Patients transferred from other hospitals, those unable to provide informed consent or with a short life expectancy, e.g. terminal cancer, were excluded (*Figure 1*). For this analysis, patients missing measurements of either cTnT or cTnI at presentation or after 3 h were excluded.

Data collection

After admittance, all patients underwent clinical assessment including clinical history, risk factors, assessment of vital parameters, physical examination, ECG, and standard blood tests. The treatment was left at the discretion of the attending physician at hospitals adhering to the ESC guidelines for the treatment of acute coronary syndromes (2015) and the third universal definition of MI (2012). Information needed to assess the risk scores was collected retrospectively based on information in electronic medical records provided by ambulance personnel, referring physicians, and hospital physicians at presentation. In cases where pre-hospital and in-hospital personnel gave conflicting information, data provided by hospital physicians were used.

Troponin analysis

Blood samples for routine measurements and biobank were collected at arrival and after 3 and 8–12 h. cTnT analysis was performed in fresh serum samples using the high-sensitivity assay from Roche Diagnostics with a limit of blank (LoB) of 3 ng/L, a limit of detection (LoD) of 5 ng/L, and a 99th percentile of 14 ng/L as described by the manufacturer. The analytical within-series coefficient of variation (CV_A) was 10% at 4.5 ng/L.

cTnI was analysed in biobanked first thawed serum samples that had been stored at -80 °C until analysis for cTnI from Abbott Diagnostics with an LoB at 0.9 ng/L, LoD at 1.7 ng/L, and a 99th percentile of 26 ng/L. [1]

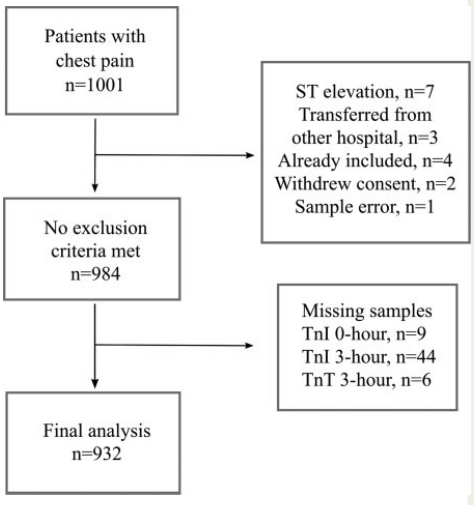

Figure 1 Patients included in final analysis.

Adjudication

The final diagnosis was adjudicated by two independent cardiologists based on all available clinical data, routine laboratory tests including high-sensitivity cTnT as described above (but not cTnI), 12-lead ECG, ultrasound, chest radiography, exercise tests, coronary computed tomography angiography, and conventional angiography. In cases of disagreement, the diagnosis was adjudicated by a third cardiologist. Totally 845 (91%) of patients had three or more cTnT measurements with the last sample drawn at least 8 h after presentation, while only 87 patients (9%) had blood samples drawn at 0 and 3 h only. Specific diagnostic criteria were predefined for 22 different medical conditions based on current guidelines (Supplementary material online).[14] Non-ST-elevation myocardial infarction was defined according to the third universal definition for MI, a definition that remain unchanged in the fourth definition that was published after planning and onset of this study. [16] A 20% (if baseline cTnT concentration were >14 ng/L) or 50% (if baseline cTnT concentration were <_14 ng/L) change in troponin concentration was regarded significant. Unstable angina pectoris was defined as angina at rest with prolonged duration (>20 min), crescendo angina, recent destabilization of stable angina, or post-MI angina, with stable serial troponin concentrations.

Follow-up and endpoints

This article is a post-hoc analysis with a primary endpoint of CV events: non-fatal MI (Type 1 and Type 2), all-cause mortality, and unplanned revascularization, including intention to treat. Secondary endpoint was NSTEMI during index hospitalization. Information on cardiac events and mortality within 30 days was collected from patient files and the Norwegian Patient Register and Norwegian Cause of Death Registry, which are under Norwegian legislation and register all hospital provided healthcare and deaths in Norway.

Troponin-based rule-out pathways

The ESC 0/3 h algorithm recommends rule-out of MI if the troponin concentration at presentation is below the gender-neutral 99th percentile, onset of symptoms >6 h before presentation and the ECG is non-ischaemic. GRACE (Global Registry of Acute Coronary Events) score is used for prognostic risk stratification, and patients with a score below 140 is eligible for stress testing and/or early discharge[7] (*Figure 2*). Serial sampling with re-testing 3 h after admittance is recommended in patients with onset of symptoms <6 h before presentation. Myocardial infarction is ruled out if cTn is below the gender-neutral 99th percentile or without significant change, defined as >50% of URL.[17]

The High-STEACS pathway rules out MI if levels of troponin I or T is <5 ng/L at presentation, onset of symptoms is >2 h before presentation and the ECG is non-ischaemic.[18] If symptoms appeared <2 h before presentation, a second blood sample is collected 3 h later, with MI ruled out if the change in troponin concentration is <3 ng/L and still below the gender-neutral 99th percentile of 14 ng/L for cTnT (Roche Elecsys) or gender-specific 99th percentiles of 16 ng/L (females) and 34 ng/L (males) for cTnI (Abbott Architect).

For the analysis of High-STEACS we used the same 99th percentiles as used in previous studies[3,19] (gender-specific for cTnI and gender-neutral for cTnT) compared with gender-neutral 99th percentiles for the ESC 0/3 h algorithms.[7]

ESC low risk of ACS criteria and clinical risk scores

According to the ESC guidelines, chest pain patients may be regarded low risk of ACS if they exhibit no very-high/high-risk criteria (haemodynamic instability, recurrent chest pain, life-threatening arrythmias, mechanical complications, acute heart failure, recurrent ST-T wave changes, Tn dynamics, or GRACE score >140) or intermediate-risk criteria (diabetes mellitus, eGFR <60, left ventricular ejection fraction <40%, early post-infarction angina, prior percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG), or GRACE score >109).

We included a wide range of standardized clinical risk scores in the evaluation; HEART,[20] CARE,[21] GRACE,[22,23] T-MACS,[24] sT-MACS,[25] TIMI,[26] EDACS,[27] sEDACS,[28] Goldman,[29] and Geleijnse–Sanchis.[30] Components are summarized in *Table 1*. Some of these scores were not developed or validated in low-risk populations (i.e. TIMI), and some are prognostic rather than diagnostic (i.e. GRACE score). Hence, a low accuracy in identifying coronary artery disease (CAD) in low-risk patients do not mean the risk score is less useful in its intended area of use.

The main criteria for being included was population size $n > 1000$ and external validation. Less established risk scores were identified using the search term 'chest pain risk score' in PubMed, but most were dismissed due to small sample size or similarity to other risk score, i.e. several varieties of the TIMI risk score have been developed. Goldman score is chosen as a representative for one of the earliest risk scores, while Geleijnse–Sanchis, although performed in a small cohort ($n = 646$) and not being validated, includes parameters found exclusively in this risk scores, including a thorough symptom evaluation score. CARE score ($n = 650$) is similar to HEART, but without troponin measurements, a potential easy-to-use tool in facilities without access to troponin assays. We also evaluated a modified HEART score (mHEART) where only patients with undetectable troponin values (i.e. cTnT <5 ng/L) would be awarded zero troponin points. Detectable (i.e. cTnT >_ 5 ng/L), but non-elevated troponins would be given 1 point and elevated troponins (i.e. >14 ng/L) 2 points.[31]

Statistics

The baseline characteristics were reported as means (±2 SD) for normally distributed data and median with 25 and 75 percentiles for non-normally distributed data. Differences between groups were compared using Pearson χ^2 test or Fisher's exact test (if $n < 5$ per group) for binominal distributed data, means with 95% confidence interval (CI) for normally distributed data and Mann–Whitney *U* test for non-normally distributed data. Safety of the troponin-based rule-out algorithms and risk scores were assessed using sensitivity and NPV, and difference in sensitivity was assessed using McNemar's test. Efficacy was quantified as proportion of patients ruled out by the different algorithms. Accuracy for the given threshold was assessed using the formula Sensitivity x Prevalence þ Specificity x (1 – Prevalence). Risk scores are continuous variables, and area under the receiver operating characteristics curve (AUROC) served as an additional indicator of accuracy. For the combination of troponin-based algorithms (categorical variable) and risk scores (continuous variable), we created a combined variable using binominal logistic regression. Differences in AUROC were evaluated using Delong test. Hypothesis testing was two-tailed, and *P*-values <0.05 were considered statistically significant. Analysis was performed using IBM SPSS Statistics version 24.0.0.1 for Windows (IBM Statistics, Chicago, IL, USA) and MedCalc version 17.6 for Windows (MedCalc Software bvba, Ostend, Belgium).

Results

Baseline characteristics

The median age of patients was 63 years, and 60% were male. Non-ST-elevation myocardial infarction was diagnosed in 13% of patients ($n = 124$), unstable angina in 11% ($n = 106$), other cardiac diseases in

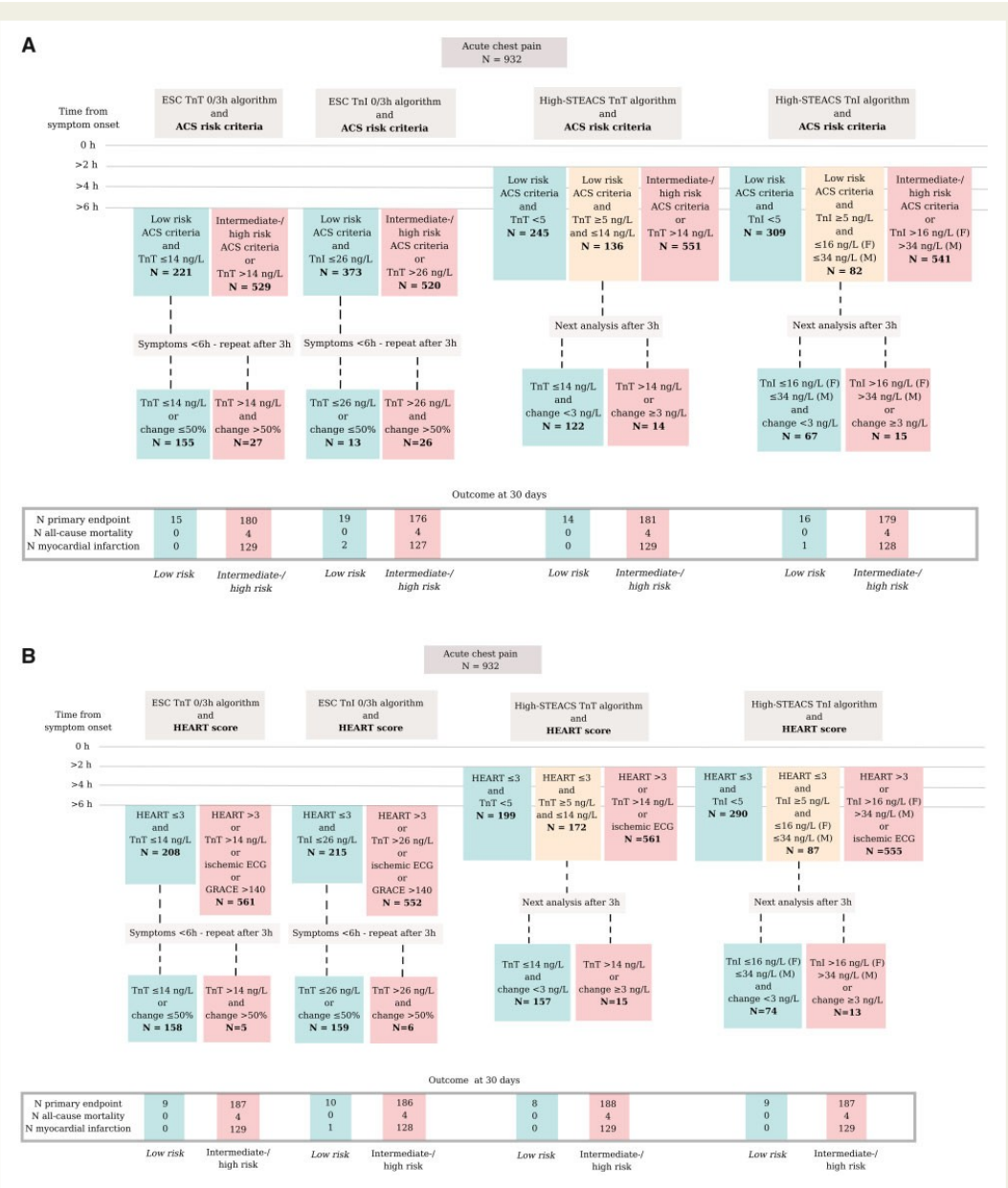

Figure 2 (*A*) Summary of the ESC 0/3 h and High-STEACS algorithms, number of patients allocated to rule-out or none-rule-out at presentation or 3 h and outcome within 30 days. (*B*) Summary of the ESC 0/3 h and High-STEACS algorithms combined with HEART score, number of patients allocated to rule-out or none-rule-out at presentation or 3 h and outcome within 30 days.

Table 1 Components of 10 different risk scores and mHEART

	History	Age	ECG	Risk factors	Troponin levels	Known CAD	Angina	SBP	Other	Low risk
HEART (2008)	Typical = 2p Atypical = 1p	>65 = 2p >45 = 1p	ST-dep = 2p Other = 1p	>_3 or CAD = 2p >_1 = 1p	>_3 x 99th = 2p >_99th = 1p					<_3p
mHEART (2017)	Typical = 2p Atypical = 1p	>65 = 2p >45 = 1p	ST-dep = 2p Other = 1p	>_3 or CAD = 2p >_1 = 1p	>_99th = 2p Measurable = 1p					<_3p
CARE (2018)	Typical = 2p Atypical = 1p	>65 = 2p >45 = 1p	ST-dep = 2p Other = 1p	>_3 or CAD = 2p >_1 = 1p						<_1p
TIMI (2000)		>65 = 1p	ST-changes >0.5 mm = 1p	>_3 = 1p	>99th = 1p	1p	Severe = 1p		Aspirin used within 7 days = 1p	<_1p
GRACE (2003/2004)		0–100p	ST-changes >0.5 mm = 17p		>99th = 14p			0–40p	Pulse = 0–34p Creatinine = 0–28p Cardiac arrest = 30p Killip class = 0–44p	<_108p <_89p
EDACS (2014)	Diaphoresis = 3p Radiation[a] = 5p Resp. pain = -4p Reproduced by palpation = -6p	2–20p		>_3 or CAD[b] = 4p					Male gender = 6p	<_15p
sEDACS (2016)	Radiation[a] = 1p	0–6p		>_3 or CAD = 1p					Male gender = 1p	<_3p
T-MACS[c] (2017)	Diaphoresis = d Radiation[e] = r Vomiting = v		Ischaemia = i		By degree of elevation = t		Crescendo = c	<100 = h		<_0.02
sT-MACS (2018)	Diaphoresis = 1p Radiation[e] = 1p Vomiting = 1p		Ischaemia = 1p		TnT >9 ng/L = 1p		Crescendo = 1p	<100 = 1p		<_0p
Geleijnse–Sanchis (2005)	>10 symptom points = 1p	>_67 = 1p		DM[d] = 2p		1p	Severe = 1p			<_1p
Goldman (1996)			Ischaemia = high risk				Crescendo = 1p	<110 = 1p	Bilateral pulmonary rales = 1p	<_1p

CAD, coronary artery disease; CARE, characteristics, age, risk factors, EGG; EDACS, Emergency Department Assessment of Chest Pain Score and MI, myocardial infarction; GRACE, Global Registry of Acute Coronary Events; HEART, History, ECG, Age, Risk factors, Troponin; mHEART, modified HEART score with troponin points given if hs-Tn is measurable; SBP, systolic blood pressure; TIMI, Thrombolysis In Myocardial Infarction; T-MACS, troponin-only Manchester Acute Coronary Syndromes.

[a]To *any* shoulder/arm/jaw.

[b]Age 18–50 years.

[c]Percentage risk of ACS calculated using the following formula: p = 1/(1 þ e^ - (1.713i þ 0.847c þ 0.607r þ 1.417v þ 2.058d þ 1.208h þ 0.089t - 4.766)), where hs-TnT is continuous and the other factors dichotomous.

[d]Demanding insulin.

[e]To *right* arm/shoulder.

7.2% (n = 67), and non-cardiac chest pain in 68% (n = 635). Patients with ACS were older, had more risk factors for cardiovascular disease and used more mediations than patients without ACS (*Table 2*). Time from symptom onset to arrival was median 8 h. About 97 patients (10.4%) had onset of symptoms <2 h before presentation. Coronary computed tomography angiography was performed in 33.0% of the patients, while 8.4% had an angiography without further treatment. About 16.5% were treated with either PCI or CABG. Revascularization was performed in 89/124 (71.8%) of patients with NSTEMI and 58/106 (54.7%) of patients with UAP (Supplementary material online, *Table S1*).

Symptoms indicating ACS (P < 0.05) were retrosternal location of chest pain, radiation to both arms, effect of nitroglycerine, debut during physical activity, and pain duration between 1 and 30 min. Symptoms indicating non-ACS was chest pain with stinging character, dependence on stature or respiration, accompanied dizziness, reproducibility upon palpation, history of chronic psychological stress, debut during rest, and pain duration >24 h. Of note, severity of pain (Numeric Rating Scale), pain described as pressing, radiation of pain to the left arm/shoulder or to the jaw, shortness of breath, and nausea were not significantly associated with ACS. A detailed description of patient characteristics including symptoms, medication at admission, and treatment during the ED stay is given in Supplementary material online, *Tables S1* and *S2*.

Patients with NSTEMI had significantly higher cTn values at admission than patients with UAP (median cTnT 50 vs. 9 ng/L, P < 0.001, cTnI 121 vs. 5 ng/L, P < 0.001; *Table 3*).

Primary endpoint

Within 30 days 194 patients (21%) experienced a composite endpoint of non-fatal MI, all-cause mortality, or unplanned revascularization. Of these, 4 patients died and 128 had a MI (*Figure 2A*). Excluding the 124 patients with index NSTEMI, 70 of 807 patients (8.7%) reached an endpoint. Three patients died, 5 had a non-fatal MI after discharge, and 62 underwent unplanned revascularization.

Troponin-based algorithms and prediction of the primary endpoint

The four troponin algorithms combined with ACS risk criteria showed similar AUC (0.70–0.71), sensitivity (90–93%), and NPV (95–96%) for the identification of MI, mortality, or unplanned revascularization, with slightly more patients regarded low risk by the ESC algorithms (40.3% vs. 39.4%, P < 0.01 for ESC cTnT vs. High-STEACS cTnT). Number of primary endpoints among patients with low risk of ACS was 4.0–4.9% (ESC 0/3 h) and 3.8–4.3% (High-STEACS), see *Figure 2A*. In total, no patients died, 0–0.5% experienced an MI (ESC 0/3 h cTnT: 0 patients; ESC 0/3 h cTnI 2 patients; High-STEACS cTnT: 0 patients; High-STEACS cTnI: 1 patient), and 3.8–4.4% underwent unplanned revascularization (ESC 0/3 h cTnT; 15 patients; ESC 0/3 h cTnI 17 patients; High-STEACS cTnT: 14 patients; High-STEACS cTnI: 15 patients).

