Novel aspects of cardiovascular biomarkers in myocardial infarction and obstructive sleep apnea

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1. SYNOPSIS

Background
In contemporary clinical practice, measurement of circulating biomarkers is an integral part of patient care. Biomarkers have many areas of application and provide diagnostic, prognostic and therapeutic information. The appropriate use of biomarkers can facilitate the understanding of underlying pathophysiological processes and may contribute to improved management of various disease states.

Aim
The aim of this thesis was to explore novel aspects of circulating biomarkers associated with cardiovascular disease, with an emphasis on myocardial infarction (MI) and obstructive sleep apnea (OSA).

Methods
In a retrospective, national, single-center, pre-post comparison study of hospital admissions, changes in non-ST-segment elevation myocardial infarction (NSTEMI) diagnostics after the implementation of a high-sensitivity cardiac troponin T (hs-cTnT) assay were examined. In a post hoc analysis of patients with acute ST-segment elevation myocardial infarction (STEMI) treated with primary percutaneous coronary intervention (PCI) who participated in a prospective, international, multi-center, randomized placebo-controlled trial, the utility of cardiac troponin I (cTnI) variables for prediction of clinical outcomes and cardiac function during three months follow-up were investigated. In a national, single-center, cross-sectional study of individuals examined for possible OSA, circulating levels of several biomarkers (myocardial stress; single-molecule cTnI [S-cTnI] and hs-cTnT, inflammation; myeloid-related protein-8/14 and C-reactive protein) and their associations to variables of disordered breathing during sleep were explored.

Results
An increase of primary NSTEMI admissions after the implementation of a high-sensitivity assay was observed, while a higher proportion of the subjects did not have pathological findings on coronary angiography and fewer had significant dynamic
cTnT changes. In patients with STEMI, cTnI levels were associated with an increased risk of clinical events and decreased cardiac function at three months independent of clinical risk factors. Furthermore, cTnI significantly improved discrimination of patients with and without endpoint. In individuals investigated for possible OSA, the application of novel cardiac troponin (cTn) assays facilitated estimation of myocardial stress in all subjects. No independent associations between apnea-hypopnea index and myocardial stress or inflammation were found after adjustment for other risk factors, whereas oxygen variables were observed to inherit a heterogeneous pattern of selected independent associations. The association between several of the variables of disordered breathing during sleep and inflammation was stronger in individuals with a higher body mass index (BMI).

**Interpretation and conclusion**

The introduction of a hs-cTnT assay enhanced NSTEMI diagnostics, but more patients will have to be considered for potentially having increased cTnT levels due to other etiologies than MI. In STEMI patients, the present findings imply that one single measurement of cTnI after PCI provides incremental information to conventional risk stratification. In individuals investigated for possible OSA, the use of S-cTnI and hs-cTnT confirmed that improved performance characteristics are broadening potential applications of cTn assays. For the diagnostic evaluation of possible OSA, the future clinical utility of all investigated biomarkers appears limited. However, the observed interaction between a higher BMI and several variables of disordered breathing during sleep may indicate activated inflammatory pathways inconsistently masked by unmeasured confounders or too moderate to be significant in this study sample. These findings expand our knowledge of the investigated biomarkers and contribute to determine their future role in patient care.
2. ACKNOWLEDGEMENTS

My interest in biomarkers and how they improve our recognition of pathophysiological activity evolved during my first years working as a doctor at Lovisenberg Diakonale Hospital. Inspired by observations in the clinic, several research ideas emerged that I contemplated could have potential to enhance patient care. After discussions with Dr. Torstein Jensen, Dr. Gudmund Nordby and Head of Department Anne Marit Tangen in 2010, I was encouraged to work further with these proposals. Their recognition stimulated me to initiate contact with Professor Dan Atar at Oslo University Hospital Ullevål, who, together with his colleagues Professor Stefan Agewall and Dr. Jonas Hallén, kindly agreed to establish a collaborative group with the intent of materializing a complete PhD research proposition. The tremendous backing received from my supervisors, Gudmund, Anne Marit and other co-workers during this developmental phase was imperative for the realization of the present thesis. I will always be thankful to them for giving me the opportunity to pursue my fascination for biomarkers and research.

The work was conducted at Lovisenberg Diakonale Hospital in collaboration with Oslo University Hospital and several centers in the USA. Lovisenberg Diakonale Hospital provided the necessary funding to plan and initiate the project, while the South-Eastern Norway Regional Health Authority granted a full-time research fellowship from 2013. The financial support from these institutions has been essential and I am very grateful for their indispensable contribution.

My main supervisor Dan has been a true mentor and an endless source of inspiration. His scientific excellence, open-mindedness, positivity, day-to-day availability and enduring confidence in my abilities have impressed me. Dan has included me in important decisions during the project and allowed me to actively participate in many of his other research activities. Evidently, his international network has also given me the opportunity to collaborate with several leading figures and institutions within the field. This magnificent generosity and kindness have made my years as a researcher very exciting and stimulated me to develop both on a personal and professional level. I am greatly indebted to Dan for everything he has taught me.
I have also been privileged to have a fantastic group of co-supervisors, who have complemented each other very well. Stefan has with his attention to details and significant scientific eminence provided critical input and constructive guidance. He has always responded to my queries swiftly and precisely, which has facilitated a steady progression without any notable delays. There have been several instances where Stefan has helped me with specific problems that I myself considered unsolvable, and through our educational discussions he has taught me a lot about how to reach a decision and then stick with it. Torstein encouraged me to enter into research from the very beginning and was in charge of the first study. He helped me structure my initial research ideas and introduced me to the writing of study protocols. Torstein has generously shared from his broad experience throughout the project and I am thankful for his valuable advice and continuous support. Jonas has been an extraordinary resource for me on a day-to-day basis. He has made himself available when needed, and through his exceptional scientific and personal qualities he has always recognized the best solutions to the challenges that I have encountered. Jonas has also been able to motivate me during difficult times and I have learnt a lot from our long and frequent discussions. He was greatly involved in the design of the PhD research proposition and provided innumerable comments during preparation of the manuscripts. I would like to express my sincere appreciation for his never-ending support.

Dan introduced me to Dr. A. Michael Lincoff, Director of the Cleveland Clinic Coordinating Center for Clinical Research. Dr. Lincoff invited me to stay as a visiting fellow at the Cleveland Clinic to learn more about the field of cardiology and work on-site with the PROTECTION AMI database. My time in Cleveland was truly inspiring and an experience I will remember with great fondness. Further, I would like to thank Danielle Brennan, Dr. Mitchell W. Krucoff and Dr. Matthew T. Roe for their significant contribution to the PROTECTION AMI project.

I have also had the great privilege to visit and work with Dr. Petr Jarolim, Director of the Biomarker Research Laboratory/TIMI Clinical Trials Laboratory. His laboratory performed some of the biomarker analyses and it was very valuable for me to be educated on the technical and instrumental aspects of biomarker sampling during my stay in Boston. Dr. Jarolim has substantial merit within the field and I would like to thank him for contributing with insightful and constructive comments during our collaboration.
I want to express a special thanks to Dr. Tobias E. Herrscher, with whom I worked closely during the obstructive sleep apnea studies. His experience with sleep disorders was a great enrichment to the group and I highly appreciated his considerable efforts. I am as well grateful for the statistical assistance that Morten W. Fagerland provided for this project, through both time-consuming pedagogic lessons and performing several of the analyses that required his expertise.

The backing from everyone at the Unger-Vetlesens Institute and other co-workers at Lovisenberg has also been essential. I would like to express my gratitude for the encouragement they have given me throughout my career. A special thanks to Gudmund, Anne Marit, Dr. Bjørn Holm and Dr. Viggo Skar for always supporting me and being available for discussions when needed, Dr. Einar Amlie for tremendous help during retrieval of information from the hospital database, Britt Øverland for her significant contribution in interpreting the sleep studies, Merete Bolstad and May Lill Madsen for performing some of the biomarker analyses and Ana Urzua Riquelme for superb administrative assistance.

I would also like to thank all my family and friends. I am significantly indebted to my father Christian Hall for sharing his experience and knowledge every time I have had questions. I have very much enjoyed our numerous long-lasting conversations about biomarker research and academic medicine. This has been complemented by valuable input and continuous encouragement from my mother Kirsten Sundby Hall and brother Andreas Sundby Hall, to whom I am very thankful.

