Unrecognized myocardial infarction and cardiac biochemical markers in patients with stable coronary artery disease
Dedication
Till min pappa Karl-Evert Berg.
Jag älskar och saknar dig.
ANNA NORDENSKJÖLD

Unrecognized myocardial infarction and cardiac biochemical markers in patients with stable coronary artery disease
Abstract

Aim: The overarching aim of the thesis was to explore the occurrence and clinical importance of two manifestations of myocardial injury; unrecognized myocardial injury (UMI) and altered levels of cardiac biochemical markers in patients with stable coronary artery disease (CAD).

Methods: A prospective multicenter cohort study investigated the prevalence, localization, size, and prognostic implication of UMI in 235 patients with stable CAD. Late gadolinium enhancement cardiovascular magnetic resonance (LGE-CMR) imaging and coronary angiography were used. The relationship between UMI and severe CAD and cardiac biochemical markers was explored. In a substudy the short- and long-term individual variation in cardiac troponins I and T (cTnI, cTnT) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) were investigated.

Results: The prevalence of UMI was 25%. Subjects with severe CAD were significantly more likely to exhibit UMI than subjects without CAD. There was a strong association between stenosis ≥70% and presence of UMI in the myocardial segments downstream. The presence of UMI was associated with a significant threefold risk of adverse events during follow up. After adjustments UMI was associated with a non-significant numerically doubled risk. The levels of cTnI, NT-proBNP, and Galactin-3 were associated with the presence of UMI in univariate analyses. The association between levels of cTnI and presence of UMI remained significant after adjustment. The individual variation in cTnI, cTnT, and NT-proBNP in subjects with stable CAD appeared similar to the biological variation in healthy individuals.

Conclusions: UMI is common and is associated with significant CAD, levels of biochemical markers, and an increased risk for adverse events. A change of >50% is required for a reliable short-term change in cardiac troponins, and a rise of >76% or a fall of >43% is required to detect a long-term reliable change in NT-proBNP.

Keywords: Unrecognized myocardial infarction, Coronary artery disease, Prevalence, Prognosis, Troponin, NT-proBNP, Galactin-3

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<th>Description</th>
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<tr>
<td>A</td>
<td>ACS</td>
<td>acute coronary syndrome, AHA; American Heart Association, AMI; acute myocardial infarction.</td>
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<td>B</td>
<td>BNP</td>
<td>brain natriuretic peptide.</td>
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<td>C</td>
<td>CABG</td>
<td>coronary artery bypass grafting, CAD; coronary artery disease, CCS; Canadian Cardiovascular Society, CI; confidence interval, CMR; cardiovascular magnetic resonance, cTn; cardiac troponin, cTnI and cTnT; cardiac troponin I and T, CV; coefficient of variation, CVa; analytical CV, CVi; intra-individual CV, CVt; total CV, CVg; inter-individual CV.</td>
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<td>E</td>
<td>ECG</td>
<td>electrocardiography, EDTA; ethylene diamine tetra acetic.</td>
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<td>F</td>
<td>FFR</td>
<td>fractional flow reserve.</td>
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<tr>
<td>G</td>
<td>Gal-3</td>
<td>Galectin-3, GEE; generalized estimating equation, GFR; glomerular filtration rate.</td>
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<tr>
<td>H</td>
<td>HF</td>
<td>heart failure, hs; high sensitivity.</td>
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<td>I</td>
<td>II</td>
<td>index of individuality, IQR; interquartile range.</td>
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<td>L</td>
<td>LAD; LBBB</td>
<td>left anterior descending artery, LBBB; left bundle branch block, LCX; left circumflex coronary artery, LDL; low-density lipoprotein, LoB; limit of blank, LoD; limit of detection, LGR; late gadolinium enhancement, LGE-CMR; late gadolinium enhancement cardiovascular magnetic resonance, LVEF; left ventricular ejection fraction.</td>
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<td>M</td>
<td>MACE</td>
<td>major adverse cardiac event, MI; myocardial infarction.</td>
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<td>N</td>
<td>NSTEMI</td>
<td>non ST-elevation myocardial infarction, NT-proBNP; N-terminal pro-B-type natriuretic peptide.</td>
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<td>O</td>
<td>OR</td>
<td>odds ratio.</td>
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<tr>
<td>P</td>
<td>PCI; PUMI</td>
<td>percutaneous coronary intervention, PUMI; Prevalence and Prognostic Value of Unrecognized Myocardial Injury in Stable Coronary Disease.</td>
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<td>R</td>
<td>RCA; RCV</td>
<td>right coronary artery, RCV; reference change value, RMI; recognized myocardial infarction.</td>
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<tr>
<td>S</td>
<td>SCAAR</td>
<td>Swedish Coronary Angiography and Angioplasty Registry, STEMI; ST-elevation myocardial infarction, SD; standard deviation.</td>
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<td>T</td>
<td>Tn; TnI</td>
<td>troponin, TnC, troponin C, TnI; troponin I, TnT; troponin T.</td>
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<tr>
<td>U</td>
<td>UMI</td>
<td>unrecognized myocardial infarction (in this thesis synonymous with LGE-CMR detected UMI), URL; upper reference limit.</td>
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INTRODUCTION