Risk scores

The risk scores with highest AUC were HEART and T-MACS (P < 0.05 compared to the other risk scores). Both had a sensitivity of 91–92% and an NPV of 96% for the primary endpoint (*Table 4*), and the percentages identified as low risk were 39–42%. Number of primary endpoints in the low-risk groups were 4.3% (HEART <_3) and 4.4% (T-MACS <_0.02). The algorithms differ in which patients they fail to identify. HEART missed more patients with NSTEMI (8 vs. 1), while T-MACS missed more patients with unplanned revascularization (15 vs. 9). None of the ruled-out patients died. HEART >3 and T-MACS >0.02 identified 85% and 76% of the 62 patients who underwent unplanned revascularization, respectively.

Combination of troponin algorithms and risk scores

When the ACS risk criteria recommended in ESC guidelines were replaced by clinical risk scores, NPV, and sensitivity increased without reduced efficacy. Troponin-based algorithms combined with HEART <_3, mHEART <_3, or T-MACS <_0.02 showed similar AUC (P > 0.05). The combinations including HEART score showed a sensitivity of 95–96% and NPV ~97.5%, see *Table 4*. mHEART, which increases at even the slightest rise in troponin values, showed sensitivity of 98%, but allocated less than one-third of patients to low risk. T-MACS had a sensitivity of 92% and allocated <40% to low risk. The combination of ESC 0/3 h algorithms or High-STEACS with any of the eight remaining risk scores showed significantly lower AUC for the primary endpoint.

Replacing the ACS low-risk criteria with HEART score <_3 resulted in 10–12 less patients being classified as low risk but reduced the number of false negatives by almost the same number (6–9 patients). Low-risk patients (9–10, 2.2–2.7%) experienced a primary event, almost exclusively unplanned revascularizations. HEART combined with the ESC 0/3 h cTnT algorithm or High-STEACS identified all MIs and deaths, while one MI was missed using the cTnI version of ESC 0/3 algorithm. Supplementary material online, *Tables S4* and *S5* for information about the ACS patients missed by the different algorithms.

Secondary endpoint

The ESC 0/3 h cTnT and High-STEACS cTnT algorithms (without evaluation of ACS low-risk criteria) missed 2–3 NSTEMIs, with sensitivity 97.6–98.4% and NPV 99.5–99.7% (*Table 5*). The proportion of low-risk patients ranged from 62% to 76% across the different algorithms. The two cTnI algorithms (ESC 0/3 h cTnI and High-STEACS cTnI) ruled out MI in a larger number of patients than the cTnT-based (ESC 0/3 h cTnT and High-STEACS cTnT) algorithms (71–76% vs. 62–64%, P < 0.001).

Most of the clinical risk scores performed worse compared to the troponin-based algorithms. The mHEART, CARE, and T-MACS scores showed comparable sensitivity of 99.2–100%, with fewer patients eligible for rule-out, between 10.3% and 39.1% (*Table 5*).

Discussion

In this post-hoc analysis of a prospective single-centre study of unselected patients presenting with chest pain, we show that the combination of troponin-based algorithms and a clinical risk score is superior to troponin-based algorithms combined with standard ACS risk criteria for detection of the combined endpoint of non-fatal MI, all-cause mortality, and unplanned revascularizations within 30 days. The

Table 2 Baseline characteristics of the study population by cause of chest pain

	All patients, *n* 5 932	ACS, *n* 5 230	Non-ACS, *n* 5 702	*P*-value
Baseline characteristics				
Age, years	63 (52–74)	68 (59–78)	61 (50–73)	<0.001
Male, %	562 (60.3)	163 (70.9)	399 (56.8)	<0.001
Symptom to arrival time, h	8.0 (3–45)	8.0 (3–38)	8.1 (3–47)	0.883
Hospital stay, h	28 (21–68)	74 (52–115)	25 (19–45)	<0.001
Risk factors				
Hypertension, %	383 (41.1)	115 (50.0)	268 (38.2)	0.001
Hyperlipidaemia, known %	180 (19.3)	61 (26.4)	119 (17.0)	0.002
Hyperlipidaemia, new[a], %	85 (9.1)	24 (10.4)	61 (8.7)	0.440
Diabetes mellitus, %	116 (12.4)	49 (21.2)	67 (9.6)	<0.001
Insulin-dependent	37 (4.0)	15 (6.5)	22 (3.1)	0.021
Family history, %	188 (20.2)	43 (18.6)	145 (20.7)	0.497
Unknown	109 (11.7)	30 (13.0)	79 (11.3)	0.481
Current smoker, %	195 (20.9)	49 (21.2)	146 (20.8)	0.901
Previous smoker, %	399 (42.8)	89 (38.5)	310 (44.2)	0.129
Medical history				
Prior MI, %	197 (21.7)	72 (31.3)	125 (17.8)	<0.001
Prior PCI, %	192 (20.6)	77 (33.5)	115 (16.4)	<0.001
Prior CABG, %	79 (8.5)	42 (18.3)	37 (5.3)	<0.001
Heart failure, %	44 (4.7)	15 (6.5)	29 (4.1)	0.143
Stroke, %	29 (3.1)	9 (3.9)	20 (2.9)	0.428
Peripheral vascular disease, %	21 (2.3)	11 (4.8)	10 (1.4)	0.001
Vital parameters at admission				
Systolic BP, mmHg	144 ± 43	149 ± 42	143 ± 42	0.016
Diastolic BP, mmHg	82 ± 26	82 ± 29	83 ± 25	0.588
Heart rate, b.p.m.	76 ± 38	74 ± 41	75 ± 36	0.789
BMI[b]	27.1 ± 9.0	26.7 ± 8.8	27.2 ± 9.0	0.220
Electrocardiography				
ST-segment depression, %	33 (3.5)	20 (8.7)	13 (1.9)	<0.001
T-wave inversion, %	30 (3.2)	15 (6.5)	15 (2.1)	0.001

Values are expressed as median (IQR), mean ± 2 SD, or *n* (%).
BMI, body mass index; BP, blood pressure; CABG, coronary artery bypass grafting; MI, myocardial infarction; PCI, percutaneous coronary intervention.
[a]Total cholesterol >6.5 ng/L at presentation.
[b]Data missing in 52.7% (491/932).

clinical risk scores alone missed a fairly large number of MIs but fewer patients with unplanned revascularization. The ESC and High-STEACS algorithms showed excellent diagnostic performance for identifying index NSTEMI, for which they were developed.

Emergency departments around the world have different flowcharts for the treatment of chest pain patients without MI. After initial work-up and exclusion of life-threatening non-cardiac diseases (pulmonary embolism, aortic disease, pneumothorax, etc.) physicians must decide whether or not to admit the patient for further cardiac examinations. Our data confirm that routine use of clinical risk scores instead of standard ACS risk criteria may improve the accuracy of this decision-making process, for one thing, by forcing the physician to structuralize their evaluation. This might prove especially useful for less experienced physicians.

The question of whether physician's gestalt rather than clinical risk scores is sufficient to identify patients with ACS is disputed, and available studies show conflicting results.[10–12,32] Nestelberger *et al*.[33] recently investigated whether clinical gestalt and ECG changes added to the ESC 0/1 h algorithm would better identify chest pain patients with NSTEMI, mortality, and revascularization within 30 days. Based on their numbers, sensitivity increased from 81% (95% CI 78–83%) to 92% (95% CI 90–94%) when gestalt was added to the troponin-based algorithm (without consideration of the ACS low-risk criteria). About 45% (95% CI 42–47%) of patients would still be ruled out as

Table 3 Median (IQR) troponin values in blood samples taken at presentation (0 h), 3 h (3 h), and 8–12 h after presentation (12 h), by adjudicated diagnosis, $n = 932$

cTnT, ng/L	0 h	3 h	12 h
NSTEMI ($n = 124$)	50 (23–180)	129 (50–307)	216 (67–635)
UAP ($n = 106$)	9 (5–18)	9 (5–19)	10 (5–20)
SAP ($n = 7$)	16 (14–48)	17 (15–47)	19 (16–45)
Other cardiac disease ($n = 60$)	18 (9–29)	19 (8–41)	20 (10–43)
Other specified diagnosis ($n = 75$)	9 (3–17)	9 (5–17)	10 (3–21)
NCCP ($n = 560$)	5 (3–9)	5 (3–9)	5 (3–10)
cTnI, ng/L			
NSTEMI ($n = 124$)	121 (27–596)	614 (136–1977)	1262 (212–6458)
UAP ($n = 106$)	5 (3–10)	5 (4–12)	6 (4–12)
SAP ($n = 7$)	11 (6–14)	12 (7–17)	12 (8–29)
Other cardiac disease ($n = 60$)	11 (4–21)	16 (6–38)	18 (7–64)
Other specified diagnosis ($n = 75$)	6 (2–13)	7 (3–14)	9 (4–13)
NCCP ($n = 560$)	3 (2–5)	3 (2–6)	3 (2–6)

NCCP, non-cardiac chest pain; NSTEMI, non-ST-elevation myocardial infarction; SAP, stable angina pectoris; UAP, unstable angina pectoris.

low risk. When using HEART >3 as the additional criteria, we found at least comparable increase in sensitivity from 76% (95% CI 69–81%) to 95% (95% CI 92–98%), and the number of patients still being ruled out was comparable (39%, 95% CI 35–44%).

The classical risk factors of CAD described both in ESC and ACC/AHA guidelines intertwine with elements of the clinical risk scores. One minor difference is history. Although highlighted as an important part of evaluation in both guidelines, the structured evaluation of history and typicality of symptoms (e.g. 0, 1, or 2 points for History in HEART score) might be a major strength, and the reason why risk scores outperform standard ACS criteria in our study.

Another important finding in our study is that assessment of individual symptoms is no definite indicator of ACS. Although some typical signs, such as retrosternal location and radiation to both arms, were significantly more often found in patients with ACS, other typical symptoms like radiation to the left arm, shortness of breath, and nausea were similar frequent in patients with non-ACS. As an example, 30% of patients with a final diagnosis of myalgia reported pain radiating to the left arm as opposed to only 18% of patients with ACS. One might speculate that the reason is a high general knowledge on symptoms of ACS. As information on internet is readily accessible, more patients might contact their general practitioner or call an ambulance if they experience classical symptoms of ACS, even though they may have low risk of coronary disease. The low discriminatory effect of some of the classical symptoms of ACS as found in our and other studies,[34,35] indicate that clinical risk scores through a balanced evaluation of history and risk factors has at least similar safety and is less dependent on physician experience, compared to clinical evaluation used to the best of physician's knowledge.

The importance of identifying all patients with UAP during ED evaluation is unclear. Patients with UAP have increased long term risk of mortality but only a moderate 5% 30-day risk of MI.[36] As high-sensitivity troponin assays identify even very small MIs, it has been argued that the term UAP may in fact disappear and be re-classified as a subgroup of severe stable CAD.[37,38] Knowing that patients with stable CAD has no prognostic benefit of coronary revascularization,[39] mistakenly discharging a UAP patient with low risk of adverse events (perhaps pending further examination) seem safe. However, risk aversion, fear of malpractice, and loss of respect from colleagues may explain why some physicians choose to admit most chest pain patients eligible for early discharge according to the ESC or High-STEACS algorithms and standard ACS risk criteria.[40,41]

The use of clinical risk scores as the sole diagnostic tool could be an option in outpatient clinics without access to high-sensitive troponin assays, as some of the scores do not include such analysis (*Table 1*). Randomized trials applying HEART score alone as a criteria for early discharge have shown more promising results compared to our study.[42,43] However, the non-adherence rate in these studies are not unignorably, and the rate of primary endpoints was much lower. The superior value of HEART over GRACE and TIMI in unselected patients with chest pain has been shown before.[3,44,45] EDACS had lower accuracy than HEART, also in line with earlier findings.[3] T-MACS performed better as compared to HEART for the secondary endpoint, but with slightly lower NPV for the primary endpoint compared to the validation performed by the group who developed the score.[24] The choice between HEART or T-MACS should therefore be done based on local clinical preference.

Although clinical risk scores identify patients in need of revascularizations, their ability to identify MI/death within 30 days is lower compared to troponin-based algorithms.[3,4] In our study, all patients with MI or death within 30 days were already identified by the cTnT versions of ESC 0/3 h or High-STEACS algorithms in combination with ACS criteria, and the additional effect of risk scores were hence non-existing for identification of NSTEMI.

Similarly, and as expected, the ESC and High-STEACS algorithms have high precision in identifying patients with NSTEMI during index hospitalization, with no difference in sensitivity and number of ruled out patients ($P > 0.05$). The results are in line with several studies showing excellent diagnostic performance of troponin-based algorithms for ruling out NSTEMI.[3,18]

Table 4 Primary endpoint

	True pos	False pos	True neg	False neg	NPV (95% CI)	Sens (95% CI)	PPV (95% CI)	Spes (95% CI)	Rule-out/ low-risk, %	Accuracy[a]	AUROC[b]
Troponin-based algorithms											
ESC 0/3 h TnT	147	190	548	47	92.1 (90.1–93.8)	75.8 (69.1–81.6)	43.6 (40.1–47.2)	74.3 (70.9–77.4)	63.8 (61–67)	74.6 (71.7–77.3)	0.75 (0.72–0.78)
ESC 0/3 h TnI	129	97	641	65	90.8 (89.0–92.3)	66.5 (59.4–73.1)	57.1 (51.9–62.2)	86.9 (84.2–89.2)	75.8 (73–79)	82.6 (80.0–85.0)	0.77 (0.74–0.80)
High-STEACS TnT	152	206	532	42	92.7 (90.6–94.3)	78.4 (71.9–83.9)	42.5 (39.1–45.9)	72.1 (68.7–75.3)	61.6 (58–65)	73.4 (70.4–76.2)	0.75 (0.72–0.78)
High-STEACS TnI	137	134	604	57	91.4 (89.5–93.0)	70.6 (63.7–76.9)	50.6 (46.1–55.0)	81.8 (78.9–84.6)	70.9 (68–74)	79.5 (76.8–82.1)	0.76 (0.73–0.79)
Troponin-based algorithms combined with low risk of ACS according to ESC guidelines											
ESC 0/3 h TnT	179	377	361	15	96.0 (93.6–97.5)	92.3 (87.6–95.6)	32.2 (30.4–34.0)	48.9 (45.3–52.6)	40.3 (37–43)	57.9 (54.7–61.1)	0.71 (0.68–0.74)
ESC 0/3 h TnI	175	371	367	19	95.1 (92.6–96.8)	90.2 (85.1–94.0)	32.1 (30.2–33.9)	49.7 (46.1–53.4)	41.4 (38–45)	58.2 (54.9–61.4)	0.70 (0.67–0.73)
High-STEACS TnT	180	385	353	14	96.2 (93.8–97.7)	92.8 (88.2–96.0)	31.9 (30.2–33.6)	47.8 (44.2–51.5)	39.4 (36–43)	57.2 (53.9–60.4)	0.70 (0.67–0.73)
High-STEACS TnI	178	378	360	16	95.7 (93.3–97.3)	91.8 (87.0–95.2)	32.0 (30.3–33.8)	48.8 (45.1–52.5)	40.3 (37–43)	57.7 (54.5–60.9)	0.70 (0.67–0.73)
Risk scores											
HEART <_3	177	360	378	17	95.7 (93.4–97.2)	91.2 (86.3–94.8)	33.0 (31.1–34.9)	51.2 (47.6–54.9)	42.4 (39–46)	59.6 (56.3–62.7)	0.83 (0.80–0.85)
mHEART <_3	189	435	303	5	98.4 (96.2–99.3)	97.4 (94.1–99.2)	30.3 (29.0–31.7)	41.1 (37.5–44.7)	33.0 (30–36)	52.8 (49.5–56.0)	0.81 (0.78–0.84)
CARE <_1	193	643	95	1	99.0 (93.0–99.9)	99.5 (97.2–100.0)	23.1 (22.6–23.6)	12.9 (10.5–15.5)	10.3 (8–12)	30.9 (28.0–34.0)	0.78 (0.75–0.80)
TIMI <_1	153	305	433	41	91.4 (88.9–93.3)	78.9 (72.4–84.4)	33.4 (31.0–36.0)	58.7 (55.0–62.3)	50.9 (48–54)	62.9 (59.7–66.0)	0.75 (0.72–0.78)
GRACE inhosp <_108[c]	102	245	491	89	84.7 (82.5–86.6)	53.4 (46.1–60.6)	29.4 (26.0–33.0)	66.7 (63.2–70.1)	62.6 (59–66)	64.0 (60.8–67.1)	0.67 (0.63–0.70)
GRACE 6 months <89[c]	91	203	533	100	84.2 (82.2–86.0)	47.6 (40.4–55.0)	31.0 (27.1–35.1)	72.4 (69.0–75.6)	68.3 (65–71)	67.3 (64.2–70.3)	0.68 (0.65–0.71)
T-MACS <_0.02	178	390	348	16	95.6 (93.1–97.2)	91.8 (87.0–95.2)	31.3 (29.6–33.1)	47.2 (43.5–50.8)	39.1 (36–42)	56.4 (53.2–59.7)	0.81 (0.79–0.84)
sT-MACS <_0	178	402	336	16	95.5 (92.9–97.1)	91.8 (87.0–95.2)	30.7 (29.1–32.4)	45.5 (41.9–49.2)	37.8 (35–41)	55.2 (51.9–58.4)	0.78 (0.75–0.80)
EDACS <_15	140	328	410	54	88.4 (85.7–90.6)	72.2 (65.3–78.4)	29.9 (27.5–32.5)	55.6 (51.9–59.2)	49.8 (47–53)	59.0 (55.8–62.2)	0.68 (0.65–0.71)
sEDACS <_3	153	397	341	41	89.3 (86.2–91.7)	78.9 (72.4–84.4)	27.8 (25.9–29.9)	46.2 (42.6–49.9)	41.0 (38–44)	53.0 (49.7–56.3)	0.64 (0.61–0.67)
Geleijnse–Sanchis <_1	69	142	596	125	82.7 (81.0–84.2)	35.6 (28.8–42.7)	32.7 (27.7–38.2)	82.7 (81.0–84.2)	77.4 (75–80)	71.4 (68.3–74.2)	0.66 (0.63–0.69)
Goldman <_1	40	55	683	154	81.6 (80.5–82.7)	20.6 (15.2–27.0)	42.1 (33.3–51.4)	92.6 (90.4–94.3)	89.8 (88–92)	77.6 (74.8–80.2)	0.63 (0.60–0.66)
Troponin-based algorithms combined with HEART score											
ESC 0/3 h TnT þ HEART <_3	185	381	357	9	97.5 (95.4–98.7)	95.4 (91.4–97.9)	32.7 (31.0–34.4)	48.4 (44.7–52.1)	39.3 (35–42)	58.2 (54.9–61.4)	0.84 (0.81–0.86)
ESC 0/3 h TnI þ HEART <_3	184	374	364	10	97.3 (95.2–98.5)	94.9 (90.7–97.5)	33.0 (31.3–34.7)	49.3 (45.7–53.0)	40.1 (37–43)	58.8 (55.6–62.0)	0.86 (0.84–0.88)
High-STEACS TnT þ HEART <_3	186	390	348	8	97.8 (95.7–98.9)	95.9 (92.0–98.2)	32.3 (30.7–33.9)	47.2 (43.5–50.8)	38.2 (35–41)	57.3 (54.1–60.5)	0.84 (0.81–0.86)
High-STEACS TnI þ HEART <_3	185	383	355	9	97.5 (95.4–98.7)	95.4 (91.4–97.9)	32.6 (30.9–34.2)	48.1 (44.4–51.8)	39.1 (36–42)	57.9 (54.7–61.1)	0.85 (0.83–0.88)
Troponin-based algorithms combined with mHEART											
ESC 0/3 h TnT þ mHEART <_3	190	445	293	4	98.7 (96.5–99.5)	97.9 (94.8–99.4)	29.9 (28.9–31.2)	39.7 (36.2–43.3)	31.9 (29–35)	51.8 (48.6–55.1)	0.83 (0.80–0.85)
ESC 0/3 h TnI þ mHEART <_3	190	444	294	4	98.7 (96.5–99.5)	97.9 (94.8–99.4)	30.0 (28.7–31.3)	39.8 (36.3–43.5)	32.0 (29–35)	51.9 (48.7–55.2)	0.86 (0.83–0.88)
High-STEACS TnT þ mHEART <_3	190	448	290	4	98.6 (96.5–99.5)	97.9 (94.8–99.4)	29.8 (28.5–31.1)	39.3 (35.8–42.9)	31.5 (29–35)	51.5 (48.2–54.8)	0.83 (0.80–0.85)
High-STEACS TnI þ mHEART <_3	190	448	290	4	98.6 (96.5–99.5)	97.9 (94.8–99.4)	29.8 (28.5–31.1)	39.3 (35.8–42.9)	31.5 (29–35)	51.5 (48.2–54.8)	0.85 (0.82–0.87)
Troponin-based algorithms combined with TIMI											