Lastly, I want to express great appreciation to my wife Elisabeth Sørdahl Hall for being immensely supportive in all ways possible, and to our son Håkon Sørdahl Hall for being there to motivate me after he came into our lives. Elisabeth has always been willing and able to discuss my successes and frustrations, which has augmented my intellectual capability and stimulated me to move forward. Your enduring patience has been truly admirable and I remain deeply grateful for your exceptional devotion.
3. ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AHI</td>
<td>Apnea-hypopnea index</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under curve</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>CHF</td>
<td>Congestive heart failure</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CPAP</td>
<td>Continuous positive airway pressure</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>cTn</td>
<td>Cardiac troponin</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>EMG</td>
<td>Electromyogram</td>
</tr>
<tr>
<td>hs-cTnT</td>
<td>High-sensitivity cardiac troponin T</td>
</tr>
<tr>
<td>IDI</td>
<td>Integrated discrimination improvement</td>
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<tr>
<td>MI</td>
<td>Myocardial infarction</td>
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<tr>
<td>MRP-8/14</td>
<td>Myeloid-related protein-8/14</td>
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<tr>
<td>NSTEMI</td>
<td>Non-ST-segment elevation myocardial infarction</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>N-terminal pro-B-type natriuretic peptide</td>
</tr>
<tr>
<td>LoB</td>
<td>Limit of blank</td>
</tr>
<tr>
<td>LoD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>LoQ</td>
<td>Limit of quantification</td>
</tr>
<tr>
<td>LVEF</td>
<td>Left ventricular ejection fraction</td>
</tr>
<tr>
<td>ODI</td>
<td>Oxygen desaturation index</td>
</tr>
<tr>
<td>OSA</td>
<td>Obstructive sleep apnea</td>
</tr>
<tr>
<td>PCI</td>
<td>Percutaneous coronary intervention</td>
</tr>
<tr>
<td>PG</td>
<td>Polygraphy</td>
</tr>
<tr>
<td>PSG</td>
<td>Polysomnography</td>
</tr>
<tr>
<td>S-cTnI</td>
<td>Single-molecule cardiac troponin I</td>
</tr>
<tr>
<td>STEMI</td>
<td>ST-segment elevation myocardial infarction</td>
</tr>
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</table>
4. LIST OF PAPERS

Paper I

Paper II

Paper III

Paper IV
5. BACKGROUND

5.1 Cardiovascular disease

Cardiovascular disease (CVD) is the number one cause of death globally (1). CVD is defined as a group of disorders involving the heart or blood vessels (2). Myocardial infarction (MI) and obstructive sleep apnea (OSA) are two distinct disease entities related to CVD; MI is a captious and potentially lethal manifestation of coronary artery disease (CAD), whereas evidence suggests that patients with OSA have an increased risk of developing CAD and several other CVDs (3-5). As the most frequent cause of death out of all CVDs, 7.3 million people died due to CAD worldwide in 2008 (6).

5.2 Myocardial infarction

History and Epidemiology

Since MI was identified as a distinct clinical entity by James B. Herrick in 1912, with the mainstay of treatment being "absolute bed rest" (7), a remarkable evolution in the understanding of MI’s pathophysiological basis has occurred. Considerable improvements have been made to prevention, detection and treatment of the disease. Despite these substantial developments, MI yearly afflicts more than 7 million people worldwide and remains associated with considerable morbidity and mortality (8).

Pathophysiology

MI is generally preceded by years of gradually progressive and asymptomatic CAD. The process is termed atherosclerosis of the coronary arteries, where plaque builds up within the vessel walls (9). Initially considered a cholesterol-storage disorder, many now regard atherosclerosis as an inflammatory disease resulting from a complex pathophysiological interplay between several factors (10). Components of lipid metabolism, inflammation and various cell types within the lesion have been identified as contributors in experimental studies (11). Risk factors for the condition include dyslipidemia, hypertension, smoking and hyperglycemia (12). When atherosclerosis progresses it may lead to narrowing of the vessel lumen and reduced blood flow to the myocardium (9). However, it is also a major pathophysiological component in the archetypal MI. In this setting, acute thrombus formation takes place...
in a coronary artery following atherosclerotic plaque disruption (13). Normal blood flow to the myocardium is either hindered by the thrombus obtruding the artery lumen or by embolic debris lodged in distal blood vessels. Subsequently, ischemia ensues and myocyte necrosis occurs (14). Nevertheless, through advances in our understanding of the underlying processes, as well as the development of refined tools to detect, quantify and visualize infarcted myocardium, it has now been established that other mechanisms than thrombus formation in an atherosclerotic artery may also contribute to MI. In the current “Third Universal Definition of Myocardial Infarction”, several distinct types of MI’s are characterized based on pathological, clinical and prognostic differences (Table 1) (14).

**Table 1.** Types of myocardial infarction.

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Spontaneous myocardial infarction related to a primary coronary event with resulting intraluminal thrombus, such as plaque rupture or dissection.</td>
</tr>
<tr>
<td>2</td>
<td>Myocardial infarction resulting from imbalance between oxygen supply and/or demand, such as coronary artery spasm or arrhythmias.</td>
</tr>
<tr>
<td>3</td>
<td>Myocardial infarction resulting in death when biomarkers are unavailable.</td>
</tr>
<tr>
<td>4a</td>
<td>Myocardial infarction related to percutaneous coronary intervention.</td>
</tr>
<tr>
<td>4b</td>
<td>Myocardial infarction related to stent thrombosis.</td>
</tr>
<tr>
<td>4c</td>
<td>Myocardial infarction related to restenosis following initially successful stent deployment or balloon angioplasty.</td>
</tr>
<tr>
<td>5</td>
<td>Myocardial infarction related to coronary artery bypass grafting.</td>
</tr>
</tbody>
</table>

**Diagnostic assessment**

An acute MI is defined by “evidence of myocardial necrosis in a clinical context consistent with myocardial ischemia” (14). Consequently, the interpretation of symptoms and signs of potential ischemia, in combination with the detection of necrosis, formulate the key elements during patient assessment. Over the past decades, the diagnostic evaluation has changed quite substantially. This has mainly been driven by extensive improvements in the laboratorial methods used to establish the presence of myocardial necrosis (15).
Observing a pattern of rising and/or falling cardiac biomarker levels, with at least one measurement being above the 99th percentile concentration value in a normal reference population, has been an essential diagnostic criterion for many years (16-18). Nevertheless, due to suboptimal precision at lower concentration levels for the earlier generations of cardiac troponin (cTn) assays, some have advocated for the use of a higher cut-off point (19). Accordingly, there has been considerable variation in applied cut-off points across centers (20). However, with the improved performance characteristics of recently developed cTn assays, it is expected that their use in clinical practice will lead to greater adherence to the 99th percentile guideline recommendation. With more widespread use of this cut-off point, it is likely that the number of patients being diagnosed with MI will increase (21-23). In parallel, the prevalence of observed cardiac biomarker elevations due to other causes is anticipated to escalate (15,21,24). Consequently, the improved diagnostic sensitivity that follows the use of these assays will be accompanied by poorer specificity. To avoid misdiagnosing patients with cardiac biomarker elevations from other mechanisms than ischemic myocardial necrosis, it is therefore important that at least one of the following additional criteria is fulfilled (14): (1) Symptoms of ischemia; (2) new (or presumably new) significant ST/T wave changes or left bundle branch block; (3) development of pathological Q waves on electrocardiogram (ECG); (4) imaging evidence of new loss of viable myocardium or regional wall motion abnormality; (5) identification of intracoronary thrombus by angiography or autopsy.

**Prognostic assessment**

Several characteristics have been associated with a poor prognosis in MI patients. This has led to the development of risk scores used to stratify individuals. The risk can be determined based on findings at admission (25-28), or upon discharge when incremental information obtained during hospitalization can be incorporated (29-32). These scores are well-validated tools used to assess in-hospital and long-term risk of mortality, or a composite of recurrent ischemia and mortality. Demographic information, prior medical history, findings from physical examination, ECG changes

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1 These criteria are used when a MI diagnosis is based on the detection of a rise and/or fall of cardiac biomarker values with at least one value above the 99th percentile upper reference limit. MI resulting in cardiac death when biomarkers are unavailable, MI related to percutaneous coronary intervention, MI related to stent thrombosis, MI related to restenosis, MI related to coronary artery bypass grafting and prior MI are diagnosed based on other criteria specific to each of these entities (14).
and biochemical biomarkers are typical important indicators. In addition, variables such as angiography findings, estimates of left ventricular function and occurrence of adverse events during the hospital admission may be included. However, they do not encompass measures of infarct size, which has been indicated as an important determinant of chronic left ventricular dysfunction and adverse events following MI (33,34). The elements of one such validated risk score for 30-day mortality in ST-segment elevation myocardial infarction (STEMI) are provided in Table 2 (25).

Table 2. TIMI Risk Score for STEMI.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 65-74</td>
<td>2</td>
</tr>
<tr>
<td>≥75</td>
<td>3</td>
</tr>
<tr>
<td>Diabetes, hypertension or angina</td>
<td>1</td>
</tr>
<tr>
<td>Systolic blood pressure &lt;100 mmHg</td>
<td>3</td>
</tr>
<tr>
<td>Heart rate &gt;100 bpm</td>
<td>2</td>
</tr>
<tr>
<td>Killip class II-IV</td>
<td>2</td>
</tr>
<tr>
<td>Weight &lt;67 kg</td>
<td>1</td>
</tr>
<tr>
<td>Anterior ST elevation or left bundle branch block</td>
<td>1</td>
</tr>
<tr>
<td>Time to treatment &gt;4 hours</td>
<td>1</td>
</tr>
<tr>
<td>Maximum score</td>
<td>14</td>
</tr>
</tbody>
</table>

5.3 Obstructive sleep apnea

History and epidemiology

While the clinical picture of alveolar hypoventilation syndromes as a character trait has been recognized for a long time, a proper understanding of their pathophysiology did not transpire until the 20th century. The “Pickwickian syndrome”, a condition closely related to OSA, was first introduced as a term by Sir William Osler in 1918 (35). Obesity, hypersomnolence, periodic breathing with hypoventilation and cor pulmonale were later defined as the classical features of this syndrome (36).

However, the interaction between respiration and sleep was not recognized to be of considerable importance until “multiple respiratory pauses during sleep” were recorded with a polysomnograph in 1965 (37). Shortly thereafter, “sleep-induced apneas” were documented in non-obese individuals and the first symposium on sleep-related respiratory problems established a new concept of “sleep-induced apnea
syndromes” in 1972 (38). The pathophysiology and clinical ramifications have since been subject to increased focus in the medical community. Today, OSA is regarded a distinct clinical entity among several different “sleep related breathing disorders” (39). It is a common condition more frequent with men and older age groups. Depending on the population studied and the definition applied for the diagnosis, the typical adult prevalence ranges from 2% to 30% (40-42).