Ischemic heart disease, and especially one of its manifestations, myocardial infarction (MI), is a major cause of morbidity and mortality worldwide. The diagnosis of acute MI is based on the combination of a change in biochemical markers of myocardial injury (currently, cardiac troponins), characteristic symptoms, and/or electrocardiography (ECG) findings typical of myocardial ischemia [1]. Occasionally the MI results in lasting ECG-changes, such as pathological Q-waves, that can be an indication of a previously experienced MI.

However, despite the tests available, the interpretation of results is not always straightforward, and the diagnosis is not always correct. Many MIs are missed, due to, along with other factors, the absence of distinct symptoms and/or misinterpretation of the ECG or measures of biochemical markers.

Recently, highly sensitive methods for detection of myocardial injury have been developed including late gadolinium enhancement cardiovascular magnetic resonance (LGE-CMR) and high-sensitivity assays for cardiac troponins.

Cardiac imaging with LGE-CMR has proven to be more sensitive for the detection of unrecognized MI (UMI) than ECG [2]. However, little information is available regarding the clinical importance and prognostic implications of an LGE-CMR detected UMI.

The high-sensitivity assays for cardiac troponins have made it possible to reliably detect very low levels of troponins in most healthy individuals and thus quantitatively small troponin elevations in cardiac patients. With the new techniques, it has become essential to be able to discriminate low levels of cardiac troponins of clinical relevance from those that reflect biological variations.

In the studies presented in this thesis, two manifestations of myocardial injury are investigated, LGE-CMR detected UMI and levels of cardiac biochemical markers, in patients with stable coronary artery disease.
BACKGROUND

This section comprises:

(i) a general introduction to coronary artery disease (CAD), including stable angina pectoris, myocardial infarction (MI), and unrecognized myocardial infarction (UMI);

(ii) characterization of the cardiac biochemical markers troponin (cTn), N-terminal pro-B-type natriuretic peptide (NT-proBNP), and Galectin-3 (Gal-3);

(iii) an overview of late gadolinium enhancement cardiovascular magnetic resonance (LGE-CMR).

Coronary artery disease

Coronary artery disease (CAD) is common in the general population [1] and the single most common cause of death worldwide. Over seven million people die from CAD annually, accounting for 13.2% of all deaths [3].

Coronary artery disease exists in an acute state; acute coronary syndrome (ACS), i.e. acute myocardial infarction (AMI) and unstable angina pectoris and as stable CAD, i.e. stable angina pectoris. CAD is unpredictable and may change over time, with periods differing in degree of stability.