Continued

Table 4 *Continued*

	True pos	False pos	True neg	False neg	NPV (95% CI)	Sens (95% CI)	PPV (95% CI)	Spes (95% CI)	Rule-out/ low-risk, %	Accuracy[a]	AUROC[b]
ESC 0/3 h TnT þ TIMI <_1	181	337	401	13	96.9 (94.8–98.1)	93.3 (88.8–96.4)	34.9 (33.0–37.0)	54.3 (50.7–58.0)	44.4 (41–48)	62.5 (59.3–65.6)	0.80 (0.78–0.83)
ESC 0/3 h TnI þ TIMI <_1	180	332	406	14	96.7 (94.3–97.8)	92.8 (88.2–96.0)	35.2 (33.2–37.2)	55.0 (51.3–58.6)	45.1 (42–48)	62.9 (59.7–66.0)	0.85 (0.82–0.87)
High-STEACS TnT þ TIMI <_1	183	345	393	11	97.3 (95.3–98.5)	94.3 (90.1–97.1)	34.7 (32.8–36.6)	53.3 (49.6–56.9)	43.3 (40–47)	61.8 (58.6–64.9)	0.80 (0.78–0.83)
High-STEACS TnI þ TIMI <_1	183	344	394	11	97.3 (95.3–98.5)	94.3 (90.1–97.1)	34.7 (32.8–36.7)	53.4 (49.7–57.0)	43.5 (40–47)	61.9 (58.7–65.0)	0.84 (0.81–0.86)
Troponin-based algorithms combined with T-MACS											
ESC 0/3 h TnT þ T-MACS <0.02	179	390	348	15	95.9 (93.4–97.4)	92.3 (87.6–95.6)	31.5 (29.8–33.2)	47.2 (43.5–50.8)	38.9 (36–42)	56.6 (53.3–59.8)	0.83 (0.80–0.85)
ESC 0/3 h TnI þ T-MACS <0.02	179	391	347	15	95.9 (93.4–97.4)	92.3 (87.6–95.6)	31.4 (29.7–33.1)	47.0 (43.4–50.7)	38.8 (36–42)	56.4 (53.2–59.7)	0.83 (0.81–0.86)
High-STEACS TnT þ T-MACS <0.02	179	396	342	15	95.8 (93.3–97.4)	92.3 (87.6–95.6)	31.1 (29.5–32.8)	46.3 (42.7–50.0)	38.3 (35–41)	55.9 (52.7–59.1)	0.83 (0.80–0.85)
High-STEACS TnI þ T-MACS <0.02	179	398	340	15	95.8 (93.3–97.4)	92.3 (87.6–95.6)	31.0 (29.4–32.7)	46.1 (42.4–49.7)	38.1 (35–41)	55.7 (52.4–58.9)	0.83 (0.81–0.86)
Troponin-based algorithms combined with EDACS											
ESC 0/3 h TnT þ EDACS <_15	180	382	356	14	96.2 (93.9–97.7)	92.8 (88.2–96.0)	32.0 (30.3–33.8)	48.2 (44.6–51.9)	39.7 (37–43)	57.5 (54.3–60.7)	0.80 (0.77–0.82)
ESC 0/3 h TnI þ EDACS <_15	175	363	375	19	95.2 (92.8–96.8)	90.2 (85.1–94.0)	32.5 (30.7–34.5)	50.8 (47.1–54.5)	42.3 (39–45)	59.0 (55.8–62.2)	0.83 (0.80–0.85)
High-STEACS TnT þ EDACS <_15	181	390	348	13	96.4 (94.0–97.9)	93.3 (88.8–96.4)	31.7 (30.0–33.4)	47.2 (43.5–50.8)	38.7 (36–42)	56.8 (53.5–60.0)	0.80 (0.77–0.82)
High-STEACS TnI þ EDACS <_15	178	374	364	16	95.8 (93.4–97.3)	91.8 (87.0–95.2)	32.3 (30.5–34.1)	49.3 (45.7–53.0)	40.8 (38–44)	58.2 (54.9–61.4)	0.82 (0.79–0.84)

Diagnostic precision of troponin-based algorithms alone, in combination with low-risk ACS criteria (ESC guidelines) and in combination with clinical risk scores.
CARE, characteristics, age, risk factors, EGG; EDACS, Emergency Department Assessment of Chest Pain Score; GRACE, Global Registry of Acute Coronary Events; HEART, History, ECG, Age, Risk factors, Troponin; mHEART, modified HEART score with troponin points given if hs-Tn is measurable; MI, myocardial infarction; T-MACS, troponin-only Manchester Acute Coronary Syndromes; TIMI, thrombolysis in myocardial infarction.
[a]Sensitivity x Prevalence þ Specificity x (1 - Prevalence).
[b]Calculations based on categorical data for troponin-based algorithms, continuous data for risk scores, and continuous data for the combination of troponin-based algorithms and risk scores.
[c]Excluding five patients with unknown GRACE score.

Table 5 Secondary endpoint

	True pos	False pos	True neg	False neg	NPV (95% CI)	Sens (95% CI)	PPV (95% CI)	Spes (95% CI)	Rule-out/ low risk, %	Accuracy[a]	AUROC[b]
Troponin-based algorithms											
ESC 0/3 h TnT	121	216	592	3	99.5 (98.5–99.8)	97.6 (93.1–99.5)	35.9 (33.3–38.7)	73.3 (70.1–76.3)	63.8 (61–67)	76.5 (73.7–79.2)	0.85 (0.83–0.88)
ESC 0/3 h TnI	117	109	699	7	99.0 (98.0–99.5)	94.4 (88.7–97.7)	51.8 (47.3–56.2)	86.5 (84.0–88.8)	75.8 (73–79)	87.6 (85.3–89.6)	0.90 (0.88–0.92)
High-STEACS TnT	122	236	572	2	99.7 (98.6–99.9)	98.4 (94.3–99.8)	34.1 (31.7–36.6)	70.8 (67.5–73.9)	61.6 (58–65)	74.5 (71.5–77.2)	0.85 (0.82–0.87)
High-STEACS TnI	121	150	658	3	99.6 (98.6–99.9)	97.6 (93.1–99.5)	44.7 (41.1–48.3)	81.4 (78.6–84.1)	70.9 (68–74)	83.6 (81.1–85.9)	0.90 (0.87–0.91)
Troponin-based algorithms combined wit h low risk of ACS accordin g to ESC guidelines											
ESC 0/3 h TnT	124	432	376	0	100.0	100.0 (97.1–100.0)	22.3 (21.2–23.4)	46.5 (43.1–50.0)	40.3 (37–43)	53.7 (50.4–56.9)	0.73 (0.70–0.76)
ESC 0/3 h TnI	122	424	384	2	99.5 (98.0–99.9)	98.4 (94.3–99.8)	22.3 (21.2–23.6)	47.5 (44.0–51.0)	41.4 (38–45)	54.3 (51.0–57.5)	0.73 (0.70–0.76)
High-STEACS TnT	124	441	367	0	100.0	100.0 (97.1–100.0)	22.0 (20.9–23.0)	45.4 (42.0–48.9)	39.4 (36–43)	52.7 (49.4–55.9)	0.73 (0.70–0.76)
High-STEACS TnI	123	433	375	1	99.7 (98.2–100.0)	99.2 (95.6–100.0)	22.1 (21.0–23.3)	46.4 (42.9–49.9)	40.3 (37–43)	53.4 (50.2–56.7)	0.73 (0.70–0.76)
Risk scores											
HEART <_3	116	421	387	8	98.0 (96.1–99.0)	93.6 (87.7–97.2)	21.6 (20.3–23.0)	47.9 (44.4–51.4)	42.4 (39–46)	54.0 (50.7–57.2)	0.85 (0.82–0.87)
mHEART <_3	123	501	307	1	99.7 (97.8–100.0)	99.2 (95.6–100.0)	19.7 (18.8–20.6)	38.0 (34.6–41.4)	33.0 (30–36)	46.1 (42.9–49.4)	0.83 (0.80–0.85)
CARE <_1	124	712	96	0	100.0	100.0 (97.1–100.0)	14.8 (14.5–15.2)	11.9 (9.7–14.3)	10.3 (8–12)	23.6 (20.9–26.5)	0.76 (0.73–0.79)
TIMI <_1	96	362	446	28	94.1 (92.0–95.7)	77.4 (69.0–84.4)	21.0 (19.0–23.1)	55.2 (51.7–58.7)	50.9 (48–54)	58.2 (54.9–61.4)	0.73 (0.70–0.76)
GRACE <_108[c]	76	271	534	46	92.1 (90.2–93.6)	62.3 (53.1–70.9)	21.9 (19.2–24.9)	66.3 (63.0–69.6)	62.6 (59–66)	65.8 (62.7–68.9)	0.69 (0.66–0.72)
GRACE <_88[c]	70	224	581	52	91.8 (90.1–93.2)	57.4 (48.1–66.3)	23.8 (20.6–27.4)	72.2 (68.9–75.3)	68.3 (65–71)	70.2 (67.2–73.2)	0.70 (0.67–0.73)
T-MACS <_0.02	123	445	363	1	99.7 (98.1–100.0)	99.2 (95.6–100.0)	21.7 (20.6–22.8)	44.9 (41.5–48.4)	39.1 (36–42)	52.2 (48.9–55.4)	0.91 (0.89–0.93)
sT-MACS <_0	123	457	351	1	99.7 (98.0–100.0)	99.2 (95.6–100.0)	21.2 (20.2–22.3)	43.4 (40.0–46.9)	37.8 (35–41)	50.9 (47.6–54.1)	0.83 (0.80–0.85)
EDACS <_15	91	377	431	33	92.9 (90.6–94.6)	73.4 (64.7–80.9)	19.4 (17.5–21.6)	53.3 (49.8–56.8)	49.8 (847–53)	56.0 (52.8–59.2)	0.68 (0.65–0.71)
sEDACS <_3	99	451	357	25	93.5 (90.9–95.3)	79.8 (71.7–86.5)	18.0 (16.5–19.6)	44.2 (40.7–47.7)	41.0 (38–44)	48.9 (45.7–52.2)	0.64 (0.61–0.67)
Geleijnse–Sanchis <_1	41	170	638	83	88.5 (87.1–89.7)	33.1 (24.9–42.1)	19.4 (15.4–24.3)	79.0 (76.0–81.7)	77.4 (75–80)	72.9 (69.9–75.7)	0.63 (0.59–0.66)
Goldman <_1	34	61	747	90	89.3 (88.2–90.3)	27.4 (19.8–36.2)	35.8 (27.7–44.8)	92.5 (90.4–94.2)	89.8 (88–92)	83.8 (81.3–86.1)	0.61 (0.58–0.64)
Troponin-based algorithms combined wit h HEART score											
ESC 0/3 h TnT þ HEART <_3	124	442	366	0	100.0	100.0 (97.1–100.0)	21.9 (20.9–23.0)	45.3 (41.8–48.8)	39.3 (35–42)	52.6 (49.3–55.8)	0.89 (0.87–0.91)
ESC 0/3 h TnI þ HEART <_3	123	435	373	1	99.7 (98.1–100.0)	99.2 (95.6–100.0)	22.0 (20.9–23.2)	46.2 (42.7–49.7)	40.1 (37–43)	53.2 (50.0–56.5)	0.93 (0.92–0.95)
High-STEACS TnT þ HEART <_3	124	452	356	0	100.0	100.0 (97.1–100.0)	21.5 (20.5–22.6)	44.1 (40.6–47.6)	38.2 (35–41)	51.5 (48.2–54.8)	0.90 (0.88–0.92)
High-STEACS TnI þ HEART <_3	124	444	364	0	100.0	100.0 (97.1–100.0)	21.8 (20.8–22.9)	45.1 (41.6–48.6)	39.1 (36–42)	52.4 (49.1–55.6)	0.93 (0.92–0.95)
Troponin-based algorithms combined wit h mHEA T											
ESC 0/3 h TnT þ mHEART <_3	124	511	297	0	100.0	100.0 (97.1–100.0)	19.5 (18.7–20.4)	36.8 (33.5–40.2)	31.9 (29–35)	45.2 (41.9–48.4)	0.88 (0.86–0.90)
ESC 0/3 h TnI þ mHEART <_3	124	510	298	0	100.0	100.0 (97.1–100.0)	19.6 (18.7–20.4)	36.9 (33.6–40.4)	32.0 (29–35)	45.3 (42.1–48.6)	0.93 (0.92–0.95)
High-STEACS TnT þ mHEART <_3	124	514	294	0	100.0	100.0 (97.1–100.0)	19.4 (18.6–20.3)	36.4 (33.1–39.8)	31.5 (29–35)	44.9 (41.6–48.1)	0.89 (0.86–0.91)
High-STEACS TnI þ mHEART <_3	124	514	294	0	100.0	100.0 (97.1–100.0)	19.4 (18.6–20.3)	36.4 (33.1–39.8)	31.5 (29–35)	44.9 (41.6–48.1)	0.93 (0.91–0.94)
Troponin-based algorithms combined with TIMI											
ESC 0/3 h TnT þ TIMI <_1	124	394	414	0	100.0	100.0 (97.1–100.0)	23.9 (22.7–25.3)	51.2 (47.7–54.7)	44.4 (41–48)	57.7 (54.5–60.9)	0.85 (0.83–0.88)
ESC 0/3 h TnI þ TIMI <_1	123	389	419	1	99.8 (98.3–100.0)	99.2 (95.6–100.0)	24.0 (22.7–25.4)	51.9 (48.4–55.4)	45.1 (42–48)	58.2 (54.9–61.4)	0.92 (0.90–0.94)

Continued

Table 5 *Continued*

	True pos	False pos	True neg	False neg	NPV (95% CI)	Sens (95% CI)	PPV (95% CI)	Spes (95% CI)	Rule-out/ low risk, %	Accuracy[a]	AUROC[b]
High-STEACS TnT þ TIMI <_1	124	404	404	0	100.0	100.0 (97.1–100.0)	23.5 (22.3–24.8)	50.0 (46.5–53.5)	43.3 (40–47)	56.7 (53.4–59.9)	0.85 (0.83–0.87)
High-STEACS TnI þ TIMI <_1	124	403	405	0	100.0	100.0 (97.1–100.0)	23.5 (22.3–24.8)	50.1 (46.6–53.6)	43.5 (40–47)	56.8 (53.5–60.0)	0.91 (0.89–0.93)
Troponin-based algorithms combined with T-MACS											
ESC 0/3 h TnT þ T-MACS <0.02	124	445	363	0	100.0	100.0 (97.1–100.0)	21.8 (20.8–22.9)	44.9 (41.5–48.4)	38.9 (36–42)	52.3 (49.0–55.5)	0.93 (0.91–0.95)
ESC 0/3 h TnI þ T-MACS <0.02	124	446	362	0	100.0	100.0 (97.1–100.0)	21.8 (20.7–22.8)	44.8 (41.3–48.3)	38.8 (36–42)	52.2 (48.9–55.4)	0.95 (0.93–0.96)
High-STEACS TnT þ T-MACS <0.02	124	451	357	0	100.0	100.0 (97.1–100.0)	21.6 (20.6–22.6)	44.2 (40.7–47.7)	38.3 (35–41)	51.6 (48.4–54.9)	0.93 (0.91–0.95)
High-STEACS TnI þ T-MACS <0.02	124	453	355	0	100.0	100.0 (97.1–100.0)	21.5 (20.5–22.5)	43.9 (40.5–47.4)	38.1 (35–41)	51.4 (48.1–54.7)	0.95 (0.93–0.96)
Troponin-based algorithms combined with EDACS											
ESC 0/3 h TnT þ EDACS <_15	123	439	369	1	99.7 (98.1–100.0)	99.2 (95.6–100.0)	21.9 (20.8–23.0)	45.7 (42.2–49.2)	39.7 (37–43)	52.8 (49.5–56.0)	0.87 (0.84–0.89)
ESC 0/3 h TnI þ EDACS <_15	121	417	391	3	99.3 (97.7–100.0)	97.6 (93.1–99.5)	22.5 (21.3–23.8)	48.4 (44.9–51.9)	42.3 (39–45)	54.9 (51.7–58.2)	0.92 (0.90–0.94)
High-STEACS TnT þ EDACS <_15	123	448	360	1	99.7 (98.1–100.0)	99.2 (95.6–100.0)	21.5 (20.5–22.6)	44.6 (41.1–48.1)	38.7 (36–42)	51.8 (48.6–55.1)	0.86 (0.84–0.89)
High-STEACS TnI þ EDACS <_15	122	430	378	2	99.5 (98.0–99.9)	98.4 (94.3–99.8)	22.1 (21.0–23.3)	46.8 (43.3–50.3)	40.8 (38–44)	53.7 (50.4–56.9)	0.91 (0.89–0.93)

Diagnostic precision of troponin-based algorithms alone and in combination with clinical risk scores.
CARE, characteristics, age, risk factors, EGG; EDACS, Emergency Department Assessment of Chest Pain Score; GRACE, Global Registry of Acute Coronary Events; HEART, History, ECG, Age, Risk factors, Troponin; mHEART, modified HEART score with troponin points given if hs-Tn is measurable; MI, myocardial infarction; T-MACS, troponin-only Manchester Acute Coronary Syndromes; TIMI, thrombolysis in myocardial infarction.
[a]Sensitivity x Prevalence þ Specificity x (1 - Prevalence).
[b]Calculations based on categorical data for troponin-based algorithms, continuous data for risk scores and continuous data for the combination of troponin-based algorithms and risk scores.
[c]Excluding five patients with unknown GRACE score.

The strength of this study is the broad inclusion criteria and no cut-off for the onset of symptoms before presentation, closely mimicking a real-life ED scenario with the object of identifying ACS as opposed to MI alone. Patients with non-ACS had a mean hospital stay of 40 h with several measurements of troponin concentrations, which makes coronary events unlikely to go undetected. Symptoms and clinical information collected from multiple sources allowed for evaluation of a variety of risk scores.