Pathophysiology
OSA is characterized by repetitive abnormal breathing giving rise to disrupted ventilation during sleep (43). A recurrent collapse of the upper airway results in partial (hypopnea) or complete (apnea) cessation of airflow despite ongoing respiratory effort (Figure 1). This leads to intermittent disturbances in gas exchange and fragmented sleep (44). One respiratory event typically lasts 20-40 seconds and can occur more than 100 times per hour in severe cases (45). Components that likely underpin the occurrence or severity of OSA include upper airway anatomy, the ability of the upper airway dilator muscles to respond to respiratory challenge during sleep, arousal threshold, body habitus, stability of the respiratory control system and the potential for state-related changes in lung volume to influence these factors (45,46). Studies have indicated that obesity, increasing age and male gender are among the more significant predictors for the condition (43,47,48).
Cardiovascular consequences

In patients with OSA, the cardiovascular system is repetitively exposed to cycles of hypoxia, exaggerated negative intrathoracic pressure and arousal (44). This conglomerate of noxious stimuli may lead to depressed myocardial contractility, activation of the sympathetic nervous system, raised blood pressure, heart rate and myocardial wall stress, depressed parasympathetic activity, provoked oxidative stress and systemic inflammation, impaired vascular endothelial function, activated platelets and hypercoagulability (5). While the specific processes are still subject to research, current understanding suggests that at least some of these mechanisms contribute to causal pathways of CVD (Figure 2) (5,44,49). This is further supported by data indicating a significant association between OSA and several CVDs (3,4). Also, studies have demonstrated a lower risk or improvement of various surrogate markers in association with continuous positive airway pressure (CPAP) treatment (50-55).
Figure 2. Possible mechanisms resulting from noxious stimuli in obstructive sleep apnea.

5.4 Biomarkers

The National Institutes of Health Biomarkers Definitions Working Group has defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention" (56). Biomarkers can be classified into categories according to their inherent properties. Consequently, a “CVD biomarker” may be used to broadly address an indicator of a pathophysiological process implicated in CVD. Alternatively, an “inflammatory biomarker” may be used to describe a mechanism more explicitly.

The term "biomarker" is usually associated with measurements of substances in tissues or body fluids (57). However, it is evident from the definition that it encompasses a wide range of characteristics. This includes anything from body weight, blood pressure and heart rate to more complex metrics of ECG recordings and echocardiograms. It is essential that a biomarker can be “objectively measured”,

Illustration: Dylan H. Hargreaves, UWO
which implies that the obtained measure is accurate and reproducible. In the case of
blood samples, the following criteria have been proposed as properties of an ideal
biomarker (58,59): (1) An accurate, reproducible assay should be available at a
reasonable cost and have acceptable turnaround time; (2) the assay should be highly
sensitive and specific for the outcome it is expected to identify, and the test result
should explain a reasonable proportion of the outcome independent of established
predictors; (3) the test should be acceptable and understandable to the patient, and the
test results should be easily interpretable by clinicians; (4) very importantly,
knowledge of the biomarker’s levels should change patient management.

Biomarkers have many areas of use, and they already do enable better
management of numerous disease states in daily clinical practice. In fact, several
established applications, such as blood pressure and cholesterol level measurements,
have contributed to a steady decline in CVD morbidity and mortality in high-income
countries over the past decades (60). Nevertheless, current projections indicate that
this group of disorders will remain the leading cause of death worldwide (61). Thus,
investigating additional aspects of biomarkers associated with CVD remains of
considerable importance given the potential to further improve patient care.

5.5 Cardiac troponin
Troponin is a protein complex of three subunits. The units are named according to
their function; the calcium binding subunit (TnC), the inhibitory subunit (TnI) and the
tropomyosin-binding subunit (TnT) (62,63). Troponin regulates contraction and
relaxation through modulation of the calcium-mediated interaction between actin and
myosin in the human striated muscle (64). With TnI and TnT being expressed as
cardio-specific isoforms generally considered unique to the myocardium (cTnT and
cTnI), they are well suited for detection of heart muscle injury (65). Experimental
data have suggested that cTn leaks from the cell only in instances of membrane
disruption following myocyte necrosis (66). However, the detection of a brief rise and
subsequent fall of cTn concentration during marathon running (67), as well as a rise
after transient stress test-induced myocardial ischemia (68), has cast doubt on the
hypothesis that cTn is released only upon irrevocable necrotic damage. In fact,
various additional mechanisms have been suggested as causes for liberation of cTn
from the myocardium. These include increased physiological cell turnover, apoptosis,
formation and release of membranous blebs, cellular release of troponin degradation products and increased cellular wall permeability (69). Cardiac troponin is currently the biomarker of choice for the diagnosis of MI due to superior sensitivity and specificity when compared to the alternative biomarkers for myocardial cell death (14). Typical release kinetics, with an early peak followed by a more prolonged, slowly abating plateau, can be observed following onset of infarction (Figure 3). While the peak is more influenced by cTn from a loosely bound pool, the second phase mainly originates from degradation of the contractile apparatus (18). A few out of many examples of other conditions associated with elevated cTn levels include heart failure, myocarditis, renal insufficiency and pulmonary embolism (Figure 4) (17,70). Thus, detection of circulating cTn molecules can also be applied more broadly as a biomarker of myocardial stress, but do not define the underlying cause (70). Consequently, in addition to being a central diagnostic aid in establishing MI, it has been shown that the biomarker possesses strong prognostic properties in several other CVDs (71).

**Figure 3.** Cardiac troponin in myocardial infarction.

Adapted and translated from Atar (15). Reproduced with permission. © Kari C. Toverud CMI (board Certified Medical Illustrator).
5.6 Myeloid-related protein-8/14 and C-reactive protein

Myeloid-related protein-8/14 (MRP-8/14) is a heterodimer with proinflammatory characteristics expressed by monocytes, neutrophils and platelets. It is a complex of two calcium-binding proteins that possess intra- and extracellular activity (MRP-8 [S100A8] and MRP-14 [S100A9]). MRP-8 and MRP-14 are displayed by monocytes upon interaction with activated endothelium and reflect phagocyte stimulation (72,73). They are members of the S100 family of proteins, which is involved in the inflammatory response through, in part, activation of the receptor for advanced glycation end-products (74,75). In animal studies, MRP-8/14 is critical for the biological response to vascular injury (76). Thus, MRP-8/14 concentrations in plasma or serum may be a useful biomarker of inflammation (77). Circulating MRP-8/14 has previously been shown to be associated with inflammatory disorders and MI and to be...
an independent predictor of cardiovascular events in healthy subjects and patients with acute coronary syndrome (72,78-83).

C-reactive protein (CRP) is a homopentamer synthesized by hepatocytes in response to factors produced by adipose tissue and inflammatory cells (84-90). There have also been reports of potential CRP expression in other locations, including fat and the coronary artery smooth muscle (91-93). CRP has been implicated in several important host defense, scavenging and metabolic functions, particularly through its capacity to activate complement after binding exogenous and autologous molecules (84,85). For many years, it was considered a nonspecific acute-phase response to most forms of inflammation, infection and tissue damage, but has later also been established as a useful biomarker of chronic low-grade inflammatory activity (84). CRP has been shown to be an independent predictor of cardiovascular events in apparently healthy individuals and of recurring events or death in patients with established CVD (94-97).
6. AIMS

The aim of this thesis was to explore novel aspects of circulating biomarkers associated with CVD, with an emphasis on MI and OSA.

Specific aims:

- To examine characteristics of non-ST-segment elevation myocardial infarction (NSTEMI) diagnostics before and after the implementation of a high-sensitivity cardiac troponin T assay (hs-cTnT) into clinical practice (paper I)

- To determine the prognostic value of various cTnI variables for prediction of clinical outcomes and cardiac function during three months follow-up after primary PCI for STEMI (paper II)

- In individuals investigated for possible OSA, to characterize myocardial stress by using a single-molecule cTnI (S-cTnI) and a hs-cTnT assay and explore the association to apnea-hypopnea index (AHI) and oxygen assessment variables recorded during polysomnography (PSG) or polygraphy (PG) (paper III)

- In individuals investigated for possible OSA, to characterize inflammation by using a MRP-8/14 and a CRP assay and explore the association to AHI and oxygen assessment variables recorded during PSG or PG, as well as to investigate whether body mass index (BMI) interacts with these associations (paper IV)
7. METHODS

7.1 Sample and design

Three study samples were used to explore novel aspects of biomarkers associated with CVD, with an emphasis on MI and OSA.

Paper I

Hospital admissions over two one-year periods were investigated in a retrospective, national, single-center, pre-post comparison study at Lovisenberg Diakonale Hospital’s medical department; the first period was from 1st of August 2007 to 31st of July 2008 (catchment’s population 145000 people) and the last period from 1st of August 2009 to 31st of July 2010 (catchment’s population 155000 people). A hs-cTnT assay used for diagnostic assessment in the last period was introduced in the hospital on the 1st of April 2009, whereas a conventional cTnT assay was used in the first period. All registered hospital stays in the two periods were evaluated to compare the occurrence of primary NSTEMI admissions and various properties of these admissions. The study was conducted according to the principles of the Declaration of Helsinki and the Uniform Requirements for Manuscripts Submitted to Biomedical Journals by the International Committee of Medical Journal Editors. It was approved by the hospital’s local ethics committee and the privacy protection act supervisor.