Risk factors

Large epidemiological studies conducted half a century ago identified several factors associated with CAD: older age, male sex, lifestyle, diet, elevated blood pressure, higher cholesterol level, and cigarette smoking [4, 5]. A more recent large case-control study investigating risk factors for AMI in subjects from 52 countries confirmed that the most important risk factors are smoking, dyslipidemia, hypertension, diabetes, abdominal obesity, psychosocial factors, diet, and physical inactivity. Combined, these factors accounted for over 90% of the risk for AMI [6]. In addition, history of development of atherosclerotic cardiovascular disease in a parent or sibling prior to age 55 in males or 65 in females is considered an important risk factor [7].
Atherosclerosis

Atherosclerosis is a dynamic process exhibiting multiple stages: intimal thickening, fibrous cap atheroma formation, thin-cap fibroatheroma formation, and plaque rupture/erosion. Atherosclerotic lesions tend to occur in proximal sections of arteries, downstream of branch points or bifurcations at flow dividers [8]. Arteries without many branches, such as the internal mammary or radial arteries, tend not to develop atherosclerosis [9]. The atherosclerotic process begins in childhood, and advanced lesions occur with increasing frequency with aging [10].

At the beginning of the atherosclerotic process, low-density lipoproteins (LDL) accumulate in the arterial wall, where they are modified by oxidation [9, 11]. Modified LDL particles activate the immune system and initiate inflammation [12]. Leukocytes adhere to the endothelium and pass between endothelial cell junctions to enter the intima [9]. The leukocytes mature into macrophages that absorb lipids and expand, forming “foam cells,” until they become unstable and may undergo apoptosis. As the lesions expand, smooth muscle cells migrate into, and proliferate within, the intima. Such smooth muscle cells are susceptible to apoptosis, which is associated with further macrophage accumulation and micro-vesicles that can calcify [13]. Large accumulations of necrotic acellular lipid-rich material may develop into confluent necrotic cores [14].

As atherosclerotic plaques develop and expand, they develop their own microvascular network extending from the adventitia through the media and into the thickened intima [15]. The thin-walled vessels are prone to disruption, leading to hemorrhage within the plaque and contributing to the progression of coronary atherosclerosis [16].

Fibrous cap atheromas are defined as plaques with a well-defined lipid core, sometimes necrotic, covered by a fibrous cap that, may be relatively acellular or may be rich in smooth muscle cells [11]. The fibrous cap atheroma may develop into a lesion causing substantial luminal stenosis.

Thin-cap fibroatheroma, also referred to as a vulnerable plaque, exhibits a large necrotic core separated from the lumen by a thin fibrous cap (Figure 1). The fibrous cap is heavily infiltrated by macrophages and, to a lesser extent, by T-lymphocytes [17]. Pathology has identified thin-cap fibroatheromas as precursor lesions to ruptured plaques [11]. A sudden plaque
rupture leads to a loss of the integrity of the protective single layer of endothelial cells. The disruption exposes the highly thrombogenic structures beneath to circulating platelets and coagulation factors, and a thrombus may form at the site of rupture [11]. Thrombus formation may lead to total or partial occlusion of the artery and development of ACS. Most of the rupture prone plaques causes initially only mild to moderate stenosis in the affected artery [18, 19]. Plaque rupture may also be silent and show no obvious clinical symptoms [20].

Healed lesions occur at sites of prior rupture with thrombus formation that may or may not have been symptomatic. Healed ruptures often exhibit multiple layers of necrotic cores interspersed with fibrous tissue [20]. Autopsy has shown that 73% of plaques associated with stenosis reducing the artery diameter >51% involved prior healed disruption, whereas 19% of plaques causing 21-50% stenosis and 16% of plaques causing less than 20% stenosis showed prior disruption [21]. Repeat silent ruptures and thrombosis, followed by wound healing, may lead to progression of atherosclerosis with an increase in plaque burden and stenosis and negative arterial remodeling [20].