Study limitations

First, estimation of risk and gathering of clinical information was performed retrospectively. Even though the study cardiologist calculating HEART score was blinded for all further examinations and treatment, objective symptoms reported in medical journals may have been coloured by assumptions made by the ED physician and ambulance personnel. The retrospective gathering of information could also have affected EDACS, T-MACS, and Geleijnse–Sanchis, since some episodes of diaphoresis and vomiting may not have been reported. Other major characteristics, like location, character, and radiation of pain was described in detail in almost all patients.

The study contains few early presenters (<2 h) (10.4%), which makes the results less applicable for this category of patients. The long median time from symptom onset to presentation (8 h) also affects the applicability in patient groups who present to ED earlier.

Another limitation is that the adjudication of diagnoses was performed using cTnT as routine test, and the performance of the cTnT compared to the cTnI algorithms may potentially be overestimated. The use of a gender-neutral 99th percentile during the adjudication could have negatively biased the performance of the High-STEACS cTnI algorithm, that used gender-specific 99th percentiles.

Lastly, the study has a single-centre design and the inclusion period is long, which may raise questions about representativeness of the data. However, the rate of NSTEMI, UAP, and patient characteristics are similar to other cohorts, and the broad inclusion criteria should ensure a representative inclusion. The generalizability of the results would greatly benefit from being validated in a prospective validation cohort, preferably performed by another study groups.

Conclusion

Troponin-based algorithms intended to identify NSTEMI should preferable be combined with a clinical risk score rather than the ACS low-risk criteria recommended by ESC to improve sensitivity and NPV for identification of patients with high risk of MI, death, or need for invasive treatment. The number of patients eligible for rule-out were maintained. For clinicians who are reluctant to discharge chest pain patients from ED due to fear of malpractice and overlooking ACS, 2.2% risk of revascularization within 30 days might be acceptable. Future studies should compare the safety and efficiency of a strategy implying treatment of low-risk ACS patients during index hospitalization to a liberal practice using out-of-hospital follow-up of ACS patients eligible for rule-out.

Supplementary material

Supplementary material is available at *European Heart Journal: Acute Cardiovascular Care* online.

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Conflict of interest: K.M.A. has served on one advisory board for Roche Diagnostics and received lecturing fees from Siemens Healthineers. T.O. has served on advisory boards for Abbott Diagnostics, Roche Diagnostics, and Novartis, and has received research support from AstraZenica, Abbott Dioagnostics, Roche Diagnostics, ThermoFisher, Singulex, and Biomedica via Akershus University Hospital, and speaker's honoraria from Roche Diagnostics and Novartis. Ø.S. has received lecture fees from Abbott Diagnostics.

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SUPPLEMENTAL FILES PAPER 3

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 cTnI) 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 were 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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Table S1. Medication at presentation and during admission, ECG findings, laboratory findings, performed examinations, risk scores and outcome.

	All patients, n=932	ACS, n=230	Non-ACS, n=702	P-value
Medication at presentation				
Aspirin, %	324 (34.8)	118 (51.1)	206 (29.4)	<0.001
Clopidogrel, %	43 (4.6)	22 (9.5)	21 (3.0)	<0.001
Ticagrelor, %	25 (2.7)	7 (3.0)	18 (2.6)	0.706
Dipyridamol, %	8 (0.9)	1 (0.4)	7 (1.0)	0.687
Warfarin, %	60 (6.4)	15 (6.5)	45 (6.4)	0.968
Apixaban	28 (3.0)	2 (0.9)	26 (3.7)	0.028
Dabigatran	0 (0.0)	0 (0.0)	0 (0.0)	-
Rivaroxaban	17 (1.8)	5 (2.2)	12 (1.7)	0.656
Dalteparin, %	3 (0.3)	2 (0.9)	1 (0.1)	0.154
Betablocker, %	318 (34.1)	101 (43.9)	217 (30.9)	<0.001
ACEi, %	99 (10.6)	35 (15.2)	64 (9.1)	0.009
A2-blockers	207 (22.2)	54 (23.4)	153 (21.8)	0.623
Diuretics, %	164 (17.6)	51 (22.1)	113 (16.1)	0.039
Aldosterone antagonists, %	0 (0.0)	0 (0.0)	0 (0.0)	-
Statins, %	357 (38.3)	112 (48.7)	245 (34.9)	<0.001
Other lipid lowering drugs, %	36 (3.9)	9 (3.9)	27 (3.9)	0.976
Ca-blocker, %	131 (14.1)	44 (19.0)	87 (12.4)	0.012
Long-acting nitrate, %	46 (4.9)	16 (7.0)	30 (4.3)	0.050
First ECG at presentation				
Sinus rhythm, %	833 (89.4)	206 (89.2)	627 (89.4)	0.915
Atrial fibrillation, %	71 (7.6)	18 (7.8)	53 (7.5)	0.891
Pacemaker, %	24 (2.6)	5 (2.2)	19 (2.7)	0.658
Other rhytm, %	2 (0.2)	1 (4.8)	1 (0.1)	0.434
ECG not available, %	2 (0.2)	0 (0.0)	2 (0.3)	1.000
No ischemia, %	574 (61.6)	104 (45.2)	470 (67.0)	<0.001
Unchanged from before, %	186 (20.0)	52 (22.5)	134 (19.1)	0.263
ST elevation, %	13 (1.4)	2 (0.9)	11 (1.6)	0.537
New LBBB, %	11 (1.2)	3 (1.3)	8 (1.1)	0.739
New RBBB, %	6 (0.6)	2 (0.9)	4 (0.6)	0.641
ST depression, %	33 (3.5)	20 (0.9)	13 (1.9)	<0.001
T-wave inversion, %	30 (3.2)	15 (6.5)	15 (2.1)	0.001
Unspecific changes, %	60 (6.4)	28 (12.1)	32 (4.6)	<0.001
ECG changes during hospitalization				
New ECGs performed, %	810 (86.9)	218 (94.8)	592 (84.3)	<0.001
Changes, %	107 (11.5)	48 (20.9)	59 (8.4)	<0.001
New atrial fibrillation, %	12 (1.3)	2 (0.9)	10 (1.4)	0.740
New LBBB, ST-dep or el., %	5 (0.5)	0 (0.0)	5 (0.7)	0.341
T wave inversion, %	30 (3.2)	27 (11.7)	3 (0.4)	<0.001
Laboratory finding				
Creatinine	81.8 ± 58.3	85.8 ± 58.5	80.5 ± 58.0	<0.001
eGFR	82.3 ± 41.4	77.5 ± 39.1	83.9 ± 41.7	<0.001
Glucose	6.4 ± 4.1	7.1 ± 5.5	6.2 ± 3.4	<0.001

HbA1C*	5.8 ± 1.6	6.0 ± 1.8	5.7 ± 1.6	<0.001
Total cholesterol	4.9 ± 2.6	5.1 ± 3.0	4.9 ± 2.5	0.166
HDL	1.4 ± 1.0	1.4 ± 1.0	1.5 ± 1.0	0.014
LDL	3.1 ± 2.4	3.3 ± 2.8	3.1 ± 2.2	0.139
Triglycerides†	1.6 ± 2.3	1.8 ± 2.1	1.6 ± 2.3	<0.001
Medical treatment given in ED				
DAPT and anticoagulation, %	381 (40.9)	163 (70.9)	218 (31.1)	<0.001
Partially treated, %‡	416 (44.6)	58 (25.1)	358 (51.1)	<0.001
No treatment, %	117 (12.6)	5 (2.2)	112 (16.0)	<0.001
Not reported, %	18 (1.9)	4 (1.7)	14 (2.0)	0.800
CT angiography index hospitalization				
Performed, %	283 (30.4)	37 (16.2)	246 (35.1)	<0.001
High ca-score, angiography not perf.,	5 (1.8)	2 (5.4)	3 (1.2)	0.129
Anatomical stenosis, %	43 (15.2)	25 (67.6)	18 (7.3)	<0.001
Atherosclerosis without stenosis, %	115 (40.6)	8 (21.6)	107 (43.5)	0.012
No stenosis, %	120 (42.4)	2 (5.4)	118 (48.0)	<0.001
CT angiography after discharge				
Performed, %	25 (2.7)	1 (0.4)	24 (3.4)	0.015
Stenosis, %	1 (4.0)	1 (100.0)	0 (0.0)	0.040
Atherosclerosis, %	4 (16.0)	0 (0.0)	4 (16.7)	1.000
Normal findings, %	20 (80.0)	0 (0.0)	20 (83.3)	0.200
Exercise ECG				
Performed, %	135 (14.5)	15 (6.5)	120 (17.1)	<0.001
Positive, %	15 (11.1)	4 (26.7)	11 (9.2)	0.065
Negative, %	71 (52.6)	1 (6.7)	70 (58.3)	<0.001
Inconclusive, %	40 (29.6)	9 (60.0)	31 (25.8)	0.006
Unknown result, %	9 (6.7)	1 (6.7)	8 (6.7)	1.000
Echocardiography				
Performed, %	367 (39.4)	171 (74.3)	196 (27.9)	<0.001
EF >50%, %	306 (83.4)	137 (80.1)	169 (86.2)	0.117
EF 30-49%, %	39 (10.6)	25 (14.5)	14 (7.2)	0.023
EF <30%, %	22 (6.0)	9 (5.2)	13 (6.7)	0.564
EF, mean	55.3 ± 22.8	54.4 ± 21.5	56.3 ± 23.9	0.921
Angiography and revascularization				
Angiography only, index hosp., %	74 (7.9)	34 (14.7)	40 (5.7)	<0.001
PCI index, %	135 (14.5)	131 (56.7)	4 (0.6)	<0.001
ACB index, %	13 (1.4)	13 (5.6)	0 (0.0)	<0.001
Angiography only, after discharge, %	4 (0.4)	1 (0.4)	3 (0.4)	0.992
PCI after discharge	5 (0.5)	5 (2.2)	0 (0.0)	<0.001
ACB after discharge	1 (0.1)	1 (0.4)	0 (0.0)	0.081
Risk scores				
HEART	4.1 ± 4.2	6.0 ± 3.7	3.4 ± 3.6	<0.001
mHEART	4.7 ± 2.5	6.5 ± 3.4	4.0 ± 4.0	<0.001
CARE	3.7 ± 3.4	5.0 ± 2.8	3.2 ± 3.1	<0.001
GRACE inhospital mortality	100.5 ± 64.3	114.8 ± 61.0	95.6 ± 62.0	<0.001
GRACE 6 months mortality	74.4 ± 62.8	89.3 ± 55.6	69.4 ± 61.5	<0.001
T-MACS percent	0.150 ± 0.543	0.389 ± 0.80	0.076 ± 0.31	<0.001
sT-MACS	0.9 ± 1.7	1.6 ± 1.7	0.7 ± 1.4	<0.001
TIMI	1.7 ± 3.1	2.9 ± 3.2	1.3 ± 2.6	<0.001

EDACS	15.3 ± 13.3	18.7 ± 11.4	14.1 ± 13.1	<0.001
sEDACS	3.8 ± 3.0	4.5 ± 2.6	3.6 ± 3.0	<0.001
Geleijnse-Sanchis	0.9 ± 1.9	1.4 ± 2.1	0.7 ± 1.7	<0.001
Goldman	0.2 ± 0.9	0.5 ± 1.1	0.2 ± 0.8	<0.001
Outcome at 30 days				
All-cause mortality, %	4 (0.4)	1 (0.4)	3 (0.4)	1.000
All-cause mortality in patients	3 (0.3)	0 (0.0)	3 (0.4)	1.000
Non-fatal MI, %	129 (13.8)	127 (55.0)	2 (0.3)	<0.001
MI or all-cause mortality, %	133 (14.3)	128 (55.4)	5 (0.7)	<0.001
Revascularization, %	152 (16.3)	147 (63.6)	5 (0.7)	<0.001
New MI after discharge, %§	8 (0.9)	6 (2.6)	2 (0.3)	0.004
First MI after hospitalization, %	5 (0.5)	3 (1.3)	2 (0.3)	0.018

Values are median (IQR), mean ± 2SD, or n (%). ACEi indicates angiotensin converting enzyme inhibitor; A2, angiotensin 2; ECG, electrocardiogram; LBBB, left bundle branch block; RBBB, right bundle branch block; GFR, glomerular filtration ratio; HDL, high density lipoprotein; LDL, low density lipoprotein, DAPT, double anti platelet treatment; HEART, History, ECG, Age, Risk factors, Troponin; CARE, characteristics, age, risk factors, EGG; GRACE, Global Registry of Acute Coronary Events; T-MACS, Troponin-only Manchester Acute Coronary Syndromes; sT-MACS, simplified Troponin-only Manchester Acute Coronary Syndromes; TIMI, Thrombolysis In Myocardial Infarction; EDACS, Emergency Department Assessment of Chest Pain Score and MI, myocardial infarction.
*Data missing in 43.3% (404/932).
†Data missing in 9.4% (88/932).
‡Treated with APT and/or AC, but not DAPT+AC.
§Including patients with MI during index hospitalization.

Table S2. Symptoms characteristics

	All patients (n=932)	ACS (n=230)	Non-ACS (n=702)	P-value
Location				
Retrosternal, %	416 (44.6)	128 (55.7)	288 (41.0)	<0.001
Precordial, %	186 (20.0)	23 (10.0)	163 (23.3)	<0.001
Right sided, %	32 (3.4)	2 (0.9)	30 (4.3)	0.013
Primarily shoulders or arms, %	19 (2.0)	2 (0.9)	17 (2.4)	0.146
Epigastrial, %	51 (5.5)	14 (6.1)	37 (5.3)	0.650
Abdominal, %	12 (1.3)	1 (0.4)	11 (1.6)	0.184
Primarily in the back, %	21 (2.3)	3 (1.3)	18 (2.6)	0.260
Located in the whole thorax, %	15 (1.6)	4 (1.7)	11 (1.6)	0.865
Other location, %	47 (5.0)	13 (5.6)	34 (4.9)	0.640
Unknown, %	163 (17.5)	49 (21.2)	114 (16.3)	0.086
Severity				
Mild (NRS 1-3), %	114 (12.2)	22 (9.6)	92 (13.1)	0.155
Moderate (NRS 4-7), %	165 (17.7)	40 (17.3)	125 (17.8)	0.859
Severe (NRS 8-10), %	205 (22.0)	55 (23.8)	150 (21.4)	0.443
Unknown, %	417 (44.7)	108 (46.8)	309 (44.1)	0.479
Character				
Pressing/squeezing, %	566 (60.7)	149 (64.8)	417 (59.4)	0.147
Burning, %	55 (5.9)	16 (6.9)	39 (5.6)	0.446
Stinging, %	130 (13.9)	10 (4.3)	120 (17.1)	<0.001
Other, %	59 (6.3)	10 (4.3)	49 (7.0)	0.150
Unknown, %	176 (18.9)	53 (22.9)	123 (17.5)	0.069
Radiation				
Both arms, %	46 (4.9)	29 (12.6)	17 (2.4)	<0.001
Left arm, %	180 (19.3)	42 (18.2)	138 (19.7)	0.615
Right arm, %	12 (1.3)	2 (0.9)	10 (1.4)	0.512
Left shoulder, %	74 (7.9)	16 (6.9)	58 (8.3)	0.511
Right shoulder, %	24 (2.6)	9 (3.9)	15 (2.1)	0.144
Jaw, %	192 (20.6)	49 (21.2)	143 (20.4)	0.791
Epigastrium, %	17 (1.8)	4 (1.7)	13 (1.9)	0.904
Abdomen, %	4 (0.4)	2 (0.9)	2 (0.3)	0.242
Back, %	129 (13.8)	34 (14.7)	95 (13.6)	0.656
Lower extremity, %	16 (1.7)	2 (0.9)	14 (2.0)	0.251
Numbness upper extremity, %	91 (9.8)	23 (10.0)	68 (9.7)	0.909
Unknown, %	17 (1.8)	10 (4.3)	7 (1.0)	0.001
Additional symptoms				
Shortness of breath, %	370 (39.7)	89 (38.5)	281 (40.1)	0.675
Not stated, %	229 (24.6)	69 (30.0)	160 (22.8)	0.028
Nausea, %	205 (22.0)	45 (19.5)	160 (22.8)	0.287
Not stated, %	459 (49.2)	120 (52.2)	339 (48.3)	0.301
Vomiting, %	33 (3.5)	10 (4.3)	23 (3.3)	0.455
Not stated, %	552 (59.2)	143 (62.2)	409 (58.3)	0.295
Diaphoresis, %	132 (14.2)	36 (15.6)	96 (13.7)	0.475
Not stated, %	472 (50.6)	114 (49.6)	358 (51.0)	0.707
Palpitations	104 (11.2)	19 (8.2)	85 (12.1)	0.102
Not stated, %	768 (82.4)	196 (85.2)	572 (81.5)	0.197

Dizziness, %	141 (15.1)	22 (9.5)	119 (17.0)	0.006
Not stated, %	721 (77.4)	188 (81.7)	533 (75.9)	0.068
Dependent of stature, %	91 (9.8)	11 (4.8)	80 (11.4)	0.003
Not stated, %	803 (86.2)	210 (91.3)	593 (84.5)	0.009
Dependent of respiration, %	109 (11.7)	7 (3.0)	102 (14.6)	<0.001
Not stated, %	724 (77.7)	200 (87.0)	524 (74.6)	<0.001
Effect of nitroglycerin, %	179 (19.2)	70 (30.3)	109 (15.5)	<0.001
Not stated, %	655 (70.3)	139 (60.4)	516 (73.5)	<0.001
Pain upon palpation, %	115 (12.3)	14 (6.1)	101 (14.4)	0.001
Not stated, %	687 (73.7)	189 (82.2)	498 (70.9)	0.001
Symptom debut				
During physical activity, %	164 (17.6)	84 (36.4)	80 (11.4)	<0.001
After physical activity, %	61 (6.5)	16 (6.9)	45 (6.4)	0.787
During acute psychologic stress, %	24 (2.6)	2 (0.9)	22 (3.1)	0.059
Chronic psychologic stress, %	43 (4.6)	3 (1.3)	40 (5.7)	0.006
During rest	636 (68.5)	122 (53.0)	514 (73.2)	<0.001
Unknown, %	13 (1.4)	3 (1.3)	10 (1.4)	0.886
Pain duration				
<1 min, %	8 (0.9)	0 (0.0)	8 (1.1)	0.103
1-30 min	192 (20.6)	64 (27.7)	128 (18.3)	0.002
30-60 min	51 (5.5)	13 (5.6)	38 (5.4)	0.905
60 min - 24 hours	359 (38.5)	85 (37.0)	274 (39.0)	0.575
>24 hours	107 (11.5)	9 (3.9)	98 (14.0)	<0.001
Terminated by NGs, %	41 (4.4)	15 (6.5)	26 (3.7)	0.073
Terminated by morphine, %	10 (1.1)	3 (1.3)	7 (1.0)	0.701
Unknown, %	164 (17.6)	41 (17.7)	123 (17.5)	0.944

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Table S3. Characteristics of patients with a CV event (primary endpoint) missed by the ESC algorithms in combination with ACS low risk criteria