Paper II

Subjects receiving PCI for STEMI in the prospective, international, multi-center, phase II, randomized placebo-controlled PROTECTION AMI trial were studied in a post hoc analysis. In this trial, patients with suspected STEMI presenting within 6 hours of pain onset were included in 114 hospitals in 18 different countries between December 2008 and June 2010. Patients were randomized to the experimental agent delcasertib or placebo infusion on top of contemporary care. Follow-up endpoints included clinical events and assessment of cardiac function at three months. As no clear treatment effect of delcasertib had been observed, the randomization groups were combined in the present analysis. ECG inclusion criteria were \( \geq 2 \) mm of ST elevation in at least two contiguous precordial leads (V1-V4) or \( \geq 2 \) mm ST elevation in two inferior leads (II, III, aVF) with ST depression in two other contiguous leads.
(total ≥8 mm ST deviation). Exclusion criteria included left bundle branch block or paced rhythm, prior coronary artery bypass grafting, persistent systolic blood pressure <90 mmHg unresponsive to intravenous fluids, fibrinolysis within 72 hours before presentation and end stage renal disease requiring dialysis. The protocol was approved at the institutional review board of each participating hospital, and all subjects provided written informed consent.

**Paper III and IV**

Consecutive individuals referred by general practitioners or Ear-Nose-Throat specialists to the sleep laboratory for evaluation of possible OSA from October 2009 through February 2010 were considered for inclusion in a single-center, cross-sectional study at Lovisenberg Diakonale Hospital. Participants were excluded if they had known OSA, if the sleep study indicated another type of sleep-disordered breathing than OSA or if there was no serum sampled for biobank (due to technical difficulties or if they did not consent). The study was conducted according to the principles of the Declaration of Helsinki and the Uniform Requirements for Manuscripts Submitted to Biomedical Journals by the International Committee of Medical Journal Editors. It was approved by the hospital’s local ethics committee, the privacy protection act supervisor, the Norwegian Directorate of Health and the regional ethics committee. All subjects provided written informed consent.

**7.2 Hospital admission data**

The hospital admission parameters evaluated in paper I were retrieved retrospectively. Discharge diagnoses had been coded for all medical department admissions by the treating physician using ICD-10 and, together with registered laboratorial data and selected demographics, this information was extracted from the hospital database by a blinded physician using Qlikview (Qlik Technologies Inc., Radnor, PA, USA).

In all admissions with a registered diagnosis of MI (ICD-10 code I21 and I22), the DIPS electronic medical record system (DIPS ASA, Bodø, Norway) was then used to review patient charts and ECG recordings. This allowed for identification of primary NSTEMI admissions and registration of relevant pre-defined variables. A primary NSTEMI admission was defined if a patient with ECG findings not consistent with STEMI was admitted directly from the ambulance service, emergency
department, outpatient clinic, surgical department or general practitioner. All other MI stays, such as transfers from other hospitals, readmissions after coronary angiography investigations and others, were not eligible for classification as a primary NSTEMI admission.

Coronary angiographies were performed at a secondary care facility (Oslo University Hospital) upon referral by the clinician, and were defined as acute if the patients were transferred directly from our hospital to the secondary care facility following a primary NSTEMI admission. The findings were classified using pre-specified criteria by evaluating the written reports from these examinations.

The highest cTnT level was defined as the highest concentration of measured cTnT during the hospital stay. To describe cTnT dynamics it was defined to calculate the relative change by using the lowest registered measurement before and after the highest concentration, with subsequent selection of the highest relative change from these two calculations. The biomarker characteristics were then categorized into pre-specified groups to allow comparison.

7.3 Clinical endpoints and cardiac function assessment

PROTECTION AMI protocol-defined outcome data were used in paper II.

Clinical endpoints (death, cardiogenic shock during the index hospitalization, congestive heart failure (CHF) or serious arrhythmia) were collected through three months follow-up. Cardiogenic shock during the index hospitalization was defined as a systolic blood pressure of <90 mmHg for >30 minutes or the need for supportive measures such as inotropic agents, in combination with evidence of end-organ hypoperfusion. CHF was defined if clinically present or developing ≥48 hours after admission (from Killip class I) or post-discharge if admitted to a health institution >12 hours for CHF. Serious arrhythmia was defined as sustained ventricular tachycardia >30 seconds occurring >48 hours after admission or ventricular fibrillation requiring pharmacologic treatment, external electrical cardioversion or cardiopulmonary resuscitation occurring >48 hours after admission. All clinical events were adjudicated by an independent committee.

Serum N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels were measured in all patients and left ventricular ejection fraction (LVEF) was estimated by multi gated acquisition scan in anterior infarctions for assessment of cardiac
function at three months. The results were analyzed and reported by central core laboratories.

### 7.4 Sleep study

Participants evaluated in paper III and IV were examined with overnight in-home PG or in-hospital PSG. The in-home, unattended PG was performed with a standard 10-channel cardiorespiratory recording device (Embleta PDS, ResMed, Høvik, Norway) or a 12-channel monitor with a nasopharyngeal/oesophageal catheter measuring flow and pressure in the upper airways and indirectly the intrathoracic pressure (Reggie, Camtech, Høvik, Norway). The attended, in-hospital PSG (Embla S4500, ResMed) included a six-channel electroencephalogram, a two-channel electrooculogram, submental electromyogram (EMG), thoracic and abdominal movements (respiratory inductance plethysmography), air flow (nasal air pressure catheter), pulse oximetry, EMG from both legs, body position and a three-channel ECG.

The data from the sleep study was scored by a qualified sleeping disorder specialist not involved in the study. A modified version of the 2007 American Academy of Sleep Medicine criteria for scoring respiratory events was used (98). Apnea was measured via transformed airflow signals from nasal pressure and defined as cessation of airflow ≥10 seconds. Hypopnea was defined as a 50% reduction in airflow with either a ≥3% oxyhemoglobin desaturation or an arousal or presumed arousal (an increase of 10% in heart rate). The AHI was calculated based on the total number of events per hour of total recording (PG) or sleep (PSG) time (movement time omitted). Oxygen desaturation index (ODI) was estimated by using the average number of desaturations of ≥3% per hour.

OSA was defined as AHI ≥5 (symptoms of sleepiness were not considered).

### 7.5 Biomarker analysis

**Cardiac troponin T (paper I and III)**

Electrochemiluminescent immunoassays on a Modular Analytics E170 platform from Roche Diagnostics (Roche, Basel, Switzerland) were used for analysis of cTnT at Lovisenberg Diakonale Hospital. The conventional assay was Elecsys® Troponin T (paper I) and the high-sensitivity assay was Elecsys® hs TnT (paper I and III). For the conventional assay, the manufacturer designates the lower detection limit at 10 ng/L,
the limit of quantification (LoQ) at 30 ng/L and the 99th percentile at <10 ng/L. For the high-sensitivity assay, the manufacturer designates the limit of blank (LoB) at <3 ng/L, the limit of detection (LoD) at 5 ng/L, the LoQ at 13 ng/L and the 99th percentile at 14 ng/L (99). During in-house quality-control analyses, the laboratory found a coefficient of variation (CV) of 8.3% at a concentration of 72 ng/L with the conventional assay, and a CV of 4.7% at a concentration of 34 ng/L with the high-sensitivity assay.

**Cardiac troponin I (paper II and III)**

A paramagnetic particle, chemiluminescent, two-site immunoenzymatic Access® AccuTnI® assay (Beckman Coulter, Fullerton, CA, USA) was used by a blinded core laboratory for analysis of cTnI (paper II). The assay has been reported to have a lower detection limit value at 0.006 μg/L, a LoQ value at 0.014 μg/L and a 99th percentile value at 0.04 μg/L (100,101). Curve fitting was performed by a separate core laboratory according to a previously described methology for generation of peak and area under curve (AUC) values (102-104). Briefly, subjects had to have at least three values, one of which had to be drawn prior to the estimated peak, to derive a curve and determine estimated peak and AUC.

An Erenna system (Singulex, Inc., Alameda, CA, USA) based on microcapillary single-molecule counting combined with a microparticle immunoassay technology located at the Biomarker Research/TIMI Clinical Trials Laboratory (Brigham and Women’s Hospital, Boston, MA, USA) was used for the single-molecule assay analysis of cTnI (paper III) (105). The assay has been standardized and validated in accordance with the National Institute of Standards and Technology material, with a reported LoB at <0.088 ng/L, a LoD at 0.091 ng/L, a LoQ at 0.88 ng/L and a 99th percentile at 10.19 ng/L in a healthy reference population (106,107).

**Myeloid-related protein-8/14 (paper IV)**

Batch analysis of MRP-8/14 was performed at Lovisenberg Diakonale Hospital using a commercially available MRP8/14 enzmelinked immunosorbent kit (Bühlmann Laboratories AG, Schönenbuch, Switzerland). According to the manufacturer, this assay has an analytical range of 0.4–24 μg/mL, an intra-assay CV of 4.3% (20 pairs of values from seven different serum samples each obtained in a single run) and an inter-
assay CV of 5.8% (four samples each in 20 different runs). For a normal population, the expected median value for serum is 1.14 µg/mL with a 95th percentile at 2.9 µg/mL (considered the upper limit of the normal reference range). These values were estimated by the manufacturer from apparently healthy male and female blood donors aged 18–70 years. All samples were analyzed in duplicates by an experienced biomedical laboratory scientist blinded to study data, with a reported average CV of 4.0%. Readings <0.4 µg/mL were interpreted as undetectable.

C-reactive protein (paper IV)
An immunoturbidimetric Tina-quant C-reactive Protein Gen. 3 (CRPL3) assay on a Modular Analytics P platform by Roche Diagnostics (Roche, Basel, Switzerland) was used for quantification of CRP at Lovisenberg Diakonale Hospital. According to the packet insert, this assay has an analytical range of 0.3–350 mg/L and a designated normal reference range <5 mg/L (108). The laboratory considered concentrations <1.0 mg/L as undetectable, and reported between-run CVs of 2.3% at 4.3 mg/L and 2.9% at 73.2 mg/L.

7.6 Statistical methods
General statistics
The data were approached statistically by a pre-defined limited set of analyses.