Plaques in coronary arteries may remain asymptomatic, they may become obstructive and bring about stable CAD, and/or they can rupture, causing ACS.

\[\text{Figure 1. Atherosclerotic plaque. Pathobiologic and local hemodynamic features of high-risk (rupture prone) plaque. Reprinted with permission from Elsevier [22].}\]
**Stable coronary artery disease**

**Symptoms**
Patients with stable CAD may or may not suffer from symptoms of stable angina pectoris. Stable angina pectoris, is typically defined as substernal chest discomfort of characteristic quality and duration provoked by exertion or emotional stress and relieved within minutes by rest and/or nitrates [23]. In addition to substernal chest discomfort, associated symptoms as dyspnea and nausea are common. Silent ischemia without symptoms may occur.

**Pathophysiology**
The clinical presentation of stable CAD is associated with underlying mechanisms that primarily include [23]

(i) plaque-related obstruction of epicardial arteries  
(ii) focal or diffuse spasm of normal or plaque-diseased arteries  
(iii) microvascular dysfunction  
(iv) left ventricular dysfunction due to prior myocardial necrosis and/or hibernating myocardium.

Exercise- and stress-related chest symptoms from CAD are considered to be associated with ≥50% narrowing of the left main coronary artery and ≥70% in one or more other major coronary arteries [23].

Hibernating myocardium is a state where some segments of the myocardium exhibit abnormalities of contractile function, usually due to chronic ischemia that is potentially reversible by revascularization. The regions of myocardium are still viable and can return to normal function.

**Prevalence**
Stable CAD is multifaceted, and the prevalence is difficult to estimate; reported numbers vary depending on the definition used. In population-based studies, the prevalence of stable angina pectoris increases with age, from 5-7% in women aged 45-64 years to 10-12% in women aged 65-84 years and from 4-7% in men aged 45-64 years to 12-14% in men aged 65-84 years [23].
Prognosis
Current information regarding prognosis for patients with stable CAD has been derived from clinical trials of anti-anginal and preventive therapy and/or revascularization; hence the data may be biased by the selective nature of the populations studied. Estimates of annual all-cause mortality range from 1.2 to 2.4\% [24-29], with an annual incidence of cardiac death from 0.6 to 1.4\% and non-fatal MI from 0.6-2.7\% [23]. These estimates are consistent with data of registries [30]. In a Swedish cohort study, the cardiovascular mortality rate in patients with stable CAD was found to be 1.3\% per year, with a non-fatal event-rate rate of 7.1\% per year [31]. A recent study, comparing two cohorts with stable CAD found the annual rate of cardiovascular events, defined as MI, stroke, or cardiovascular death, to be 2.2\% in one cohort and 3.4\% in the other [32].

The aim of the management of stable CAD is to reduce symptoms, prevent cardiovascular events and improve prognosis. Guideline recommends lifestyle modification, control of CAD risk factors and evidence-based pharmacological therapy [23].

Coronary intervention
Meta-analyses and randomized trials involving subjects with stable CAD have shown that percutaneous coronary intervention (PCI) in addition to medical therapy does not reduce the risk of death, MI, or other major cardiovascular events compared to medical management alone [24, 33, 34]. However, in the mentioned studies, the subjects were highly selected. Individuals with high grade proximal stenosis in the left anterior descending coronary artery (LAD), heart failure, or previous revascularization were excluded, making it difficult to generalize the results to all subjects with stable CAD. Nevertheless, coronary interventions with PCI and coronary artery bypass grafting (CAGB) reduce symptoms of stable CAD [23, 35].
Myocardial infarction

Definition
In current guidelines, the term acute myocardial infarction (AMI) is used when there is evidence of myocardial necrosis in a clinical setting consistent with acute myocardial ischemia [1]. Q waves or QS complexes in the absence of QRS confounders are considered to be evidence of prior MI in patients with ischemic heart disease, regardless of symptoms [1] (Table 1). Additional definitions exist for MIs related to PCI, stent thrombosis and CABG [1].