Age	Gender (male/ female)	Symptom duration, hours	TnT 0h	TnT 3h	TnT 8-12h	TnI 0h	TnI 3h	TnI 8-12h	History suspicious for CAD	Risk factors	Exami-nations	HEA-RT	T-MACS	TIMI	EDA-CS	Adj diagnosis	Endpoint	Missed by
77	M	2.2	9	13	12	7	15	17	Moderately	HTN, HC	Bicycle test, invasive angiography	4	0.034	1	23	UAP	Revasc.	ESC TnT and ESC TnI
67	M	1.8	6	20	6	4	21	4	Moderately	None	Invasive angiography	3	0.033	2	18	NSTEMI	NSTEMI	ESC TnI
76	F	2.5	3	6	3	2	7	4	Slightly	HTN	CCTA, invasive angiography	3	0.011	1	16	UAP	Revasc	All
48	M	97.7	3	3	3	2	2	2	Moderately	HC, smoking, heredity	Bicycle test, invasive angiography	4	0.045	2	14	UAP	Revasc	All
54	M	22.9	3	5	5	4	4	N/A	Highly	HTN, HC, obesity, heredity	Invasive angiography	5	0.025	3	12	UAP	Revasc	All
59	M	2.7	4	5	6	3	5	5	Moderately	HTN	CCTA, invasive angiography	3	0.012	0	14	UAP	Revasc (CABG)	All
68	F	11.8	5	5	5	5	2	3	Moderately	HTN, obesity, smoking, heredity	CCTA, angiography	5	0.013	3	6	UAP	Revasc	All
69	M	502.8	7	7	7	5	5	6	Highly	HTN, smoking	CCTA, invasive angiography	5	0.036	2	23	UAP	Revasc	All

67	M	173.8	6	6	7	3	4	3	Moderately	Smoking	CCTA, angiography	4	0.026	2	18	UAP	Revasc	All
75	F	5.1	6	7	5	5	5	6	Highly	HTN	Invasive angiography	5	0.026	2	13	UAP	Revasc	All
51	F	2.0	3	3	3	2	2	2	Highly	Smoking, heredity	CCTA, invasive angiography	4	0.011	0	11	UAP	Revasc	All
65	M	239.8	8	8	9	7	7	7	Moderately	HC	CCTA, invasive angiography	4	0.039	2	21	UAP	Revasc	All
48	M	4.7	21	14	8	12	10	4	Highly	None	Invasive angiography	4	0.114	2	10	NSTEMI	NSTEMI	ESC TnI and H-S TnI
69	M	215.4	16	13	11	9	10	6	Moderately	Smoking, heredity	CCTA, invasive angiography	6	0.034	2	23	UAP	Revasc	ESC TnI and H-S TnI
61	M	683.1	4	5	6	2	3	N/A	Highly	HTN, HC, smoking	CCTA, invasive angiography	5	0.012	1	16	UAP	Revasc	All
58	M	338.5	4	4	3	2	2	N/A	Slightly	None	CCTA, invasive angiography	1	0.012	1	14	UAP	Revasc	All
67	M	7.8	5	5	6	2	2	3	Moderately	HC	CCTA, invasive angiography	4	0.013	2	23	UAP	Revasc	All
66	M	26.9	11	10	11	3	4	4	Moderately	Smoking	CCTA, invasive angiography	4	0.151	1	21	UAP	Revasc	All
63	M	80.8	16	16	17	23	28	28	Moderately	HTN, heredity	CCTA, invasive angiography	5	0.034	3	10	UAP	Revasc (CABG)	ESC TnI

CAD means coronary artery disease; HTN: hypertension; HC: hypercholesterolemia; CCTA: coronary computed tomography angiography; UAP: unstable angina pectoris; NSTEMI: non-ST elevation myocardial infarction; ESC TnT: European society of cardiology Troponin T algorithm; ESC TnI: European society of cardiology Troponin I algorithm; H-S TnT: High-STEACS algorithm for TnT; H-S TnI: High-STEACS algorithm for TnI.

Table S4. Characteristics of patients with index NSTEMI (secondary endpoint) missed by the troponin-based algorithms.

Age	Gender	Symptom debut, hours	TnT 0h	TnT 3h	TnT 8-12h	TnI 0h	TnI 3h	TnI 8-12h	History suspicious for CAD	Risk factors	Exami-nations	HEA-RT	T-MACS	TIMI	EDACS	Adj Diagnosis	Procedure performed	Missed by
72	F	3.4	11	12	52	2	2	29	Slightly	DM, smoking, previous MI	Invasive angiography	4	0.151	2	17	NSTEMI	PCI	All
67	M	1.8	6	20	6	4	21	4	Moderately	None	Invasive angiography	3	0.033	2	18	NSTEMI	PCI	ESC TnI
65	F	73.9	4	71	37	2	80	82	Moderately	Previous PCI, heredity	Invasive angiography	6	0.028	3	15	NSTEMI	OMD	All
61	M	2.0	11	14	23	17	30	82	Moderately	HC, DM, previous PCI	Invasive angiography	5	0.050	3	16	NSTEMI	PCI	ESC TnT and ESC TnI
72	F	2.3	10	63	141	5	2	N/A	Moderately	HTN, HC	CCTA	5	0.065	1	14	NSTEMI	OMD	ESC TnI
78	M	6.8	23	51	72	14	213	255	Highly	HTN, DM, CABG	Invasive angiography	8	0.062	4	22	NSTEMI	OMD	ESC TnI
48	M	4.7	21	14	8	12	10	4	Highly	None	Invasive angiography	4	0.114	2	10	NSTEMI	PCI	ESC TnI and H-S TnI

CAD means coronary artery disease; HTN: hypertension; HC: hypercholesterolemia; CCTA: coronary computed tomography angiography; UAP: unstable angina pectoris; NSTEMI: non-ST elevation myocardial infarction; ESC TnT: European society of cardiology Troponin T algorithm; ESC TnI: European society of cardiology Troponin I algorithm; H-S TnT: High-STEACS algorithm for TnT; H-S TnI: High-STEACS algorithm for TnI.

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Diagnostic Performance of Novel Troponin Algorithms for the Rule-Out of Non-ST-Elevation Acute Coronary Syndrome

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BACKGROUND: The European Society of Cardiology (ESC) rule-out algorithms use cutoffs optimized for exclusion of non-ST elevation myocardial infarction (NSTEMI). We investigated these and several novel algorithms for the rule-out of non-ST elevation acute coronary syndrome (NSTE-ACS) including less urgent coronary ischemia.

METHOD: A total of 1504 unselected patients with suspected NSTE-ACS were included and divided into a derivation cohort (n ¼ 988) and validation cohort (n ¼ 516). The primary endpoint was the diagnostic performance to rule-out NSTEMI and unstable angina pectoris during index hospitalization. The secondary endpoint was combined MI, all-cause mortality (within 30 days) and urgent (24 h) revascularization. The ESC algorithms for high-sensitivity cardiac troponin T (hs-cTnT) and I (hs-cTnI) were compared to different novel low-baseline (limit of detection), low-delta (based on the assay analytical and biological variation), and 0–1-h and 0–3-h algorithms.

RESULTS: The prevalence of NSTE-ACS was 24.8%, 60.0% had noncardiac chest pain, and 15.2% other diseases. The 0–1/0–3-h algorithms had superior clinical sensitivity for the primary endpoint compared to the ESC algorithm (validation cohort); hs-cTnT: 95% vs 63%, and hs-cTnI: 87% vs 64%, respectively. Regarding the secondary endpoint, the algorithms had similar clinical sensitivity (100% vs 94%–96%) but lower clinical specificity (41%–19%) compared to the ESC algorithms (77%–74%). The rule-out rates decreased by a factor of 2–4.

CONCLUSION: Low concentration/low-delta troponin algorithms improve the clinical sensitivity for a combined endpoint of NSTEMI and unstable angina pectoris, with the cost of a substantial reduction in total rule-out rate. There was no clear benefit compared to ESC for diagnosing high-risk events.

Introduction

Atherosclerotic cardiovascular disease is an important health challenge and a common cause of death worldwide (1). Patients with symptoms suggestive of acute coronary syndrome are frequently referred to the emergency department (ED) and impose a high workload on hospitals (2, 3). Since 2009, high-sensitivity troponin (hs-cTn) assays have become a crucial ED tool for differentiating between patients with and without non-ST-elevation myocardial infarction (NSTEMI) (4, 5).

Accordingly, the European Society of Cardiology (ESC) recommends 0–1-h algorithms that use hs-cTn for rule-out and rule-in of NSTEMI (6). The algorithms for hs-cTnT from Roche Diagnostics and hs-cTnI from Abbott Diagnostics are fairly well validated, shown to be safe, and of high efficiency (7–10).

The ESC algorithms are based on 2 important characteristics found in healthy individuals: (*a*) normal baseline troponin concentrations a few hours after symptom onset, and (*b*) low-delta values after 1 h of observation. A drawback with these algorithms is that they were not developed to identify patients with unstable angina pectoris (UAP) (6). Accordingly, the 2020 ESC guidelines recommend the use of clinical judgment and

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imaging for identification of UAP (6), and the diagnostic workflow of this group is debated (11, 12).

The cutoffs in the ESC algorithms are pragmatically selected from research datasets. Earlier studies indicate that lower baseline concentrations than those used by the ESC 0–1-h algorithms may predict short- and long-term risk of major adverse cardiovascular events (MACE) in patients with chest pain (13–16). Furthermore, all consecutive biomarker measurements are subjected to uncertainty, due to biological variation (i.e., biomarkers measured in clinically stable individuals show homeostatic variation around a set point) and analytical variation. The combination of these variances is the reference change value (RCV) (17). The currently used ESC delta values exceed those calculated from RCV's (18). It is possible that patients with UAP, who have nonnecrotic ischemia and are in a clinically unstable situation, show larger variation in hs-cTn concentrations compared to patients with noncardiac chest pain (NCCP), who have a healthy myocardium and therefore should show troponin variation similar to or lower than the RCV (11, 19). Currently, it is unknown whether the use of delta values based on RCV could differentiate between patients with UAP and NCCP.

In this study we tested the hypothesis that the use of algorithms that combine very low baseline concentrations (similar to the limit of detection of the assay) with delta values derived from RCVs might improve the diagnostic performance for NSTE-ACS in the ED and also identify patients with UAP who have less urgent disease, and whether such algorithms could provide an improved segregation between patients with UAP and NCCP.

Methods

STUDY DESIGN

The WESTCOR study (Clinical Trials number NCT02620202) is a two-center cross-sectional prospective observational study that has been described in detail previously (15, 20). The current article reports data from the WESTCOR derivation and internal validation cohorts (as prespecified in the study protocol) including 988 and 516 patients from Haukeland University Hospital. The inclusion period lasted from September 2015 to May 2019. All patients in the validation cohort were offered computed tomographic coronary angiography unless contraindicated. The study and biobank were approved by the Regional Committees for Medical and Health Research Ethics (2014/1365 REK West and 2014/1905 REK West).

STUDY ENROLLMENT AND BIOBANKING

Patients were eligible for inclusion if they had chest pain or symptoms suspicious of NSTE-ACS. Patients with STEMI were excluded. Included patients were ≥18 years, did not have a coexisting clinical condition that would affect life expectancy, and were able to provide informed consent. The inclusion was performed in the ED (20) where the patients had 12 mL of full blood drawn into serum tubes (Greiner Bio-One, Austria) on arrival and after 3 h and 8–12 h as part of routine clinical care. Samples coagulated for 30–60 min and were centrifuged at 2200*g* for 10 min. Serum was used for measurement of hs-cTnT (fresh samples) with results reported to the attending clinician. Additional serum was aliquoted (1 mL) into cryotubes from Sarstedt (Sarstedt, Norway) and stored in a biobank at −80°C. After an implementation period, an additional biobank sample was drawn 1 h after admission without results being reported to the attending clinicians (20).

BIOCHEMICAL ANALYSIS

Details of the biochemical analyses are provided in the Methods in the online Data Supplement. Briefly, samples were measured for hs-cTnT (Roche Diagnostics) in fresh material using 9 different reagents and calibrator lots. Hs-cTnI were measured (biobanked samples) using the Abbott Diagnostics hs-cTnI assay using reagent lot 71164V100 and calibrator lot 65294V100 for the derivation cohort, and reagent lot 11151UI00 and calibrator lot 09906 UI00 for the validation cohort.

ENDPOINTS AND ADJUDICATION

The primary endpoint was a diagnosis of NSTEMI or UAP during index hospitalization. The secondary endpoints were MACE defined as combined myocardial infarction or all-cause mortality during the first 30 days after hospitalization or urgent (within 24 h after admission) revascularization. The adjudicating process (15, 20) was undertaken by 2 independent cardiologists (definitions provided in the Supplemental Methods) based on all available clinical, routine laboratory results (hs-cTnT), electrocardiogram (ECG), ultrasound, and imaging findings. A third adjudicator resolved disagreements. NSTE-ACS was defined as NSTEMI and UAP (21). NSTEMI and UAP was defined according to the third universal definition for MI (22). Delta values of 20% (baseline hs-cTnT concentration >14 ng/L) or 50% (baseline hs-cTnT concentration ≤14 ng/L) in serial hs-cTnT measures were regarded as clinically significant, as suggested by the ESC (23). UAP was defined as myocardial ischemia at rest or on minimal exertion, in the absence of acute myocardial injury/necrosis (21); a baseline concentration of hs-cTn above the 99th percentile of the assay did not exclude the patient from an UAP diagnosis if clinical assessment or imaging findings confirmed myocardial ischemia (11).

DEVELOPMENT OF NOVEL ALGORITHMS

As baseline concentration we chose the limit of detection of the assays (Supplemental Table 1), because these concentrations have been validated as rule-out cutoffs for admission samples (21), and are associated with low long-term risk of MACE (15, 24–26). The delta values were based on approximate RCV values for the hs-cTnT and hs-cTnI assays at low concentrations. Current assays have an analytical variation at low concentrations of approximately 61 ng/L (27–29). Biological variation studies have shown that the short time biological variation at low concentrations is negligible in clinically stable individuals, as compared to the analytical variation (18, 30). Accordingly, an absolute delta value of 61 ng/L or larger should be clinically sensitive for identification of minor but clinically significant variations in troponin concentrations, as could be evident in patients with UAP (18, 31).

Furthermore, from a clinical point of view the optimal novel rule-out algorithms should have: (*a*) clinical sensitivity for NSTE-ACS of 2:95.0% and 2:99% for the secondary endpoint (32), and (*b*) the maximum possible specificity. The cutoff for the primary endpoint was chosen a priori as there was no literature reporting cardiologists view on an acceptable rule-out rate for patients with UAP.

COMPARATOR ALGORITHMS

The novel algorithms were compared to the recently updated 0–1-h algorithms for rule-out of NSTEMI from the ESC. Accordingly, patients were eligible for early discharge if the baseline concentration (cTnT < 12 ng/L or cTnI < 5 ng/L) and the 1-h delta value (cTnT < 63 ng/L and cTnI < 62 ng/L) was below the prespecified concentration specific for the applicable troponin assay (Supplemental Table 1).

STATISTICAL ANALYSIS

The baseline characteristics are reported as medians with interquartile ranges for continuous data and percentages for categorical data. The data were analyzed using the nonparametric Kruskal–Wallis and Mann–Whitney *U*-test for continuous variables, and the Chi-square and Fisher's exact test for categorical variables, as appropriate. Statistical analyses included calculation of clinical sensitivity, specificity, negative predictive value, and positive predictive value for the cutoffs used in the different algorithms. Differences in sensitivity and specificity between algorithms were compared using McNemar test. Efficiency (defined as percentage of patients ruled out) was calculated for all algorithms. Prognosis regarding MACE (secondary endpoint) were estimated using Kaplan–Meier curves. We performed one subgroup analysis calculating the diagnostic performance of the 2 endpoints in early presenters (defined as S3h since onset of symptoms). A second subgroup analysis compared the baseline and delta values, and calculated the rule-out rate in the two patient groups that are of large clinical interest to separate, i.e., the patients with UAP and NCCP. Investigations during index hospitalization, and 30-day all-cause mortality, myocardial infarction, or revascularization were calculated for all patients with NSTE-ACS and after stratifying as NSTEMI and UAP (index diagnosis), and furthermore, as shown for patients with UAP who were ruled out by the ESC or the novel 0–3-h algorithm, differences were tested using the McNemar test.

We used SPSS Statistics v.24/26 and MedCalc for the statistical analyses.

Results

Biobank admission samples were available from 1504 patients, and a 1-h sample was available from 984 patients (n ¼ 479 in the derivation and n ¼ 505 in the validation cohort).

Patient characteristics for the derivation and validation cohort are shown in Table 1. The prevalence of NSTE-ACS in the derivation cohort (n ¼ 988) was 24.8%, while 60.0% were diagnosed with NCCP, and 15.2% had other diseases. Other diseases included non-cardiac diseases such as pneumonia or cholecystitis, and other cardiac diseases such as atrial fibrillation or heart failure. Median age was 63 years, and 60% were male. The validation group (n ¼ 516) had a prevalence of NSTE-ACS of 25.8%, NCCP was diagnosed in 62.9% and 11.4% had other diseases and similar median age and percentage of males. The prevalence of NSTEMI was lower (13.2% vs 8.7%) (Table 1). Less than 7% of NSTEMIs were type 2 NSTEMI.

BASELINE CONCENTRATIONS, AND 1- AND 3-HOUR ABSOLUTE DELTA VALUES

Table 2 shows troponin concentrations at baseline, and the absolute delta values at 1 h and 3 h stratified according to the adjudicated diagnosis. The baseline concentrations were similar across cohorts for hs-cTnT (samples were analyzed continuously using 9 different reagent and calibrator lots), while the hs-cTnI baseline concentrations were significantly lower in the validation compared to the derivation cohort for all diagnoses except NSTEMI (Supplemental Table 2). This was due to samples being analyzed in batches, using one reagent/calibrator lot for each cohort, with the last lot returning lower concentrations.

The patients with UAP had significantly higher ($P < 0.001$) baseline hs-cTnT and hs-cTnI concentrations (Table 2) and delta values compared to the patients with NCCP (Fig. 1 and Supplemental Table 3).

Table 1. Patient characteristics. Values are *N* (%) or median (25th and 75th percentiles).