Continuous data are presented as mean ± standard deviation or median (quartile 1-3) depending on the distribution. Categorical data are presented as number and/or percent. Comparisons of continuous data were done using the two-sample t-test (if normally distributed) or the Mann-Whitney U-test (if skewed). Categorical data were compared by the chi-square test, the Fisher’s exact test, the Fisher-Mid-P-test or the Cochran-Armitage trend test.

Spearman rank correlation or simple median regression was used to assess univariable associations of variables. A cox proportional hazard, logistic or median regression was used to determine if variables were independent predictors of a dependent variable in multivariable models. The discriminative value of variables was explored by use of logistic regression models through comparison of C-statistics and evaluation of integrated discrimination improvement (IDI).
A P-value of <0.05 was regarded statistically significant, and all hypothesis testing was two-tailed.

Specific statistics
In paper I, the denominators in categorical comparisons were the total number of medical department admissions not classified as a primary NSTEMI admission during each period (for the number of primary NSTEMI admissions and the number of acute angiographies), the total number of acute angiographies in each period (for the angiography findings) and the total number of primary NSTEMI admissions in each period (for the biomarker criteria). The statistical analyses were performed with OpenEpi (http://www.openepi.com/oe2.3/menu/openepimenu.htm) and PASW Statistics 18 (IBM SPSS Inc., Chicago, USA).

In paper II, multivariable models were applied to determine if cTnI variables (log,-transformed) were independent predictors of clinical outcomes, NT-proBNP and LVEF. Clinical risk factors comparable to the TIMI Risk Score assessment were used for adjustment and included age, female gender, hypertension, diabetes, prior heart failure, prior MI, systolic blood pressure, heart rate, weight, Killip class category, anterior infarct location and time from symptom onset to PCI (25). A clinical event composite (death, cardiogenic shock during the index hospitalization, CHF or serious arrhythmia), NT-proBNP >118 pmol/L (approximate threshold associated with increased risk in chronic heart failure) and LVEF <40% (moderate to severe reduction in left ventricular function) were used as dependent variables (109,110). Cox proportional hazards regression models were used to estimate hazard ratios and 95% confidence intervals (CI) for the time to clinical events. Logistic regression models were used to estimate odds ratios with 95% CIs for NT-proBNP >118 pmol/L or LVEF <40%. The discriminative properties of cTnI were explored by C-statistics of binary logistic regression models for the dependent variables (111). Each model containing a cTnI variable was compared to a model only containing clinical risk factors and bootstrapping was used to generate P-values. The IDI and relative IDI were also computed to quantify and test the contribution to predictive ability provided from each cTnI variable assessed (112,113). The statistical analyses were performed with the SAS System, version 9.2 (SAS Institute Inc., Cary, North Carolina, USA).

In paper III, multivariable median regression analyses were performed to investigate simultaneous associations of several predictors of S-cTnI or hs-cTnT and...
in a final step AHI or an oxygen variable was forced into the model. The covariates used were age, male sex, BMI, hypertension, CVD, diabetes and estimated glomerular filtration rate. The statistical analyses were performed with PASW Statistics 18 (IBM SPSS Inc., Chicago, USA) and STATA version 12.1 (StataCorp LP, Texas, USA).

In paper IV, multivariable median regression analyses were performed to investigate simultaneous associations of several predictors of MRP-8/14 or CRP. Three models were designed to evaluate the characteristics of the potential associations between variables of disordered breathing during sleep and inflammation: (1) A baseline model to account for age and sex; (2) a BMI model to account for age, sex and BMI; (3) a final model to account for age, sex, BMI, systolic blood pressure, cholesterol:high-density lipoprotein ratio, glycosylated haemoglobin, smoking and CVD. The potential interaction between BMI and variables of disordered breathing during sleep on inflammation was probed by addition of an interaction term (variable of disordered breathing during sleep x BMI) to the final models. Undetectable values of MRP-8/14 (<0.4) and CRP (<1.0) were imputed using uniformly distributed random values on the intervals 0.0-0.4 (MRP-8/14) and 0.0-1.0 (CRP). This imputation method was preferred after no noteworthy disagreements were observed when compared to sensitivity analyses using extreme case scenarios. The statistical analyses were performed with PASW Statistics 18 (IBM SPSS Inc., Chicago, IL, USA) and STATA version 12.1 (StataCorp LP, College Station, TX, USA).
8. RESULTS

8.1 Paper I
Characteristics of NSTEMI diagnostics before and after the implementation of a hs-cTnT assay into clinical practice were examined. There was a significant increase in primary NSTEMI admissions when using the hs-cTnT assay (225 vs. 341, risk ratio 1.57, 95% CI 1.33 to 1.85), and the number of coronary angiographies was higher (58 vs. 81, P < 0.05). Furthermore, significantly more patients were examined without signs of coronary artery disease (CAD) (0% vs. 8.6%, P < 0.05), and a smaller proportion had significant dynamic cTnT changes between the highest and lowest cTnT measurement during each admission (96.2% vs. 88.7%, P < 0.01).

8.2 Paper II
The prognostic values of various cTnI variables for prediction of outcomes in 1066 STEMI patients treated with primary PCI were determined. In adjusted models, all post-PCI single-points, peak and AUC were found to be independently associated with clinical events, NT-proBNP >118 pmol/L or LVEF <40% (P for all < 0.001). When cTnI was added individually to a baseline risk factor model for prediction of clinical events, the C-statistic improved from 0.779 to 0.846 (16-24 hours) and 0.859 (70-80 hours). Quantified by the IDI, the addition of cTnI significantly augmented prediction ability (relative IDI 44-154%, P for all ≤ 0.001). Consistent improvements in discrimination of NT-proBNP >118 pmol/L and LVEF <40% were observed.

8.3 Paper III
Myocardial stress and its association to disordered breathing during sleep were characterized by use of novel cTn assays. All 222 (100%) individuals had detectable levels using either assay, and the patients with OSA (AHI ≥5, n = 161) had a different distribution of S-cTnI (P = 0.036) and hs-cTnT (P = 0.002) compared to those without (AHI <5, n = 61). However, in multiple median regression analyses adjusted for conventional predictors, neither S-cTnI (P = 0.57) nor hs-cTnT (P = 0.80) was significantly associated with AHI. Substituting oxygen variables with AHI in the multivariable models did not change the overall result for S-cTnI, whereas average
oxygen saturation (P = 0.009) and time <90% oxygen saturation (P = 0.017) remained statistically significantly associated with hs-cTnT.

**8.4 Paper IV**

Inflammation and its association to disordered breathing during sleep were characterized by use of MRP-8/14 and CRP assays. In baseline models (adjusted for age and sex), AHI was independently associated with MRP-8/14 (P = 0.025) and CRP (P < 0.001). The associations were attenuated after the addition of BMI, but remained statistically significant for CRP (P = 0.025). However, in final models adjusted for additional factors, only average oxygen saturation for MRP-8/14 (P = 0.028) and ODI for CRP (P = 0.037) remained independent predictors of inflammation, whereas AHI lost its predictive value (MRP-8/14; P = 0.30 and CRP; P = 0.092). The association between several variables of disordered breathing during sleep and inflammation was stronger in individuals with a higher BMI (P for interaction < 0.05 for AHI, nadir oxygen saturation and time <90% oxygen saturation).
9. DISCUSSION

9.1 Discussion of methods

Sample and design

Hospital admissions from two one-year periods were evaluated in a retrospective, national, single-center, pre-post comparison study in paper I. The retrospective nature with no parallel control group is a design with limitations, and the presence of spurious findings cannot be excluded. On the other hand, one may argue that ethical constraints associated with the superior performance characteristics of the hs-cTnT assay would have made it difficult to perform a prospective, adequately powered, controlled trial with similar research questions. Furthermore, our study represents a unique evaluation of true clinical practice that has quality assessment value and provides relevant information to the process of optimizing care of patients. We consider the sample sufficiently sized for the majority of the characteristics evaluated. However, some degree of interpretive prudence is warranted due to the unexpectedly low number of acute angiographies, which increases the potential for incidental observations in the classification of angiographic findings. The fact that a large proportion of primary NSTEMI admissions were older individuals who likely had co-morbidities could explain the modest referral rate to angiography. Evidently, this characteristic also represents a restriction to both external and internal validity.

Moreover, while the external validity of the study was enhanced by its reasonably large scale and realistic setting (114), it was conducted in one center which further limits generalizability. The internal validity was strengthened by exogenous exposure to the new assay, causing reduced propensity to interventional selection bias and the Hawthorne effect (115). This must be weighed against unmeasured properties potentially influencing the components unequally in the two periods. Most notably, these include unevaluated patient characteristics, natural temporal trends and bias being introduced by physicians being more cognizant of measuring cTn concentrations after the new assay and revised cut-off point had been implemented (116,117).

Subjects receiving PCI for STEMI in a randomized trial were studied in a post hoc analysis in paper II. The nature of performing an analysis post hoc increases the probability of finding false relationships (118). At the same time, randomized trials
often give researchers a unique opportunity to explore additional scientific questions beyond its primary hypothesis. In fact, it has been argued that investigators have an ethical and financial obligation to design and conduct trials in a fashion that maximizes its scientific capacity (119). A significant strength of the present study was the sample size, which was adequate for assessment of the predictive value of cTnI for clinical endpoints. Nevertheless, we appreciate that the total number of clinical events was modest and that extrapolating our findings to STEMI populations with higher event rates may not be appropriate. Indeed, randomized trials tend to include motivated patients with less co-morbidity, which imposes limitations to both external and internal validity. Our sample did not include NSTEMIs, there were fewer women than men, they were relatively young and the number of inferior infarctions was small, all of which restrict generalization even further. On the other hand, the described STEMI cohort reflects in a high degree a typical STEMI population encountered in daily clinical practice (120). If implementation into clinical practice is considered, one must also acknowledge the cTnI between-assay variation (121). In parallel, it remains evident that the strict inclusion and exclusion criteria facilitated a more streamlined and comparable hospital flow for all subjects, which, in turn, reduces the influence of background noise. This must be contrasted to numerous opposing threats to internal validity, including unexplored potential discrepancies in discharge medication, unknown effects of the experimental agent on the variables that were investigated and that the models for prediction of cardiac function did not account for the competing risk of death during follow-up.