Classification
MI is classified based on pathological, clinical, and prognostic factors [1] (Table 2).
Table 1. Definition of myocardial infarction

Criteria for acute myocardial infarction

The term acute MI should be used when there is evidence of myocardial necrosis in a clinical setting consistent with acute myocardial ischemia. Under these conditions any one of the following criteria meets the diagnosis for MI:

- Detection of a rise and/or fall of cardiac biomarker values [preferably cTn] with at least one value above the 99th percentile URL and with at least one of the following:
  - symptoms of ischemia,
  - new or presumed new, significant ST-segment–T wave (ST–T) changes or new LBBB.
  - development of pathological Q waves in the ECG.
  - imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.
  - identification of an intracoronary thrombus by angiography or autopsy.

- Cardiac death with symptoms suggestive of myocardial ischemia and presumed new ischemic ECG changes or new LBBB, but death occurred before cardiac biomarkers were obtained, or before cardiac biomarker values would be increased.

Criteria for prior myocardial infarction

Any one of the following criteria meets the diagnosis for prior MI:

- pathological Q waves with or without symptoms in the absence of non-ischemic causes
- imaging evidence of a region of loss of viable myocardium that is thinned and fails to contract, in the absence of a non-ischemic cause
- pathological findings of a prior MI

cTn; cardiac troponin, ECG; electrocardiography, LBBB; left bundle branch block, MI; myocardial infarction, URL; upper reference limit Adapted with permission from Oxford University Press [1].
Table 2. Classification of myocardial infarction

<table>
<thead>
<tr>
<th>Type 1: Spontaneous myocardial infarction</th>
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<td>Spontaneous MI related to atherosclerotic plaque rupture, ulceration, fissuring, erosion, or dissection with resulting intraluminal thrombus in one or more of the coronary arteries leading to decreased myocardial blood flow or distal platelet emboli with ensuing myocyte necrosis. The patient may have underlying severe CAD but occasionally non-obstructive or no CAD.</td>
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<th>Type 2: Myocardial infarction secondary to an ischemic imbalance</th>
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<td>Myocardial injury with necrosis in which a condition other than CAD contributes to an imbalance between myocardial oxygen supply and/or demand, e.g. coronary endothelial dysfunction, coronary artery spasm, coronary embolism, tachy-/brady-arrhythmias, anemia, respiratory failure, hypotension, or hypertension with or without left ventricular hypertrophy.</td>
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<th>Type 3: Myocardial infarction resulting in death when biomarker values are unavailable</th>
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<td>Cardiac death with symptoms suggestive of myocardial ischemia and presumed new ischemic ECG changes or new LBBB, with death occurring prior to obtaining blood samples, before rise in cardiac biomarkers, or in rare cases in which cardiac biomarkers were not collected.</td>
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<th>Type 4a: Myocardial infarction related to percutaneous coronary intervention</th>
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<td>MI associated with PCI is arbitrarily defined by elevation of cTn values &gt;5 x 99th percentile URL in patients with normal baseline values or a rise of cTn values &gt;20% if the baseline values are elevated and are stable or falling. In addition, (i) symptoms suggestive of myocardial ischemia, or (ii) new ischemic ECG changes or new LBBB, or (iii) angiographic loss of patency of a major coronary artery or a side branch or persistent slow- or no-flow or embolization, or (iv) imaging demonstration of new loss of viable myocardium or new regional wall motion abnormality are required.</td>
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<th>Type 4b: Myocardial infarction related to stent thrombosis</th>
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<td>MI associated with stent thrombosis is detected by coronary angiography or autopsy in the setting of myocardial ischemia and with a rise and/or fall of cardiac biomarkers values with at least one value above the 99th percentile URL.</td>
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<th>Type 5: Myocardial infarction related to coronary artery bypass grafting</th>
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<td>Arbitrarily defined by elevation of cardiac biomarker values &gt;10 x 99th percentile URL in patients with normal baseline cTn values. In addition, either (i) new pathological Q waves or new LBBB, or (ii) angiographic documented new graft or new native coronary artery occlusion, or (iii) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.</td>
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CAD, coronary artery disease; ECG, electrocardiogram; LBBB, left bundle branch block; MI, myocardial infarction; PCI, percutaneous coronary intervention; URL, upper reference limit. Adapted with permission from Oxford University Press [1].
Symptoms
Myocardial infarction may produce a variety of symptoms. Chest pain with or without referred pain into arms, jaw, or the back are common, and may be accompanied by dyspnea and fatigue [1]. An MI can pass without distinct symptoms, and may be ignored or misinterpreted by patients and health care professionals. Such clinically unrecognized MIs (UMI) are common [36, 37] and may be discovered at a later stage by changes in the ECG, on imaging or at autopsy.