Derivation cohort					
	Total *N* = 988	NSTE-ACS *N* = 242	Other diseases *N* = 156	NCCP *N* = 590	*P* value
Age, years	63.0 (52.0-74.0)	69.5 (59.0-78.0)	70.0 (58.0-80.0)	59.0 (49.0-70.0)	<0.001
Male, %	600 (60.7)	172 (71.1)	94 (60.3)	334 (56.6)	0.001
Symptom to arrival time, hours	8.0 (2.9-47.8)	8.2 (2.8-48.8)	8.6 (3.5-53.8)	7.4 (2.9-46.2)	0.539
Hospital stay, hours	29.0 (21.0-69.0)	73.5 (49.8-117.3)	43.5 (24.0-86.5)	24.0 (19.0-35.0)	<0.001
Risk factors					
Hypertension, %	413 (41.8)	124 (51.2)	66 (42.3)	223 (37.8)	0.002
Hypercholesterolemia* %	394 (39.9)	121 (50.0)	63 (40.4)	210 (35.6)	0.001
Diabetes mellitus, %	121 (12.4)	51 (21.1)	16 (10.3)	54 (9.2)	<0.001
Family history, %	195 (19.7)	45 (18.6)	25 (16.0)	125 (21.2)	0.468
Unknown	121 (12.1)	35 (14.1)	17 (10.7)	69 (11.6)	0.507
Ever smoker, %	628 (63.6)	145 (59.9)	102 (65.4)	381 (64.6)	0.392
Medical history					
Prior MI, %	211 (21.4)	77 (31.8)	34 (21.8)	100 (16.9)	<0.001
Prior PCI, %	209 (21.2)	82 (33.9)	27 (17.3)	100 (16.9)	<0.001
Prior CABG, %	83 (8.4)	45 (18.6)	12 (7.7)	26 (4.4)	<0.001
Heart failure, %	47 (4.7)	15 (6.0)	14 (8.8)	18 (3.0)	0.005
Stroke, %	30 (3.0)	9 (3.7)	7 (4.5)	14 (2.4)	0.254
Peripheral vascular disease, %	22 (2.2)	11 (4.5)	2 (1.3)	9 (1.5)	0.027
Vital parameters at admission					
Systolic BP, mmHg	142.5 (129.0-158.0)	147.0 (133.0-160.0)	133.0 (122.3-154.8)	142.0 (129.0-158.0)	<0.001
Diastolic BP, mmHg	81.0 (73.0-91.0)	81.0 (74.0-90.8.0)	80.0 (72.3-91.0)	82.0 (74.5-90.0)	0.326
Heart rate, bpm	72.0 (64.0-83.0)	72.0 (64.0-84.0)	82.0 (66.3-100.0)	70.0 (63.8-80.0.0)	<0.001
BMI, kg/m^2 for 461 patients	26.4 (24.2-29.5)	25.9 (24.2-29.1)	27.2 (25.5-29.1)	26.3 (24.1-29.7)	0.259
Electrocardiography					
ST segment depression, %	34 (3.4)	21 (8.7)	7 (4.5)	6 (1.0)	<0.001
T-wave inversion, %	31 (3.1)	16 (6.6)	5 (3.2)	10 (1.7)	0.002
Validation cohort					
	Total *N* = 516	NSTE-ACS *N* = 133	Other diseases *N* = 58	NCCP *N* = 325	*P* value
Age, years	60.0 (51.0-70.0)	66.0 (57.0-74)	65.0 (56.0-72.5)	56.0 (47.0-67.0)	<0.001
Male, %	308 (59.7)	91 (68.4)	33 (56.9)	184 (56.4)	0.048
Symptom to arrival time, hours	11.4 (3.5-71.8)	9.9 (3.1-81.5)	15.0 (4.7-77.5)	11.5 (3.8-71.4)	0.588
Hospital stay, hours	27.0 (22.0-69.0)	73.0 (48-143.0)	33.5 (22.0-70.8)	24.0 (21.0-30.0)	<0.001
Risk factors					

Continued

Table 1. (continued)

Derivation cohort					
Hypertension, %	202 (39.1)	70 (52.2)	23 (41.8)	109.0 (34.0)	<0.001
Hypercholesterolemia*, %	191 (37.0)	66 (49.6)	21 (36.2)	104 (32.0)	0.002
Diabetes mellitus, %	60 (11.6)	26 (19.5)	8 (13.8)	26 (8.0)	0.002
Family history, %	80 (15.5)	21 (15.8)	8 (13.8)	51 (15.7)	0.469
Unknown	21 (4.1)	9 (6.3)	2 (3.4)	10 (3.1)	0.469
Ever smoker, %	312 (60.5)	87 (64.9)	31 (54.4)	196 (60.1)	0.368
Medical history					
Prior MI, %	78 (15.1)	30 (22.6)	8 (13.8)	40 (12.3)	0.020
Prior PCI, %	84 (16.3)	37 (27.6)	6 (10.3)	41 (12.6)	<0.001
Prior CABG, %	28 (5.4)	17 (12.7)	4 (6.9)	7 (2.2)	<0.001
Heart failure, %	5 (1.0)	1 (0.8)	0	4 (1.2)	0.649
Stroke, %	12 (2.3)	6 (4.5)	1 (1.7)	5 (1.5)	0.151
Peripheral vascular disease, %	7 (1.4)	5 (3.7)	0	2 (0.6)	0.020
Vital parameters at admission					
Systolic BP, mmHg	147.0 (134.0-161.0)	148.0 (136.0-161.5)	149.0 (128.5-167.3)	147.0 (133.0-161.0)	0.666
Diastolic BP, mmHg	86.0 (78.0-95.0)	85.0 (77.5-96.0)	90.0 (82.0-98.3)	85.0 (78.0-94.0)	0.113
Heart rate, bpm	71.0 (63.0-81.0)	72.0 (63.5-81.0)	74.0 (61.0-87.3)	70.0 (63.0-80.0)	0.361
BMI, kg/m^2 for 281 patients	27.7 (25.0-31.1)	27.7(24.8-30.9)	29.1 (25.2-31.4)	27.5 (25.1-31.2)	0.797
Electrocardiography					
ST segment depression, %	13 (2.5)	8 (6.0)	0	5 (1.5)	0.019
T-wave inversion, %	16 (3.1)	11 (8.3)	3 (5.2)	2 (0.6)	<0.001

*Hypercholesterolemia is defined as treatment with lipid lowering drugs.
NSTE-ACS, non-ST-elevation acute coronary syndrome; NCCP, noncoronary chest pain; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft.

DIAGNOSTIC PERFORMANCE OF THE NOVEL AND ESC ALGORITHMS FOR NSTE-ACS AND MACE

Overall, the low concentration/low-delta value algorithms showed superior clinical sensitivity for the primary endpoint (NSTEMI or UAP) compared to the ESC algorithms (Table 3). In the validation cohort, the novel hs-cTnT 0–1-h and 0–3-h algorithms had clinical sensitivities of 95.4% and 97.5%, respectively, compared to the significantly lower 62.8% for the ESC 0–1-h algorithm ($P < 0.001$). This was at the expense of significantly lower clinical specificity ($P < 0.001$), the algorithms showed up to a 4.2× reduction in rule-out rate compared to the ESC 0–1-h algorithm (Table 3).

The findings were less clear for the novel hs-cTnI algorithms. The 95% clinical sensitivity criterion was not met in the validation cohort, with a clinical sensitivity of 86.9% (0–1-h algorithm) and 87.6% (0–3-h algorithm). This cohort was analyzed using a reagent/calibrator lot measuring overall lower hs-cTnI concentrations compared to the derivation cohort (Table 2). The ESC 0–1-h hs-TnI algorithm had a significantly lower clinical sensitivity of 63.9% ($P < 0.001$). Again, the novel algorithms showed less efficacy, and the rule-out rate was reduced by a factor of 1.8.

The low concentration/low-delta value algorithms did not show any clear advantage compared to the ESC algorithms for the secondary endpoint [MI or all-cause mortality within 30 days or urgent (24 h) revascularization] (Table 4, Supplemental Fig. 1). The clinical sensitivity of the novel algorithms was similar to the ESC (100% vs 94%–96%), but the clinical specificity was substantially lower compared to ESC, reducing overall diagnostic efficiency.

The analysis in early presenters showed similar but overall slightly lower clinical sensitivity for all algorithms (Supplemental Table 4, A), and the novel 0–3-h algorithm for cTnT was the only one fulfilling the 95% clinical sensitivity criterion. Again, this was at the expense of significantly lower specificity, where the novel 0–1-h algorithms showed a 2–6× reduction

Table 2. Troponin concentrations (ng/L), median, and 25th and 75th percentiles. D; derivation cohort. V; validation cohort.

	NSTEMI	UAP	Other diseases	NCCP	*P* value
Baseline concentrations					
hs-cTnT$_D$	48.0 (22.8-172.0)	9.0 (5.0-18.0)	13.0 (5.8-24.0)	5.0 (3.0-9.0)	<0.001
hs-cTnT$_V$	56.5 (23.0-161.5)	9.0 (6.0-17.0)	10.5 (5.8-16.3)	5.0 (3.0-8.0)	<0.001
hs-cTnI$_D$	118.9 (26.5-560.1)	4.7 (3.1-9.9)	8.1 (3.2-17.7)	2.7 (1.7-5.2)	<0.001
hs-cTnI$_V$	102.2 (28.2-578.3)	3.3 (1.7-9.3)	3.6 (1.4-10.6)	1.5 (0.8-3.1)	<0.001
Absolute 1-h delta					
hs-cTnT$_D$	12.5 (6.0-28.3)	1.0 (0-1.0)	1.0 (0-2.0)	0 (0-1.0)	<0.001
hs-cTnT$_V$	8.0 (2.4-22.5)	0.7 (0.1-1.0)	0.7 (0-1.0)	0 (0-1.0)	<0.001
hs-cTnI$_D$	72.5 (17.8-261.3)	0.6 (0.2-1.4)	0.6 (0-1.9)	0.4 (0.1-0.7)	<0.001
hs-cTnI$_V$	37.5 (10.4-132.7)	0.9 (0.3-2.3)	0.7 (0.3-1.8)	0.5 (0.2-1.2)	<0.001
Absolute 3-h delta					
hs-cTnT$_D$	47.5 (14.0-142.3)	1.0 (0-2.0)	1.0 (0-3.0)	0 (0-1.0)	<0.001
hs-cTnT$_V$	23.0 (6.0-90.0)	1.0 (0-2.0)	1.0 (0-2.0)	0 (0-1.0)	<0.001
hs-cTnI$_D$	315.8 (47.2-1360.0)	0.8 (0.4-1.8)	1.6 (0.4-4.4)	0.6 (0.2-1.2)	<0.001
hs-cTnI$_V$	59.5 (15.6-489.3)	0.9 (0.2-2.7)	1.1 (0.2-1.9)	0.8 (0.3-1.6)	<0.001

NSTEMI, non-ST elevation myocardial infarction; UAP, unstable angina pectoris; NCCP, noncoronary chest pain.

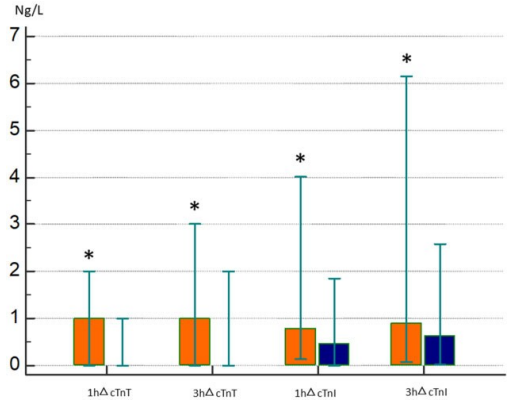

Figure 1. Absolute delta values (ng/L) for hs-cTnT and hs-cTnI in patients with unstable angina pectoris (orange) and noncardiac chest pain (no color/blue) in the total cohort. The bars show median values, poles show the 10th and 90th percentiles. Note that the median value for hs-cTnT deltas in noncardiac chest pain patients was 0 ng/L, similar to the 10th percentile and is therefore shown without color. **P* value <0.001. See color figure online at clinchem.org.

in rule-out rate compared to the ESC 0–1-h algorithms. The novel algorithms showed no benefit regarding the secondary high-risk endpoint (Supplemental Table 4, B).

RULE-OUT RATES FOR THE DIFFERENT ALGORITHMS

Patients were stratified according to index diagnosis and the number being ruled out by the different algorithms were calculated (Supplemental Table 5). All patients with

Table 3. Diagnostic performance (95% confidence intervals) and efficacy (total rule-out, percentages in brackets) for the primary endpoint combining NSTEMI and UAP during index hospitalization for the different algorithms. European Society of Cardiology algorithms are shown on a gray background.

	Sensitivity	NPV	Specificity	PPV	Rule-out rate
1-h algorithms					
hs-cTnT <5 ng/L and $D_{0\text{-}1h}$ <1 ng/L					
Derivation cohort *N* ¼ 479	95.8 (90.5-98.6)	95.7 (90.2-98.1)	30.6 (25.8- 35.6)	31.3 (29.7-33.0)	115 (24.0)
Validation cohort *N* ¼ 505	95.4 (90.2-98.3)	92.9 (85.5-96.7)	21.0 (17.0-25.5)	29.3 (28.0-30.6)	85 (16.8)
hs-cTnT <12 ng/L and $D_{0\text{-}1h}$ <3 ng/L					
Derivation cohort *N* ¼ 479	71.4 (62.7-79.7)	89.0 (85.8-91.5)	76.4 (71.7-80.7)	50.0 (44.6-55.4)	309 (64.5)
Validation cohort *N* ¼ 505	62.8 (53.8-71.1)	86.5 (83.6-88.9)	81.7 (77.4-85.4)	54 (47.7-60.2)	355 (70.3)
hs-cTnI <2 ng/L and $D_{0\text{-}1h}$ <1 ng/L					
Derivation cohort *N* ¼ 474	93.3 (87.2- 97.1)	92.7 (86.4- 96.2)	28.5 (23.8-33.5)	30.4 (28.7-.32.2)	109 (23.0)
Validation cohort *N* ¼ 507	86.9 (79.9-92.2)	90.9 (86.4-94.1)	45.1 (40.0-50.3)	35.3 (32.8-37.9)	187 (36.8)
hs-cTnI <5 ng/L and $D_{0\text{-}1h}$ <2 ng/L					
Derivation cohort *N* ¼ 474	72.3 (63.3-80.1)	87.7 (84.1-90.6)	66.5 (61.3-71.4)	42.0 (37.6-46.5)	269 (56.0)
Validation cohort *N* ¼ 507	63.9 (55.0-72.1)	86.3 (83.3-88.9)	78.5 (74.0-82.6)	50.6 (44.8-56.4)	343 (67.7)
3-h algorithms					
hs-cTnT <5 ng/L and $D_{0\text{-}3h}$ <1 ng/L					
Derivation cohort *N* ¼ 982	96.7 (93.6- 98.6)	96.5 (93.3 - 98.2)	30.0 (26.7-33.4)	31.1 (30.0- 32.3)	230 (23.4)
Validation cohort *N* ¼ 482	97.5 (92.9-99.5)	97.2 (91.9-99.1)	29.1 (24.5-34.1)	31.6 (30.0-33.1)	108 (22.4)
hs-cTnI <2 ng/L and $D_{0\text{-}3h}$ <1 ng/L					
Derivation cohort *N* ¼ 936	95.7 (92.2-97.9)	94.9 (91.0-97.2)	26.6 (23.3-30.0)	30.0 (28.9-31.2)	197 (20.2)
Validation cohort *N* ¼ 483	87.6 (80.4-92.9)	90.3 (85.1-93.9)	38.6 (32.4-42.5)	32.3 (30.1-34.7)	155 (32.1)

NPV, negative predictive value; PPV, positive predictive value; NSTEMI, non-ST elevation myocardial infarction; UAP, unstable angina pectoris.

NSTE-ACS who were ruled out were patients with UAP. A detailed description of patients missed for the secondary endpoint is given in the Supplemental Results.

The subgroup analysis undertaken in patients with UAP and NCCP (combining both cohorts), indicated better identification of UAP by the 0–3-h compared to the 0–1-h algorithms (Fig. 2). Overall, 6% of patients with UAP would be ruled out if the low-delta 0–3-h hs-cTnT algorithm was used, with a simultaneously rule-out rate >34% in patients with NCCP. Somewhat higher rule-out rates of approximately 13% (UAP) and 35% (NCCP), respectively, were shown for the hs-cTnI 0–3-h algorithm. Corresponding rates for the 0–1-h ESC algorithms were significantly higher; 56% (cTnT) and 55% (cTnI) for UAP patients, and 85% (cTnT) and 79% (cTnI) for the patients with NCCP. Results were overall similar when analyzed separately in the derivation and validation cohort (Supplemental Table 6).

INVESTIGATIONS, REVASCULARIZATIONS, AND 30-DAYS FOLLOW UP IN THE NSTE-ACS GROUP

The number of investigations, urgent revascularizations (24 h), 30-day MIs, all-cause mortality, and revascularizations for the patients with NSTE-ACS and stratified as

Table 4. Diagnostic performance (95% confidence intervals) and efficacy (total rule-out, percentages in brackets) for the combined secondary endpoint of MACE defined as 30 days MI, 30 days all-cause mortality, or urgent (24 h) revascularization, for the different algorithms. ESC algorithms are shown on a gray background.

	Sensitivity	NPV	Specificity	PPV	Rule-out rate
1-h algorithms					
hs-cTnT <5 ng/L and $D_{0\text{-}1h}$ <1 ng/L					
Derivation cohort *N* ¼ 479	100.0 (94.6-100.0)	100	27.9 (23.6-32.4)	18.1 (17.3-19.4)	115 (24.0)
Validation cohort *N* ¼ 505	100.0 (92.5-100.0)	100	18.6 (15.1-22.4)	11.2 (10.8-11.6)	85 (16.8)
hs-cTnT <12 ng/L and $D_{0\text{-}1h}$ <3 ng/L					
Derivation cohort *N* ¼ 479	100.0 (94.6-100.0)	100	74.8 (70.3-78.9)	38.8 (34.9-42.9)	309 (64.5)
Validation cohort *N* ¼ 505	93.6 (82.5-98.7)	99.2 (97.2-100.0)	77.0 (72.7-80.6)	29.3 (25.7-33.1)	355 (70.3)
hs-cTnI <2 ng/L and $D_{0\text{-}1h}$ <1 ng/L					
Derivation cohort *N* ¼ 474	100.0 (94.6-100.0)	100	26.7 (22.5-31.3)	18.1 (17.2-19.0)	109 (23.0)
Validation cohort *N* ¼ 507	100.0 (92.6-100.0)	100	40.7 (36.2-45.4)	15.6 (14.1-16.0)	187 (36.8)
hs-cTnI <5 ng/L and $D_{0\text{-}1h}$ <2 ng/L					
Derivation cohort *N* ¼ 474	100.0 (94.6-100.0)	100	65.9 (61.0-70.5)	32.2 (29.3-35.3)	269 (56.0)
Validation cohort *N* ¼ 507	95.8 (85.8-99.5)	99.4 (97.8-99.9)	74.3 (70.0-78.2)	28.1 (24.8-31.5)	343 (67.7)
3-h algorithms					
hs-cTnT <5 ng/L and $D_{0\text{-}3h}$ <1 ng/L					
Derivation cohort *N* ¼ 982	100.0 (97.5-100.0)	100	27.4 (24.4-30.6)	19.0 (18.4-19.7)	230 (23.4)
Validation cohort *N* ¼ 482	100.0 (92.5-100.0)	100	24.8 (20.8-29.2)	12.6 (12.0-13.2)	108 (22.4)
hs-cTnI <2 ng/L and $D_{0\text{-}3h}$ <1 ng/L					
Derivation cohort *N* ¼ 936	100.0 (97.3-100.0)	100	24.6 (21.7-27.8)	18.4 (17.8-19.0)	197 (20.2)
Validation cohort *N* ¼ 483	100.0 (92.5-100.0)	100	35.6 (31.1-40.2)	14.3 (13.5-15.2)	155 (32.1)

NPV, negative predictive value; PPV, positive predictive value.

NSTEMI and UAP are shown in Supplemental Tables 7 and 8, which show the same variables in the subgroup of patients with UAP who were ruled out by the ESC and the most sensitive of the novel algorithms (0–3 h). None of the ruled-out patients died or experienced an MI within 30 days (Supplemental Results), although a significantly higher proportion of patients who needed revascularization within 30 days were ruled out by the ESC algorithms ($P < 0.001$).