Individuals referred to the sleep laboratory for evaluation of possible OSA were examined in a single-center, cross-sectional study in paper III and IV. A single-center study has limited external applicability and the cross-sectional design precludes interpretation of causal relations. We consider the size of the sample reasonably large. Nevertheless, due to uncertainties related to background noise and temporal relations between the independent predictors included in the models, it is still difficult to rule out the possibility of type 2 errors. A potential selection bias, reducing both external and internal validity, may have been introduced by participants made ineligible for inclusion if they had known OSA or if there was no serum sampled for biobank (due to technical difficulties or if they did not consent). It would also have been preferable to have a more balanced gender and age distribution. Moreover, since the subjects in the investigation were referred by a doctor based on a suspected sleeping disorder, we
cannot exclude that those who were found not to have OSA had a higher prevalence of undiagnosed medical conditions when contrasted to the general population. This may have influenced the results of our analyses and reduces the validity of our findings.

Data collection and quality
There are aspects of the data reported in paper I warranting consideration. First, we sought to limit information bias through implementing standardized procedures with few investigators involved in data collection. Additionally, a staff member not involved in the study validated all inputs during database building. Despite these efforts, we appreciate that the retrospective design, with researchers aware of the subjects exposed to the new assay, could have incited bias during data processing. Moreover, since classification of primary NSTEMI admissions was based on patients registered in the database upon discharge, it is possible that some admissions were omitted due to miscoding and that the extent of such errors was inconsistent across the two periods. Also, variations in biomarker sampling properties and differences between doctors performing the angiographies may have been present. For biomarker characteristics one should note that if a patient presented >24 hours after onset of symptoms, a rise and/or fall pattern was not absolutely required for a diagnosis of MI (17). As this was not considered in the study, it could be a source of error. Furthermore, if a time limit had been defined between the two measurements used for calculating dynamic changes, a higher proportion could have failed to qualify for the 20% relative change criterion. Thus, some of the values may have had more hours between them than what would be appropriate.

The post hoc analysis of patients from a randomized trial in paper II has a significant strength in the sense that the data quality is quite robust. All data points had been prospectively defined and registered by an independent research organization. Moreover, independent central core laboratories were used for estimation of biochemical and cardiac function indices, while an independent committee adjudicated all clinical events. Nevertheless, the international and multi-center feature imposes vulnerability to variations in both biomarker and LVEF sampling properties, as well as differences in how clinical events were assessed. Also, the analytical limitations of the utilized assay represent a possible source of inaccurate
cTnI data (122), while a potentially unbalanced distribution of missing values might have influenced the results for the cTnI variables explored.

Paper III and IV evaluate cross-sectional data collected at only one time point susceptible to individual variability and random errors. Similar to the other studies, we cannot rule out the presence of pre-analytical differences during biomarker sampling. Since it was possible to perform multivariable regression analyses without undetectable cTn values and contrast the results of two independent methods, we consider the quantification of myocardial stress through the use of novel assays an advantage. Even so, we acknowledge the more limited reliability of the hs-cTnT assay in the lower concentration range. Moreover, the release kinetics of cTn molecules after myocardial stress may have caused the peak concentration to be reached later than the time of blood sampling, which could have induced a bias in cTn results. For the MRP-8/14 measurements, current knowledge of serum sampling in adults investigated for OSA is limited. We therefore consider the concomitant analyses of CRP beneficial, because it allowed us to assess the MRP-8/14 values in the context of a well-established marker of inflammation. The method used for estimation of MRP-8/14 concentrations has been validated by the manufacturer with acceptable performance characteristics, and the analyses were performed by an experienced biomedical laboratory scientist blinded to study data. Nevertheless, we cannot exclude the presence of analytical errors, especially given limited experience with analyses of MRP-8/14 after storage at −80 °C (123). We tried to limit inconsistency by having a qualified sleeping disorder specialist not involved in the study scoring the sleep studies. Still, several potential sources of error remain, including differences in sleep quality, total recording time and favored body position between PG and PSG. Additionally, since the electroencephalogram from a PSG allows for exact determination of sleep time and respiratory arousals, it is possible that the calculated indices were underestimated in individuals examined with a PG.

Statistical methods
In paper I, the statistics were based on conventional methods; we performed a basic comparison of means, medians or proportions with appropriate significance tests. Since our statistical aim was to solely describe and compare pre-defined observations in two periods, the use of more advanced statistical methodologies were not deemed necessary.
Odds ratio, hazard ratio, C-statistics and IDI were used to assess prognostic information obtained from various cTnI variables presented in paper II. Multivariable logistic or Cox proportional hazards regression models are well-established methods used to explore if increasing biomarker levels signify a statistically significant increment in risk of an adverse outcome. However, the distributions of a biomarker in subjects with and without an adverse outcome often overlap. Increased attention has therefore recently been put on additional methods to assess whether a biomarker provides incremental information to overall prediction (57,124). The C-statistic is a rank-order statistic of a model that is interpreted through a pair of subjects with and without the outcome in question. It corresponds to the ROC-AUC (area under the receiving operating curve) of a test, which indicates the probability across the spectrum of cut-off values that a case will have a higher value than a non-case (111). In addition, the discrimination slope of a model can be calculated as the absolute difference in average predictions for those with and without the outcome (125). This difference in mean of predictions is a simple measure of how well subjects with and without an outcome are separated by a model, i.e. the discriminatory ability is quantified by a degree of separation from average predictions. The IDI is equivalent to the difference in discrimination slopes between two models, and it attempts to add to C-statistics by quantifying the change in separation of probabilities in cases and non-cases after addition of a new variable (112). The IDI measure is dependent on the rate of cases and is in some instances perceived as an abstract number to comprehend. Therefore, the relative IDI can be used as an alternative measure. This corresponds to how much predictive ability that has been added to the discrimination slope of the model that includes the variable, described as a percent value of the discrimination slope of the model without the variable (hence "relative") (113). Statistical methods are available to compare the C-statistics and the difference in discrimination slopes of two models (for example with and without a biomarker). On this background, estimations and tests of C-statistics and IDI measures were performed, and the results were presented along with hazard ratios or odds ratios in paper II.

Median regression was used to explore associations of variables presented in paper III and IV. Median regression is similar to linear regression. Both models describe the association(s) between a continuous dependent variable and one or more explanatory variables. Linear regression makes inference about the mean of the dependent variable, whereas median regression makes inference about the median.
Model-building techniques and general regression features, such as the ability to study interactions and non-linear effects, are available for median regression. Thus, due to the distributions of the variables in the dataset, it was considered preferable to perform the analyses of associations presented in paper III and IV by use of median regression.

9.2 Discussion of results

_Cardiac troponin_

Since assays for detection of cTnI and cTnT were developed in the 1980s, the role of cTn in identifying necrosis following infarction of the myocardium has become firmly established (126,127). Several other applications of cTn measurements have since been discovered. Among the more recent are its uses in risk prediction when sampled after PCI and the assessment of subjects with other disorders or from the general population (71,128-136). In the present work, we evaluated characteristics of NSTEMI diagnostics after a hs-cTnT assay was implemented into clinical practice, and explored the value of different cTnI variables for prediction of clinical events and cardiac function following PCI for STEMI. Furthermore, we demonstrated the enhanced ability of novel cTn assays to identify myocardial stress in subjects investigated for possible OSA, and examined the associations between these biomarkers and variables of disordered breathing during sleep. Thus, the thesis entails a broad spectrum of findings adding new knowledge relevant to each of the abovementioned applications.

Paper I describes several imperative changes in NSTEMI diagnostics that followed the introduction of a new hs-cTnT assay. The study illustrates a continuous improvement in the biomarker field, which, in turn, facilitates an ongoing innovation to the operational characteristics of an MI. We found that an increased number of NSTEMI admissions occurred in the last period. This was accompanied by more coronary angiographies being performed, and a higher proportion of these showed no signs of CAD. Moreover, significant dynamic cTnT changes were somewhat less frequent. The increase in NSTEMI diagnoses associated with the use of hs-cTnT and a lower cut-off point is supported by findings from other types of populations (21,22,137). However, by evaluating diagnostics in a large and more unselected group over two one-year periods, the present study is distinctive in that it scrutinizes the
increase in primary NSTEMI admissions following the introduction of this assay into real world hospital practice. Although it was to be predicted that the use of a lower cut-off point would result in more NSTEMIs, the extent of change was difficult to foresee, especially since the difference in assay calibration would likely also contribute to the increase. This latter additive component implies that the high-sensitivity assay uniformly shows higher values in the lower concentration range, resulting in more subjects qualifying for MI diagnosis at a certain cut-off level (99). Thus, if the same cut-off concentration had been applied in the two periods, it is probable that this positive bias would still have led to more patients being diagnosed with NSTEMI in the period when hs-cTnT was used (138). The exact fraction of the increment observed in our study potentially attributable to this assay disparity remains unknown. In any event, if the hospital were to lower the cut-off point further to the international guideline-recommended 99th percentile concentration of the hs-cTnT assay, our findings support that the intensified detection of potential ischemic myocardial necrosis would likely result in an even higher number of patients being diagnosed with NSTEMI (23).