Incidence
The incidence of MI in Sweden in 2013 was 400 per 100,000 inhabitants [38]. MIs are conventionally divided into two groups according to the initial presentation on ECG: ST-segment elevation MI (STEMI) and non ST-segment elevation MI (NSTEMI). The estimated proportion of STEMIs is 30% of all MIs [39].

Prognosis
The mortality in patients with MI is highest within the first week of the event and gradually declines, reaching a more stable level of risk after 1-2 months. When controlled for age, there are no clear sex-related differences in mortality [39]. There is a higher mortality rate in the acute stage of STEMI than in NSTEMI. In the longer term, the survival trends cross over, resulting in a slightly poorer prognosis for NSTEMI compared to STEMI [39].

According to the Swedish National Board of Health and Welfare, 19% of all individuals with MI die within 24 hours following an event, the majority before arrival at a hospital [38]. A further 9% die within 28 days [38]. In patients admitted to hospital and recorded in the SWEDEHEART registry, the mortality rate after 30 days was 1.5% of patients below age 60 years, 3% at 60-70 years, 6% at 70-80 years, and 14% of those over the age of 80 years [39].

The Swedish National Board of Health and Welfare has reported that 36.2% of all patients with MI (33% of men and 41% of women) die within one year [38]. In patients recorded in the SWEDEHEART registry, the mortality rate was 3% of those below age 60, 7% at 60-70 years, 14% at 70-80 years, and 32% of those over the age of 80 years [39].
**Pathological characteristics**

Myocardial infarction is defined as myocardial cell death due to prolonged ischemia. The consequences of ischemia occur in a sequence that includes increased hydrogen and potassium concentrations in the venous blood that drains the ischemic area; signs of ventricular diastolic and, subsequently, systolic, dysfunction along with regional wall motion abnormalities; development of ST-T changes; cardiac ischemic pain; and necrosis [23]. Myocardial cell death develops within 20 minutes of onset of myocardial ischemia. Complete necrosis of myocardial cells at risk requires 2-4 hours or longer, depending on the collateral circulation to the ischemic area, persistent vs. intermittent coronary artery occlusion, the sensitivity of the myocardium to ischemia, preconditioning, and individual myocardial cell demand for oxygen and nutrients [1]. Irreversible injury begins in the subendocardium and progresses to the subepicardial layer. This reflects the higher oxygen consumption of the subendocardium and the redistribution of collateral flow to the outer layers of the heart at reduced coronary pressure [9]. The relationship between the area at risk of ischemia is inversely related to the collateral flow [9]. The size of MIs may vary considerably. Large MIs are associated with an increased risk for arrhythmia and death. In addition to the necrosis due to ischemia, reperfusion may cause further myocyte necrosis and sarcoplemmal disruption, with leakage of cell contents into the extracellular space [9]. At later stages, myocytes initially salvaged can undergo programmed cell death (apoptosis), which can contribute to further myocardial injury [9]. Inflammatory cells and myofibroblasts invade the infarct area. The inflammatory cells release proteases and contribute to removal of necrotic tissue. Myofibroblasts reconstruct a new collagen network and after 5-6 weeks, a solid scar forms with a stable collagen structure and overall low cellularity, but with some myofibroblasts remaining in the scar tissue [40].
Unrecognized myocardial infarction

Unrecognized MI, or silent MI, is defined as MI that is undetected during the acute phase, but is eventually discovered by detection of pathological Q waves on ECG, myocardial imaging revealing evidence of a loss of viable myocardium, or pathological findings on autopsy [1, 36].