Discussion

Our study has several important findings. First, the use of algorithms combining a low baseline concentration with delta values derived from RCVs may improve the segregation between patients with UAP and NCCP and avoid rule-out of patients who need a recent revascularization. This was particularly clear for algorithms developed for the hs-cTnT assay. Second, the timing of the sampling seems important, as 0–3-h algorithms performed overall better compared to 0–1-h algorithms. Third, reagent or calibrator lots that return lower concentrations may change the overall diagnostic performance of algorithms using low concentrations and deltas, as was demonstrated for the hs-cTnI assay. Fourth, compared to the ESC algorithms, the novel algorithms showed a substantial reduction in patients eligible for rule-out. Last, all

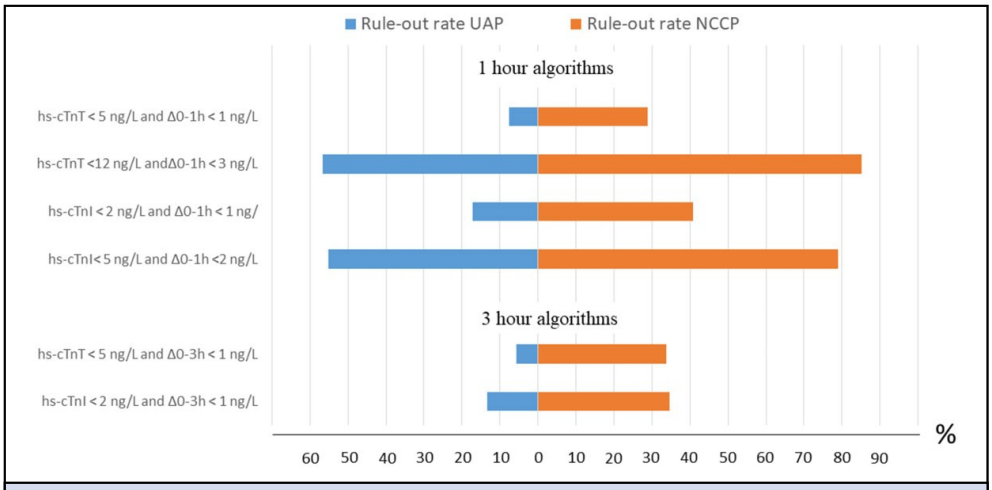

Figure 2. Percentage rule-out for patients with unstable angina pectoris (UAP) and noncardiac chest pain (NCCP) in the total cohort. See color figure online at clinchem.org.

evaluated algorithms showed similar good prognosis for a combined endpoint of 30-day all-cause mortality and MI or urgent (24 h) revascularization.

The most recent guideline from the ESC stress that even if patients are ruled out for NSTEMI, they still may have UAP and may require follow up or treatment within a recent time frame (6). Our data show that the sensitivity for less urgent NSTE-ACS could be increased from approximately 60% to 87%–95%, if the cutoffs applied are based on baseline and delta values that are derived from individuals without apparent underlying myocardial disease. Patients with UAP have increased risk of death and cardiovascular events (11, 19) and revascularization reduces symptom burden and improve quality of life (33). The prognosis is still far better compared to patients with NSTEMI and it is uncertain if rule-out of patients with UAP compromises patient safely as long as invasive treatment is offered during outpatient follow up. It should be noted that the rule-out rate for some of the novel algorithms was as low as 17% (0–1-h cTnT) compared to 60% for the cTnT ESC algorithm (10). This is an important drawback. EDs that have implemented the ESC algorithms may find the novel approach to conservative allocating too many patients to the observational zone. The rule-out rate was somewhat better in the NCCP subgroup, correctly ruling out around 30%–40% of patients with NCCP. Accordingly, the novel algorithms may be useful in EDs that aim to reduce low risk admissions but need high "safety margins" and hospitalize patients with less urgent NSTE-ACS, e.g., UAP.

Future studies, including long-term outcomes, are needed to conclude whether the low concentration/low-delta algorithms identify a subpopulation within the NCCP cohort who may be safely discharged (16).

Our study used hs-cTn delta values that were based on RCV values to identify patients with UAP, who by definition have "stable" troponin concentrations (6). It is biologically plausible that troponin concentrations are slightly increased and/or show larger variations in this group compared to participants who have a completely stable myocardial perfusion (11, 19, 34). Indeed, a recent publication demonstrated that hs-cTn concentrations increased (time dependent) when reversible myocardial ischemia was induced by a 30–90 s balloon occlusion of the left anterior descending coronary artery (35). Patients with UAP had higher baseline concentrations, indicative of a situation of low-grade chronic or acute myocardial injury, combined with larger delta values, consistent with intermittent myocardial leakage of troponins (35). The observation that 3-h deltas separated better between UAP and NCCP, compared to 1-h deltas, strengthens this assumption. It should be noted that our NSTE-ACS cohort had an overall time from symptom onset to first sampling of 8–10 h. The subgroup analysis showed lower sensitivity in patients with NSTE-ACS with S3 h since onset of symptoms, and usability in this group is uncertain. Overall, if confirmed

in other studies, our data could have consequences for the logistics in the ED, including duration of observation. Future assays with lower analytical variation could have potential for even further improved diagnostic differentiation between patients with UAP and NCCP.

Finally, our data demonstrate how the analytical performance of the assays may influence the diagnostic performance of rule-out algorithms (29). We used 2 different lots of the hs-cTnI assay, 1 in the derivation and 1 in the validation cohort. The lot used in the validation cohort returned lower troponin results (Supplemental Table 2). Consequently, more patients with NSTE-ACS showed concentrations below the limit of detection, resulting in higher rule-out of patients with UAP in this cohort (Supplemental Table 6). The patients with NCCP in the validation cohort also experienced larger delta values, similar to those observed in patients with UAP (Table 2 and Supplemental Table 3), likewise due to more measurements being done at the lowest concentrations (higher analytical variability). In sum, this led to an overall lower diagnostic performance for the cTnI algorithms in the validation cohort (Table 3). Similar systematic evaluation of lot variations could not be done for hs-cTnT because measurements were done on fresh samples during the whole inclusion period, using a larger number of reagent and calibrator lots in both cohorts. The current observations highlight the need of robust validation of algorithms, using several different clinical cohorts and reagent and calibrator lots, before implementation into clinical practice; this calls for laboratories to monitor lot variations closely, and for manufacturers to strive to reduce such variations and develop assays with incremental analytical performance.

STRENGTH AND LIMITATIONS

The study has several strengths. The inclusion criteria are broad, mimicking real-life practice. The study encompassed a derivation and a validation cohort, and evaluated 2 different high-sensitivity troponin assays. The derivation and validation cohort were slightly divergent. This should not affect the clinical sensitivity and specificity of algorithms and the diagnostic performances for hs-cTnT were similar across cohorts, in line with this assumption. The difference observed between cohorts for hs-cTnI is explained by lot variations, as outlined previously.

Our data lack validation in an external cohort; this is a limitation and our findings should therefore be seen as hypothesis generating. Another important limitation in our study is that not all eligible patients with chest pain were included, an important reason for the NSTEMI incidence being lower in the validation compared to the derivation cohort. This was due to logistical problems in the ED, a common problem in this kind of study. Even so, the NSTE-ACS incidence was similar across cohorts and the patient characteristics were also similar to other comparable studies (36, 37). It should be noted that the adjudication was based on routine hs-cTnT measurements, which could positively bias the results for the hs-cTnT algorithms. The use of all-cause mortality instead of cardiovascular mortality as an endpoint may underestimate the performance of the algorithms. Our NSTEMI adjudication was based on the third definition of MI, since this is very similar to the fourth definition it is unlikely to affect results. Finally, the clinical sensitivity was lower in early presenters, questioning the applicability in this group. The cohort of early presenters is quite small and further validation is necessary.

Conclusion

The current study shows that troponin algorithms using low baseline concentrations and delta values show improved clinical sensitivity for NSTE-ACS by improved differentiation between patients with UAP and NCCP. A major drawback was that the overall rule-out rate of patients investigated for NSTE-ACS was reduced with a factor of 2–4 compared to the ESC algorithms, which is substantial and may result in a less efficient patient flow through the ED. Our study demonstrates that timing of samples, lot variations, and analytical variability may substantially influence the diagnostic performance of rule-out algorithms that encompass low hs-cTn concentrations and deltas. This study demonstrates that high-sensitivity assays could play a role in identifying patients with UAP and NCCP in the ED, and that even further improvement of the analytical performance of troponin assays may have a clear clinical benefit.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard Abbreviations: ESC, European Society of Cardiology; NSTEMI, non-ST-elevation myocardial infarction; NSTE-ACS, non-ST-elevation acute coronary syndrome; hs-cTnT, high-sensitivity cardiac troponin T; hs-cTnI, high-sensitivity cardiac troponin I; UAP, unstable angina pectoris; NCCP, noncoronary chest pain; ED, emergency department; MACE, major cardiovascular events; RCV, reference change value; CVA, coefficient of variation; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; ECG, electrocardiogram.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design,*

acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

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SUPPLEMENTAL METHODS PAPER 4

Biochemical analysis

All samples were centrifuged after 30 min, and material for the biobank was aliquoted and frozen at -80ºC. Routine and 1-h samples were measured for hs-cTnT (Roche Diagnostics) with limit of blank of 3 ng/L, limit of detection of 5 ng/L, 99th percentile of 14 ng/L and measurement range of 4 – 10 000 ng/L *(1)*. The 10% analytical within-series coefficient of variation (CV_A) was at 4.5 ng/L, with CV_A <5% for concentrations 10 ng/L or higher. The analysis was done continuously on fresh material using 9 different reagents and calibrator lots. For hs-cTnI, biobanked samples were measured using the Abbott Diagnostics hs-cTnI assay. The assay has a limit of blank of 0.9 ng/L, limit of detection of 1.7 ng/L, and 99th percentile of 26 ng/L *(1)*. The measurement range was 2-50 000 ng/L and the 10% CV_A was 4.6 ng/L. The CV_A was <4% for concentrations above 15 ng/L. The analysis was done using reagent lot 71164V100 and calibrator lot 65294V100 for the derivation cohort, and reagent lot 11151UI00 and calibrator lot 09906 UI00 for the validation cohort. The glomerular filtration rate was estimated using the Chronic Kidney Disease Epidemiology Collaboration formula using an enzymatic isotope dilution-mass spectrometry traceable creatinine assay (Roche Diagnostics) with a CV_A <3% for concentration above 60 µmol/L.

Diagnostic definitions

Myocardial infarction was defined according to the third universal definition of myocardial infarction *(2)*.

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 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

•Identification of an intracoronary thrombus by angiography or autopsy

•Imaging evidence of new loss of viable myocardium or a new regional wall motion abnormality

Prior myocardial infarction was defined by Q waves or QS complexes in the absence of QRS confounders in patients with ischemic heart disease regardless of symptoms *(2)*

Unstable angina pectoris (UAP) was defined as symptoms suggestive of an ACS without elevation in biomarkers with or without ECG changes indicative of ischemia *(3)*.

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 *(4)*.

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 *(5)*.

Myocarditis was diagnosed according to the ESC's 2013 position statement *(6)*.

Takotsubo cardiomyopathy was diagnosed with the modified criteria suggested by The Mayo Clinic in 2008 *(7)*.

Heart failure was defined according to the 2016 ESC diagnostic criteria *(8)*.

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 were diagnosed in accordance with echocardiographic results and a history supporting the valve disease as cause of the symptoms *(9)*.

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

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

Cholecystitis was defined by the Tokyo Guidelines of 2006 while other abdominal diseases where defined according to operative, endoscopic or radiological findings *(10)*.

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

Chronic obstructive pulmonary disease was defined in accordance with the 2008 criteria of Stephens et al *(11)*, while chest pain without any specific clinical, radiologic or biochemical findings where defined as non-specific chest pain.

Definition of risk factors

Diabetes was defined by the use of insulin, oral antidiabetic, or diet to lower the concentration of blood glucose.

Hypertension was based on the use of antihypertensive medication.

Hypercholesterolemia was defined by the use of statin or other lipid-lowering drugs.

Chronic kidney disease was defined as an estimated glomerular filtration rate <60 mL/min/1.73 m^2.

Family history of cardiovascular disease was defined as cardiovascular disease in first-degree relatives, before 55 y of age in men and 65 y of age in women.

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Supplemental results paper 4

Diagnostic performance of novel troponin algorithms for the rule-out of NSTE-ACS

Supplemental Table 1. Overview of the different rule-out algorithms that were evaluated.

	Novel algorithms	ESC algorithms
hs-TnT Roche		
1 hour	hs-cTnT < 5 ng/L and $\Delta_{0\text{-}1h} < \pm 1$ ng/L	hs-cTnT <12 ng/L and $\Delta_{0\text{-}1h} < \pm 3$ ng/L
3 hour	hs-cTnT < 5 ng/L and $\Delta_{0\text{-}3h} < \pm 1$ ng/	
hs-TnI Abbott		
1 hour	hs-cTnI < 2 ng/L and $\Delta_{0\text{-}1h} < \pm 1$ ng/L	hs-cTnI < 5 ng/L and $\Delta_{0\text{-}1h} < \pm 2$ ng/L
3 hour	hs-cTnI < 2 ng/L and $\Delta_{0\text{-}3h} < \pm 1$ ng/L	

ESC, European Society of Cardiology; hs-cTnT, high-sensitivity troponin T; hs-cTnI, high-sensitivity troponin I.

Supplemental Table 2. Comparison of baseline troponin concentrations (ng/L, median, 25 and 75 percentile) in the two cohorts after stratification according to diagnosis adjudicated during hospitalization. A significant calibrator shift was identified for the hs-cTnI measurements (p-value for difference were ≤ 0.01 for all groups except NSTEMI).

	hs-cTnT			**hs-cTnI**		
Baseline	Derivation cohort	Validation cohort	p-value	Derivation cohort	Validation cohort	p-value
Total	7.0 (3.0-18.0)	7.0 (4.0-13.0)	0.07	4.0 (2.1-11.6)	2.2 (1.0-5.2)	<0.001
NSTEMI	48.0 (22.8-172.0)	56.5 (23.0-161.5)	0.73	118.9 (26.5-560.1)	102.2 (28.2-578.3)	0.58
UAP	9.0 (5.0-18.0)	9.0 (6.0-17.0)	0.57	4.7 (3.1-9.9)	3.3 (1.7-9.3)	0.01
Other diseases	13.0 (5.8-24.0)	10.5 (5.8-16.3)	0.08	8.1 (3.2-17.7)	3.6 (1.4-10.6)	<0.001
NCCP	5.0 (3.0-9.0)	5.0 (3.0-8.0)	0.81	2.7 (1.7-5.2)	1.5 (0.8-3.1)	<0.001

Supplemental Table 3. Median, 10, 90 percentile and significance level for the 1 h and 3 h absolute delta concentrations in UAP and NCCP patients.

	hs-cTnT			**hs-cTnI**		
	UAP	NCCP	p-value	UAP	NCCP	p-value
1 h delta $_D$	1.0 (0-2.0)	0 (0-1)	0.002	0.6 (01.-3.6)	0.4 (0-1.5)	0.008
1 h delta $_V$	0.7 (0-2.3)	0 (0-1.1)	0.008	0.9 (0.2-5.6)	0.5 (0.1-2.1)	<0.001
3 h delta $_D$	1.0 (0-3.0)	0 (0-2.0)	<0.001	0.8 (0.1-4.7)	0.6 (0-2.5)	0.001
3 h delta $_V$	1.0 (0-2.9)	0 (0-2.0)	<0.001	0.9 (0.1-7.2)	0.8 (0.1-2.7)	0.19

UAP, unstable angina pectoris; NCCP, none cardiac chest pain; D, deviation cohort; V, validation cohort.

Supplemental Table 4A. Diagnostic performance (95% confidence intervals) and efficacy (total rule-out, percentages in brackets) for the primary endpoint combining NSTEMI and UAP during index hospitalization for the different algorithms in early presenters (≤ 3 hour since symptom onset). ESC algorithms are shown on a grey background.

	Sensitivity	NPV	Specificity	PPV	Rule-out rate
1-hour algorithms					
hs-cTnT < 5 ng/L and $\Delta_{0\text{-}1h}$ < 1 ng/L					
Derivation cohort N=97	91.7 (73.0-99.0)	91.3 (72.6-97.7)	28.8 (18.8-40.6)	29.7 (25.9-33.8)	23 (23.7)
Validation cohort N=94	92.3 (74.9-99.1)	88.2 (64.6-96.8)	22.1 (12.9-33.8)	31.2 (27.7-34.9)	17 (18.1)
hs-cTnT <12 ng/L and $\Delta_{0\text{-}1h}$ < 3 ng/L					
Derivation cohort N= 97	83.3 (62.6-95.3)	92.3 (82.9-96.8)	65.8 (53.7-76.5)	44.4 (35.7-53.5)	52 (53.6)
Validation cohort N= 94	61.5 (40.6-79.8)	85.5 (78.2-90.6)	86.8 (76.4-93.8)	64.0 (47.4-77.8)	69 (73.4)
hs-cTnI < 2 ng/L and $\Delta_{0\text{-}1h}$ < 1 ng/L					
Derivation cohort N=97	91.7 (73.0-99.0)	90.5 (70.5-97.4)	26.3 (16.5-37.6)	29.0 (25.4-32.8)	21 (21.6)
Validation cohort N=94	80.8 (60.7-93.5)	87.2 (74.9-93.9)	50.0 (37.6-62.4)	38.2 (31.3-45.5)	39 (41.5)
hs-cTnI < 5 ng/L and $\Delta_{0\text{-}1h}$ < 2 ng/L					
Derivation cohort N=97	83.3 (62.6-95.3)	92.1 (82.5-96.7)	64.4 (52.3-75.3)	43.5 (35.0-52.4)	51 (52.6)
Validation cohort N =94	57.7 (36.9-76.7)	84.3 (77.2-89.5)	86.8 (76.4-93.8)	62.5 (45.5-76.9)	70 (74.5)
3-hour algorithms					
hs-cTnT < 5 ng/L and $\Delta_{0\text{-}3h}$ < 1 ng/L					
Derivation cohort N=214	98.2 (90.3-99.9)	98.0 (87.4-99.7)	30.8 (23.8-38.6)	32.9 (30.6-35.4)	50 (23.4)
Validation cohort N=90	95.7 (78.1-99.9)	96.0 (77.5-99.4)	35.8 (24.5-48.5)	35.9 (29.5-38.4)	25 (27.8)
hs-cTnI < 2 ng/L and $\Delta_{0\text{-}3h}$ < 1 ng/L					
Derivation cohort N=206	96.2 (87.0-99.5)	95.5 (84.0-98.8)	27.5 (20.6-35.2)	31.9 (29.1-33.9)	44 (21.4)
Validation cohort N=90	87.0 (66.4-97.2)	90.6 (76.5-96.6)	43.3 (31.2-56.0)	34.5 (28.8-40.6)	32 (35.6)

UAP, unstable angina pectoris; NSTEMI, non-ST elevation myocardial infarction; NPV, negative predictive value; PPV, positive predictive value.

Supplemental Table 4B. Diagnostic performance (95% confidence intervals) and efficacy (total rule-out, percentages in brackets) for the secondary endpoint combining 30 days MI and all-cause mortality and urgent (24 hour) revascularization for the different algorithms in early presenters (≤ 3 hour since symptom onset). European Society of Cardiology algorithms are shown on a grey background.