The study also sheds light on other aspects by examining angiography and biomarker characteristics. In association with the greater number of individuals clinicians had to evaluate due to elevated cTnT levels, it was a concern in the medical community that the high-sensitivity assay would result in more patients being misdiagnosed. Therefore, since additional properties of these admissions could elucidate features indicative of deficient diagnostic accuracy in the last period, they were of considerable interest. Through our assessment of angiographies, we demonstrated a larger proportion without signs of CAD, but noted that the number was small and that these patients may have had a “Myocardial Infarction with Normal Coronary Arteries” (139). Irrespective of this uncertainty, many would uphold that the most noteworthy finding was that the majority in both periods actually was consistent with pathological changes. However, in further reviewing biomarker characteristics, we also identified a higher number of patients with smaller dynamic cTnT changes in the last period. This may imply that more patients lacked a significant rise and/or fall pattern, which, according to the guidelines, was a prerequisite for the diagnosis (17). The extent of dynamic change required is an ongoing debate (140-143). Also, since it may improve sensitivity and specificity, some have promoted the use of absolute instead of relative changes (144). We chose a relative concentration change of 20% in
our study, consistent with the level used in some previous studies (22,145). Others have promoted the use of a higher differential, especially at low baseline cTn levels (146). When calculating relative changes, these studies applied serial measurements taken a few hours after admission. We used the highest and lowest registered values on each admission, without considering the timeline between measurements. No consensus currently exists with regard to the level of dynamic change representing ischemic myocardial necrosis with certainty. While the abovementioned findings may support that a higher number of patients were misdiagnosed in the last period, the use of the high-sensitivity assay may still be preferable since a larger number of patients were identified and subsequently referred to a higher level of care. This, in turn, presumably led to reduced morbidity and mortality (137). Moreover, our investigation suggests that more patients will have to be considered for potentially having increased cTnT levels due to other etiologies than MI. Consequently, the results from the study underscore the importance of meticulous attention and coherence to the diagnostic criteria for MI during assessment of patients, to minimize the risk of misdiagnoses (14).

In paper II, an evaluation of the prognostic value of cTnI is presented. The study strongly supports the perception that cTnI is a clinically useful risk stratification tool. In exploring the relationship between multiple cTnI measurements and comprehensive three-month outcome assessments in contemporary PCI-treated STEMI patients, it is the largest of its kind. Previous work has established that infarct size is a main determinant of future risk following MI (33,147), and shown that single-point or derived measures of cTn are easy available surrogates for infarct size (148-151). Thus, we designed various models to determine the prognostic value of different cTnI variables in prediction of adverse clinical events and follow-up cardiac dysfunction. The results do validate other investigations that have explored the association between cTn and clinical outcomes or left ventricular indices (149,151-156). At the same time, the study adds to previous findings through its extensive evaluation of multitudinous cTnI variables. Moreover, it demonstrates the significant incremental discriminative value of the biomarker and examines the relationship between index event cTnI values and NT-proBNP concentrations sampled at three months. NT-proBNP is currently being tested as a tool in biomarker-guided treatment of chronic heart failure in a prospective, large, multi-center, randomized controlled trial (GUIDE-IT), with a chosen threshold of 118 pmol/L. Our analyses indicate that
cTnI significantly improves discrimination of follow-up NT-proBNP concentrations above this threshold value. The present work therefore represents provisional evidence for a potential future application in identifying at-risk individuals possibly qualifying for biomarker-guided heart failure treatment following STEMI.

For the purpose of risk prediction in STEMI patients treated with primary PCI, the overall pattern of the results presented in paper II may be interpreted to support the superiority of single-point measurements from 16-24 hours and beyond compared to earlier time points. This is in agreement with previous publications that have reported comparable findings using other assays (149,152). The difference among the derived variables were less distinct, but the results for AUC were consistently better than peak in all models. The time curve of cTnI concentrations after reperfusion is characterized by a peak at \( \leq 12 \) hours as the cytosolic pool is rapidly washed out, followed by a slowly abating plateau as the structural pool is gradually liberated (129,149). It is plausible that variables reflecting degradation of the structural pool are more closely correlated to permanent myocardial injury. Thus, measures more influenced by cTnI from the cytosolic pool may perform poorer in predicting outcomes. Since CIs were broad and no direct statistical comparisons between cTnI variables were made, this explanation remains speculative and not specifically tested in our investigation. Many would however contend that the clinical applicability is ultimately determined by the accuracy of the prognostic information, the time at which this information is available and how feasible it is to obtain. The results from the present analyses are therefore also of great value in this respect, since they suggest that the majority of the explored cTnI variables can be applied to significantly enhance risk stratification of these patients. As such, to advance more informed decisions regarding patient management to the initial phase of hospital admission, earlier single-points may be preferred by some clinicians.

In recent years, following several investigations that have determined the significant predictive value of cTn in numerous non-ischemic acute and chronic disorders, its role in prognostic assessment in other diseases beyond MI has evolved substantially (71,134,135). Furthermore, through the use of refined assays that facilitate recognition of miniscule myocardial injuries previously undetectable with earlier generation assays, it has been shown that cTn can be identified in a much larger proportion of the general population and is independently associated with the risk of adverse outcomes in such subjects (130-132). As a consequence of this
enhanced ability to delineate myocardial status in more healthy individuals, the potential utility in other areas has become a developing field of intense research. In paper III, an accession to this innovative application is reported through analyses of circulating cTnI and cTnT in individuals investigated for possible OSA. The study examines the presence of myocardial stress through utilization of S-cTnI and hs-cTnT assays and explores the associations to variables of disordered breathing during sleep. It is to the best of our knowledge the first description of quantified cTn concentrations in 100% of subjects evaluated for the disorder.

OSA is associated with several phenotypical risk indicators previously linked to cTn, such as heart failure, BMI, CAD and diabetes (3,4,43,157-159). Thus, since other concomitant factors likely contributed to the degree of myocardial stress present, it was unsurprising that our findings were consistent with OSA patients having a different distribution of cTn. Nevertheless, there are several additional mechanisms discussed in literature conceivably making the myocardium more vulnerable to repetitive minor stress in OSA. These include hypoxia, increased left ventricular transmural pressure, pulmonary hypertension, sympathetic activation, sub-clinical arrhythmia, blood pressure surges, tachycardia, oxidative stress, inflammation, endothelial dysfunction, platelet activation and hypercoagulability (49). Still, an independent association between severity of OSA and increasing cTn levels had not been demonstrated in comparable study samples when our investigation was initiated (160,161), and none had reported concurrent cTnI and cTnT measurements. Importantly, the quantification of cTn molecules in these previous studies had been hampered by assay limitations at lower concentration levels. On this background, to allow for a more exhaustive and definite evaluation of a potential association with worsening OSA, a better measure of myocardial stress was desirable. Through the increased sensitivity and precision of the S-cTnI assay, we were able to overcome this impediment. The assessment was further reinforced by the hs-cTnT analyses, albeit somewhat less definite, since a higher proportion of values were below the assay’s LoQ. Despite the use of innovative assays, which facilitated a more rigorous estimation of myocardial status in all participants, our investigation was consistent with earlier studies in not demonstrating an independent relationship between AHI and cTn levels (160,161).

Provided that the abovementioned mechanisms were operational in our study sample and are truly causative of significant myocardial stress in OSA, it is evident
that they were not adequately reflected by AHI if one assumes that other predictors were appropriately accounted for in the multivariable analyses. Hypoxic exposure is a component where such a concern is reasonable; potentially being a key determinant of myocardial cell stress not directly proportional with disordered breathing *per se*. The duration of the apneas and hypopneas may also be of importance and this information is not provided by the AHI measure. Alternatively, upholding that it is a true association between the two variables, it may have been inconsistently masked by unmeasured confounders or too weak to be identified. Nevertheless, recently published studies have reported conflicting evidence for the potential link between increasing AHI and myocardial stress; some have demonstrated an independent relationship in multivariable models (162,163), while another did not find a significant association after adjustment for confounding factors (164). Moreover, when contrasted to ODI and nadir oxygen saturation, stronger associations of average oxygen saturation and time below 90% oxygen saturation to cTn were found in our study. This may suggest that chronic rather than intermittent hypoxia is more closely related to myocardial cell stress. Interestingly, a possible protective effect through ischemic preconditioning of the myocardium in OSA patients with an established MI has been posited in a recent report (165). The theory is supported by experimental animal studies (166). Intermittent phases of hypoxia or other unknown effects of disordered breathing have been hypothesised as stimulators of collateral blood flow through angiogenic mediators, possibly facilitating improved blood flow and myocardial salvage (167,168). Studies exploring cTn in relation to possible myocardial stress in other transient settings such as exercise stress testing have also revealed conflicting results (68,169-171). It is conceivable that related mechanisms may have been operative in our study sample, thus precluding a pattern of increasing cTn levels in patients with more severe AHI. However, this interpretation must be appraised with great caution, as the degree of confounding was not sufficiently investigated. Nevertheless, the paradigms described support a more thorough exploration of different categories of hypoxia and its subsequent effects in the future.

Similar to previous data from other types of populations, we observed discordance between measured cTnI and cTnT concentrations in our study of individuals investigated for possible OSA (133,169,172). The same finding has also recently been reported in an analysis of subjects examined for possible OSA from the general population (162). Different release kinetics due to a smaller molecular size of
cTnI versus cTnT, together with unequal post-translational modification, could be contributing factors to disharmonious cTn concentrations at a given time point (70,173). Furthermore, the disparate properties of the assays may in itself explain the discordance observed. However, there may be subtle differences in etiologies underpinning liberation of cTnI versus cTnT from the myocyte and clearance mechanisms from the circulation (133). Hence, one cannot exclude that the variation at least in part reflects true biological heterogeneity. We also observed that more patients measured above the 99th percentile with the S-cTnI assay. This finding is in agreement with current evidence, suggesting that cut-off points differ depending on the characteristics of the group examined to obtain them (107,174,175). Nonetheless, for both assays applied in our study, it was documented that most individuals had cTn levels within the normal reference range.