	Sensitivity	NPV	Specificity	PPV	Rule-out rate
1-hour algorithms					
hs-cTnT < 5 ng/L and $\Delta_{0\text{-}1h}$ < 1 ng/L					
Derivation cohort N=97	100 (85.2-100.0)	100	21.6 (12.9-32.7)	28.4 (26.0-30.9)	16 (16.5)
Validation cohort N=94	100 (80.5-100.0)	100	15.6 (8.3-25.6)	20.7 (19.2-22.4)	12 (12.8)
hs-cTnT <12 ng/L and $\Delta_{0\text{-}1h}$ < 3 ng/L					
Derivation cohort N=97	100 (79.4-100.0)	100	64.2 (52.3-74.6)	33.6 (29.2-42.5)	52 (53.6)
Validation cohort N=94	100 (73.5-100.0)	100	82.2 (74.4-91.3)	48.0 (35.9-60.3)	69 (73.4)
hs-cTnI < 2 ng/L and $\Delta_{0\text{-}1h}$ < 1 ng/L					
Derivation cohort N=97	100 (79.4-100.0)	100	25.9 (16.8-36.9)	21.1 (19.0-23.8)	21 (21.6)
Validation cohort N=94	100 (73.5-100.0)	100	47.6 (36.4-58.9)	21.8 (18.5-25.5)	39 (41.5)
hs-cTnI < 5 ng/L and $\Delta_{0\text{-}1h}$ < 2 ng/L					
Derivation cohort N=97	100 (79.4-100.0)	100	63.0 (51.5-73.4)	34.8 (28.7-41.5)	51 (52.6)
Validation cohort N =94	100 (73.5-100.0)	100	85.4 (75.8-92.2)	50.0 (37.2-62.8)	70 (74.5)
3-hour algorithms					
hs-cTnT < 5 ng/L and $\Delta_{0\text{-}3h}$ < 1 ng/L					
Derivation cohort N=214	100 (91.2-100.0)	100	28.7 (22.1-36.1)	24.4 (22.7-26.2)	50 (23.4)
Validation cohort N=90	100 (71.5-100.0)	100	31.6 (21.6-43.1)	16.9 (14.9-19.1)	25 (27.8)
hs-cTnI < 2 ng/L and $\Delta_{0\text{-}3h}$ < 1 ng/L					
Derivation cohort N=206	100 (90.8-100.0)	100	26.2 (19.7-33.5)	23.5 (21.9-25.1)	44 (21.4)
Validation cohort N=90	100 (71.5-100.0)	100	40.5 (29.6-52.2)	19.0 (16.3-21.9)	32 (35.6)

NPV, negative predictive value; PPV, positive predictive value.

Supplemental Table 5. Absolute rule-out numbers (percentages in brackets) for the different algorithms, patients are stratified according to the diagnosis adjudicated during index hospitalization. European Society of Cardiology algorithms are shown on a grey background.

	NSTE-ACS	Other diseases	NCCP	Total
1-hour algorithms				
hs-cTnT < 5 ng/L and $\Delta_{0\text{-}1h}$ < 1 ng/L				
Derivation cohort	5 (4.2)	8 (11.8)	102 (34.9)	115 (24.0)
Validation cohort	6 (4.7)	4 (7.1)	75 (23.4)	85 (16.8)
hs-cTnT <12 ng/L and $\Delta_{0\text{-}1h}$ < 3 ng/L				
Derivation cohort	34 (28.6)	30 (44.1)	245 (83.9)	309 (64.5)
Validation cohort	48 (37.2)	31 (55.4)	276 (86.3)	355 (70.3)
hs-cTnI < 2 ng/L and $\Delta_{0\text{-}1h}$ < 1 ng/L				
Derivation cohort	8 (6.7)	7 (10.3)	94 (32.8)	109 (23.0)
Validation cohort	17 (13.1)	17 (29.8)	153 (47.8)	187 (36.8)
hs-cTnI< 5 ng/L and Δ0-1h < 2 ng/L				
Derivation cohort	33 (27.7)	20 (29.4)	216 (75.3)	269 (56.0)
Validation cohort	47 (36.2)	33 (57.9)	263 (82.2)	343 (67.7)
3-hour algorithms				
hs-cTnT < 5 ng/L and $\Delta_{0\text{-}3h}$ < 1 ng/L				
Derivation cohort	8 (3.5)	19 (13.5)	203 (34.6)	230 (23.4)
Validation cohort	3 (2.5)	6 (11.5)	99 (32.0)	108 (22.4)
hs-cTnI< 2 ng/L and $\Delta_{0\text{-}3h}$ < 1 ng				
Derivation cohort	10 (4.2)	12 (7.7)	175 (31.2)	197 (20.2)
Validation cohort	15 (12.4)	13 (24.5)	127 (41.1)	155 (32.1)

NSTE-ACS, non-ST-elevation acute coronary syndrome; NCCP, non-coronary chest pain.

Supplemental Table 6. Rule-out rate for the different algorithms in the sub-groups of patients with unstable angina pectoris (UAP) and non-cardiac chest pain (NCCP) (diagnosis adjudicated during index hospitalization). Percentages and Confidence intervals in brackets. European Society of Cardiology algorithms are shown on a grey background.

	UAP	NCCP
	1-hour algorithms	
hs-cTnT < 5 ng/L and $\Delta_{0\text{-}1h}$ < 1 ng/L		
Derivation cohort	8.8 (1.5-16.2)	34.9 (25.7-44.2)
Validation cohort	6.8 (1.6-12.1)	23.4 (18.8-28.0)
hs-cTnT <12 ng/L and$\Delta_{0\text{-}1h}$ < 3 ng/L		
Derivation cohort	59.6 (46.9-72.3)	83.9 (79.7-88.1)
Validation cohort	54.5 (44.1-65.0)	86.3 (82.5- 90.1)
hs-cTnI < 2 ng/L and $\Delta_{0\text{-}1h}$ < 1 ng/L		
Derivation cohort	14.0 (5.0-23.0)	32.8 (27.4-38.2)
Validation cohort	19.5 (9.2-29.8)	47.8 (42.3-53.3)
hs-cTnI< 5 ng/L and Δ0-1h < 2 ng/L		
Derivation cohort	57.9 (45.1-70.7)	75.3 (70.3-80.3)
Validation cohort	53.4 (43.0-63.8)	82.2 (78.0-86.4)
	3-hour algorithms	
hs-cTnT < 5 ng/L and $\Delta_{0\text{-}3h}$ < 1 ng/L		
Derivation cohort	7.1 (2.3-11.9)	34.6 (30.8-38.5)
Validation cohort	3.8 (0-8.8)	32.0 (26.8-37.2)
hs-cTnI< 2 ng/L and $\Delta_{0\text{-}3h}$ < 1 ng		
Derivation cohort	9.3 (3.8-14.8)	31.2 (27.4-35.0)
Validation cohort	18.8 (10.2-27.3)	41.1 (35.6-46.6)

Supplemental Table 7. The table shows the number of investigations, revascularizations and 30 days major cardiac adverse events (MACE) in the different groups, stratified by index diagnosis. MACE was defined as death, myocardial infarction or revascularization. The increased numbers of CCTA in the validation cohort was in accordance with the study protocol (see method section).

	NSTE-ACS	**NSTEMI**	**UAP**
Derivation cohort	N=242	N=130	N=112
Investigations			
Echocardiography	180 (74.4)	109 (83.8)	71 (63.4)
CCTA*	39 (16.1)	5 (3.8)	34 (30.4)
Coronary angiography	187 (77.3)	112 (86.2)	75 (67.0)
Revascularization			
PCI‡ within 24 hours	38 (15.7)	34 (26.2)	4 (3.6)
PCI >24 hours after admission	96 (39.7)	49 (37.7)	47 (42.0)
CABG£	14 (5.8)	8 (6.2)	6 (5.3)
30 days all-cause mortality, MI or revascularization			
Total	195 (80.6)	130 (100.0)	65 (58.0)
Deaths	1 (0.4)	1 (0.8)	0
MI	133 (55.0)	130 (100)	3 (2.7)
Revascularization	157 (64..9)	93 (71.5)	64 (57.1)
Validation cohort	N=133	N=44	N=89
Investigations			
Echocardiography	110 (82.7)	38 (86.4)	72 (80.9)
CCTA	42 (31.6)	6 (13.6)	36 (40.4)
Coronary angiography	104 (78.2)	38 (86.4)	66(74.2)
Revascularization			
PCI within 24 hours	15 (11.3)	11 (25.0)	4 (4.5)
PCI >24 hours after admission	48 (36.1)	14 (31.8)	34 (38.2)
CABG	11 (8.3)	6 (13.6)	5 (5.6)
30 days all-cause mortality, MI or revascularization			
Total	98 (73.7)	44 (100.0)	54 (60.7)
Deaths	1 (0.8)	0	1 (1.1)
MI	45 (33.8)	44 (100)	1 (1.1)
Revascularization	88 (66.2)	34 (77.3)	54 (60.7)

CCTA, Coronary computed tomography angiography; NSTE-ACS, non-ST-elevation acute coronary syndrome; NSTEMI, non-ST elevation myocardial infarction; UAP, unstable angina pectoris; PCI, Percutaneous coronary intervention.

Supplemental Table 8. The investigations, revascularization and 30 days major cardiac adverse events (MACE) defined as death, myocardial infarction or revascularization in the group of patients with unstable angina pectoris (UAP) who were rule-out by the European Society of Cardiology algorithms and the most favorable of the novel algorithms (0-3 hour). Percentages (in brackets) are calculated using all patients with UAP in the nominator (n=57 (in the derivation cohort only the 57/112 patients who had a 1-hour sample were included) and n=88 (validation cohort)).

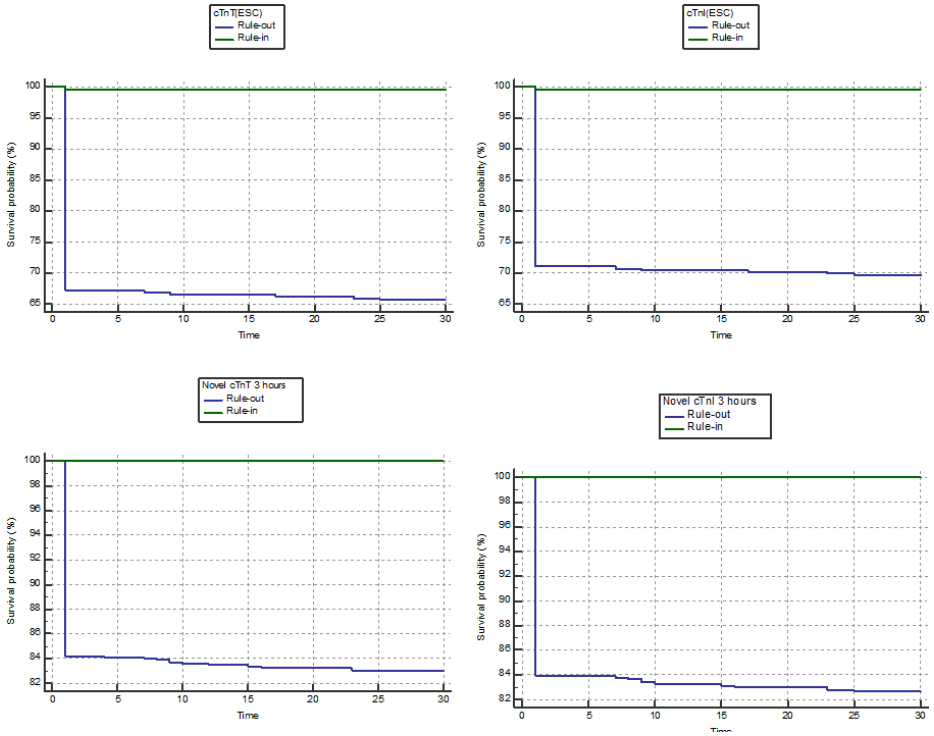
	UAP ruled-out $cTnT_{ESC}$	UAP ruled-out $cTnT_{\Delta 0\text{-}3}$	P-value	UAP ruled-out $cTnI_{ESC}$	UAP ruled-out $cTnI_{\Delta 0\text{-}3}$	P-value
Derivation cohort	**N=34/57**	**N=3/57**	<0.001	**N=33/57**	**N=7/53**	<0.001
Investigations						
Echocardiography	21 (36.8)	1 (1.8)	<0.001	22 (38.6)	4 (7.0)	<0.001
CCTA*	17 (29.8)	2 (3.5)	<0.001	15 (26.3)	6 (10.5)	0.002
Coronary angiography	22 (38.6)	1 (1.8)	<0.001	23 (40.4)	6 (10.5)	<0.001
Revascularization						
PCI‡ within 24 hours	0	0	NA	0	0	NA
PCI >24 hours but during admission	15 (26.3)	1 (1.8)	<0.001	14 (24.6)	4 (7.0)	0.01
CABG£ during admission	0	0	NA	0	0	NA
30 days all-cause mortality, MI or revascularization						
Total	21 (36.8)	2 (3.5)	<0.001	19 (33.3)	4 (7.5)	<0.001
Deaths	0	0		0	0	
MI	0	0		0	0	
Revascularization	21 (36.8)	2 (3.5)	<0.001	19 (33.3)	4 (7.5)	<0.001
Validation cohort	**N=48/88**	**N=3/79**	<0.001	**N=47/88**	**N=15/79**	<0.001
Investigations						
Echocardiography	38 (43.1)	2 (2.2)	<0.001	35 (39.8)	12 (13.6)	<0.001
CCTA	23 (26.1)	1 (1.1)	<0.001	20 (22.7)	6 (6.8)	0.002
Coronary angiography	34 (38.6)	2 (2.2)	<0.001	34 (38.6)	12 (13.6)	<0.001
Revascularization						
PCI within 24 hours	3 (3.4)	0	0.5	2 (2.3)	0	1.0
PCI >24 hours but during admission	15 (17.0)	0	<0.001	15 (17.0)	3 (3.4)	0.04
CABG during admission	2 (2.3)	0	0.5	2 (2.3)	1 (1.1)	1.0
30 days all-cause mortality, MI or revascularization						
Total	28 (31.8)	1 (1.1)	<0.001	28 (31.8)	8 (10.1)	<0.001
Deaths	0	0	NA	0	0	NA
MI	0	0	NA	1 (1.1)	0	NA
Revascularization	26 (29.5)	1 (1.1)	<0.001	28 (31.8)	8 (10.1)	<0.001

*Coronary computer tomography angiography

‡ Percutaneous coronary intervention

£ Coronary artery bypass graft

Supplemental Figure 1. Kaplan-Meier curves showing 30 days all-cause mortality, 30 days MI or 24 hours revascularization for patients ruled-in and ruled-out by the European Society of Cardiology and the novel 3-hour algorithms.



Review of "missed" patients

The list include an overview of patients who were missed by the algorithms and developed an MI or died within 30 days after admission or were treated with an urgent (24 hour) revascularization.

Review of "missed" UAP patients

Patient 1 and 2 are the same patients in both groups

ESC cTnT algorithm (hs-cTnT <12 ng/L and $\Delta_{0\text{-}1h}$ < ±3 ng/L)

Validation cohort

Patient 1

60 year old male with previous STEMI, admitted with a four hour history of chest pain. Had PCI 21 hours after admittance with a stent in CX. Diagnosed with UAP.

Hs-TnT_{0h} 7 ng/l, TnT_{1h} 8 ng/l and TnT_{3h} 7 ng/L

Patient 2

70 year old female with known atherosclerotic heart disease, admitted with a 16 hour history of chest pain. PCI at 24 hours, stented in LAD. Diagnosed with UAP.

Hs-TnT_{0h} 6 ng/l, TnT_{1h} 6 ng/l and TnT_{3h} 6 ng/L

Patient 3

50 year old male, previously healthy, admitted with two weeks history of chest pain, PCI at 24 hours, stented in LAD. Diagnosed with UAP.

Hs-TnT_{0h} 8 ng/l, TnT_{1h} 8 ng/l and TnT_{3h} 8 ng/L

ESC cTnI algorithm (hs-cTnI < 5 ng/L and $\Delta_{0\text{-}1h}$ < ±2 ng/L)

Patient 1: Hs-TnI_{0h} 3 ng/L, TnI_{1h} 2 ng/L and TnI_{3h} 3 ng/L

Patient 2: Hs-TnI_{0h} 3 ng/L and TnI_{1h} 4 ng/L TnI_{3h} 3 ng/L

Errata for

Aiming towards evidence based interpretation of cardiac biomarkers in patients presenting with chest pain

The WESTCOR study

Hilde Lunde Tjora

Thesis for the degree philosophiae doctor (PhD)
at the University of Bergen

25/2-23

(date and sign. of candidate)

(date and sign. of faculty)

Errata

The cover page and last page of the thesis is embedded in the beginning of the document, and this was a mistake, and I want them removed from the PDF.

Page 22 Missing a reference: "Myocardial injury may be acute ischemic (MI, as described above), acute non-ischemic, and chronic myocardial injury (19) (**Figure 1**)." – corrected to "Myocardial injury may be acute ischemic (MI, as described above), acute non-ischemic, and chronic myocardial injury (19, 34) (**Figure 1**)."

Page 26 Missing bold: "followed by release of cTn into the blood in a ternary or binary complex form or as free cTn fragments (43) (Figure 3)." - corrected to "followed by release of cTn into the blood in a ternary or binary complex form or as free cTn fragments (43) **(Figure 3)**."

Page 29 Missing a reference: "With the development of cTn assays, the definition of MI has changed several times over the two last decades, and the latest and fourth definition was published in 2018 (**Table 2**)." – corrected to "With the development of cTn assays, the definition of MI has changed several times over the two last decades, and the latest and fourth definition was published in 2018 (19) (**Table 2**)."

Page 44 Missing brackets in a sentence: "Patients could be followed for at least 10 years through different national health care registers such as the Norwegian Patient Registry NPR," – corrected to ""Patients could be followed for at least 10 years through different national health care registers such as the Norwegian Patient Registry (NPR),"

Page 49 Wrong reference: "In paper 4, the novel rule-out algorithms had baseline values corresponding to the LOD and delta values that were based on RCVs (105)." – corrected to "In paper 4, the novel rule-out algorithms had baseline values corresponding to the LOD and delta values that were based on RCVs (109)."

Page 51 Missing space between a word and a reference: "It was thought to be clinically meaningful to have a difference between two different algorithms or protocols of 5% for sensitivity, 5% for specificity, and 0.03 for the AUROC(110)." – corrected to "It was thought to be clinically meaningful to have a difference between two different algorithms or protocols of 5% for sensitivity, 5% for specificity, and 0.03 for the AUROC (110)."

Page 61 Missing reference of a figure: "In a subgroup analysis of UAP and NCCP patients, the 0/3-hour rule-out algorithm showed the best performance." – corrected to "In a subgroup analysis of UAP and NCCP patients, the 0/3-hour rule-out algorithm showed the best performance **(Figure 7)**."

Page 63 Missing reference: "The STARD instrument was developed to increase the quality of diagnostic performance studies (114) and provides a detailed description of how diagnostic studies should be performed" – corrected to "The STARD instrument was developed to increase the quality of diagnostic performance studies (83, 114) and provides a detailed description of how diagnostic studies should be performed"

Page 75 Missing a comma: "Adding the hs-cTnT value as a continuous variable above LOD, however improved the discrimination ability for a one-year outcome of MACE in both early and late presenters (C-statistics: 0.754, p=0.007: and 0.847, p=0.001), " – corrected to "Adding the hs-cTnT value as a continuous variable above LOD, however, improved the discrimination ability for a one-year outcome of MACE in both early and late presenters (C-statistics: 0.754, p=0.007: and 0.847, p=0.001),"

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