Myeloid-related protein-8/14 and C-reactive protein

Paper IV reports a shift in focus from myocardial stress to inflammation. Inflammation is a component implicated as a possible causal pathway of CVD in OSA. While previous work has demonstrated that children with OSA have elevated levels of MRP-8/14 (176,177), data from adults is lacking. Conversely, CRP has been studied quite extensively in adults with OSA, but with conflicting results (178-181). The present investigation is therefore of interest as it evaluates, in a reasonably large sample of subjects investigated for possible OSA, the associations between variables of disordered breathing during sleep and two disparate indicators of inflammatory activity. In addition, it provides new knowledge by probing potential interactions between variables of disordered breathing during sleep and BMI on inflammation.

Recurrent bouts of hypoxia, arousal and increased negative intrathoracic pressure represent a conglomerate of noxious stimuli with detrimental effects in patients with OSA (5,44,49). Thus, it is theoretically plausible that the disorder may promote immune system activation and increased levels of inflammatory biomarkers. In agreement with this conception, AHI was observed to be associated with both MRP-8/14 and CRP in our baseline models, and it remained a predictor of CRP also after adjustment for BMI. However, the association between AHI and biomarker concentrations did not remain statistically significant in final models, which included additional components previously linked to inflammation (182-184). Different study designs and sample sizes may have contributed to the diverse results of prior studies.
exploring inflammation in OSA (178-181). In particular, the role of potential confounders has proven evasive, since the specific causal pathways potentially linking apneas and hypopneas, inflammation, other components and CVD remain complex and not fully elucidated. The results from our cross-sectional investigation must therefore be interpreted with great caution, as we cannot be certain that the factors included for adjustment were the appropriate selection for this specific setting. Moreover, intermittent hypoxia is currently believed to be the main activator of inflammatory pathways in OSA (5,44,49). Hence, with this constituent not directly proportional to the number of apneas and hypopneas \textit{per se}, our lack of association may be due to inaccuracy of the AHI variable in reflecting intermittent hypoxic exposure in each of the subjects. Furthermore, given that the AHI variable does not reflect the duration of each apnea and hypopnea, we cannot exclude the possibility that inflammatory activity is more dependent on the length than number of events.

The associations of various metrics of oxygen saturation with inflammatory biomarker levels differed between MRP-8/14 and CRP. In the final models, average oxygen saturation was an independent predictor of MRP-8/14 levels, while ODI was a predictor of CRP. These findings support that MRP-8/14 and CRP have different pathways of expression and/or breakdown patterns, which is further endorsed by their modest correlation. Nuclear factor kappa-B mediated pathways have been proposed as possible triggers of inflammation in patients with OSA (44). MRP-8/14 is secreted mainly by neutrophils and monocytes (185), but the underlying mechanisms remain less understood. Traditionally, CRP has been regarded synthesized by hepatocytes in response to factors produced by adipose tissue and inflammatory cells (84,86-90). Intriguingly, extra-hepatic tissues such as smooth muscle and fat have also been demonstrated to express CRP (91,93). In light of these observations, it is interesting that we found a quantitative interaction between BMI and several variables of disordered breathing during sleep on both biomarkers. A recent publication has described a possible interaction between obesity and OSA on interleukin-6 and CRP (178). Thus, these findings support the hypothesis that adipose tissue may be involved in CRP production, but one cannot exclude that it is an indirect effect promoting increased synthesis from hepatocytes. Why a similar phenomenon was observed for MRP-8/14 is not known. Nonetheless, the results from our analyses support that individuals with higher BMI demonstrated an amplified inflammatory response in association with worsening levels of several of the variables of disordered breathing.
during sleep. Thus, patients with OSA may have had activated inflammatory pathways inconsistently masked by unmeasured confounders or too diminutive to be significant in this study sample.
10. CONCLUSION

The aim of this thesis was to provide further insight into novel aspects of circulating biomarkers associated with CVD, with an emphasis on MI and OSA.

- The thesis indicates that the introduction of a hs-cTnT assay enhanced NSTEMI diagnostics, while in parallel implying that more patients will have to be considered for potentially having increased cTnT levels due to other etiologies than MI.

- The dissertation demonstrates that a single measurement of cTnI after PCI provides incremental information to prediction of clinical events and cardiac function following STEMI.

- Through quantification of myocardial stress in all individuals investigated for possible OSA, the thesis confirms that the improved performance characteristics of S-cTnI and hs-cTnT are broadening potential applications of cTn assays.

- The observed interaction between a higher BMI and several variables of disordered breathing during sleep may indicate activated inflammatory pathways inconsistently masked by unmeasured confounders or too moderate to be significant in this study sample. Still, the dissertation suggests that the future clinical utility of the investigated biomarkers appears limited for the diagnostic evaluation of possible OSA.

The findings expand our knowledge of these biomarkers and contribute to determine their future role in patient care.
Measurements of circulating biomarkers are increasingly being implemented as essential tools in the detection of disease, assessment of prognosis and monitoring of responses to interventions. The high rate of new discoveries and refinements in assay technology are accompanied by a continuous search for innovative clinical applications. This thesis has attempted to provide clinically relevant insights into several aspects of circulating biomarkers associated with CVD. First, it has illustrated salient issues related to NSTEMI diagnostics following the use of a high-sensitivity assay, which are highly relevant to the assessment and care of patients in current hospital practice. Our results suggest that the introduction of more sensitive cTn assays increases the demands on the physician in the diagnostic process. Thus, meticulous attention to the criteria for MI, as well as sound clinical judgement, has become even more important. Second, the dissertation demonstrates that a single measurement of cTnI after PCI for STEMI provides significant incremental information to risk prediction. We consider the findings of this study of great value to doctors involved in prognostication and treatment of such patients today. Potentially, cTn may also be used to aid in selecting individuals to be considered for inclusion in clinical trials aimed to improve outcomes in similar populations (a principle termed “trial population enrichment”). Third, through estimation of myocardial stress in all individuals who participated in a study where they were investigated for possible OSA, the thesis has exemplified that the enhanced performance characteristics of S-cTnI and hs-cTnT are broadening the potential applications of cTn assays. Although the future clinical utility of all the biomarkers explored appears limited for the diagnostic assessment of the disorder, our comprehension of possible pathophysiological mechanisms have been improved through the contemporary descriptions of myocardial stress and inflammation in the sample studied.

An ideal biomarker should aid the clinician in diagnosis, prognosis and treatment. It should be readily available and adequately tested, have an established reference value compared to a “gold standard”, have a known sensitivity and specificity level, a rapid turnaround time and not be costly (186). Our work has provided results that expand our knowledge base and help to define the future potential applications of the biomarkers investigated. Recognizing that the presented
findings are one piece of a larger puzzle, there are additional aspects related to our research questions that will be important to investigate in subsequent studies. In our exploration of hospital admissions, we demonstrated that the introduction of a refined assay and a lower cut-off point was associated with several changes relevant to NSTEMI diagnostics. A while after the paper was published and following updated recommendations from a national expert group, our hospital along with several others in Norway further reduced the cut-off point for MI to the 99th percentile concentration of the hs-cTnT assay. To maximize the quality of care offered to patients, the implications of such a change in real world hospital practice will be important to examine in future studies. Our findings in STEMI patients, while novel, fit very well with previous analyses. The results are biologically plausible and consistent with current mechanistic insights. Thus, prospective trials in more unselected populations, validating the use of cTn for prediction of clinical events and determining the exact implementation strategy to risk scoring tools, are needed before introducing the present risk prediction concept on a broader scale. Moreover, we envision that testing a cTnI biomarker-guided approach, for purposes of implementing an aggressive anti-remodeling strategy before discharge in patients at increased risk of heart failure at three months, could prove beneficial. The relative value of the predictive information provided by cTnI, when considered with additional outcome-related biomarkers, also remains uncertain. For example, it will be important to simultaneously assess cTnI with markers such as angiographic flow and blush, NT-proBNP, ST-segment recovery and reperfusion ventricular arrhythmia “bursts”. Additionally, our study of individuals investigated for possible OSA has confirmed that the development of novel assays has expanded the potential area for use of cTn measurements in the field of medicine. However, the value of stratifying subjects based on cTn values within the normal reference range will vary between populations. Specific studies addressing these differences will be essential to better define the clinical utility. Evidently, the cross-sectional design of the study makes it challenging to establish causal relationships and conclusions concerning pathophysiological pathways in OSA. Therefore, one cannot rule out that mechanisms entailing myocardial stress or inflammation are operative and contribute to an increased risk of CVD events. In view of two recent publications demonstrating an independent relationship between OSA and myocardial stress (162,163), specific mechanistic studies of pathophysiological pathways and the potential protectiveness of ischemic
preconditioning would be important to increase our understanding (187). Similarly, in light of meta-analyses indicating that inflammatory biomarkers are higher in OSA and may partially be suppressed by CPAP treatment (50,188), it appears additional studies are needed before drawing definite conclusions. The clinical ramifications of the potential interaction with BMI on inflammation also remain unknown and clarification of this possible phenomenon would be helpful. Indisputably, meticulous attention to study design, size, participant selection and the methods used to evaluate parameters will be essential in upcoming trials to clearly establish whether noxious stimuli resulting from disordered breathing during sleep are truly causative of myocardial stress and inflammatory activity in patients with OSA.
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