In this thesis UMI is synonymous with LGE-CMR detected UMI unless otherwise stated.

UMI detected by ECG

In large cohort studies, in which the presence of pathological Q-waves in the ECG was considered evidence of a previous MI, such ECG-detected UMIs have accounted for 5-44% of all MIs [36, 37, 41]. In individuals with stable CAD, the prevalence of ECG-detected UMIs has been reported to be 8-36% [2, 42, 43].

Large cohort studies have shown that an ECG-detected UMI carries a poor prognosis, similar to that of a clinically recognized MI [36]. In subjects with known CAD, ECG-detected UMIs were found to be associated with a 55% increase in all-cause death rates, nonfatal MI, and stroke combined [43]. Two available ECG-based studies showed a significantly better prognosis for UMI than for clinically recognized MI [44, 45].

The rate of ECG-detected UMIs may underestimate or misjudge the true incidence and prevalence of UMI, due to the uncertainty of ECG readings. First, the accepted ECG criteria for MI have changed over time. Secondly, not all MIs result in pathological Q waves. Thirdly, several cardiac and non-cardiac conditions can produce ECG changes mimicking those associated with MI and confound the diagnosis: pre-excitation, cardiomyopathy, left bundle branch block (LBBB), right ventricular hypertrophy, and hyperkalemia may be associated with Q waves or QS complexes in the absence of an MI [1]. Fourthly, an ECG may change over time, and a pathological Q-wave can be missed [46]. It has been estimated that ECG features of MI disappear within two years in 10% of subjects with anterior MI and in 25% of those with an inferior MI [47]. In 13.4% of individuals surviving Q wave MI, the Q wave had disappeared within four years after the event [48].
UMI detected by LGE-CMR
Late gadolinium enhancement cardiovascular magnetic resonance imaging has made it possible to detect small scars resulting from MI [49, 50], and has been shown to be more sensitive in the detection of UMI than an ECG [2, 51-53], conventional echocardiography [54], or nuclear scintigraphic techniques [52, 55]. With LGE-CMR both Q-wave and non-Q-wave UMIs may be detected.

LGE-CMR-detected UMIs are often found in the inferior-lateral segments of the left ventricle, a region in which ECG shows low sensitivity [51]. The prevalence of UMI as detected by ECG and those found by LGE-CMR differ significantly [2, 41]. Kwong et al. [2], found that a majority of subjects exhibiting ECG-detected UMIs showed no LGE-CMR-detected scarring to indicate MI.

UMI detected by LGE-CMR in the general population
Barbier et al. [51] examined 248 randomly selected 70-year-old subjects using LGE-CMR. Unrecognized MIs were found in 19.8% (49/248). The same group [56] also reported UMIs in 30% (120) of 394 randomly selected 75-year-old subjects examined with both cerebral and cardiac LGE-CMR. In a study of 936 individuals from the general population aged 67-93 years using ECG and LGE-CMR, previously clinically recognized MIs (RMs) were present in 9.7% (91/936), and UMIs were detected by LGE-CMR in 17% (157/936) and by ECG in 5% (46/936) [41]. Over a median of 6.4 years, 33% of subjects with RMI died, 28% of those with LGE-CMR-detected UMIs died, 16% of those with ECG detected UMI died and 17% of subjects with no MI died. Individuals with RMI and LGE-CMR-detected UMI had a statistically significant higher mortality rate than those without MI, while those with ECG-detected UMIs did not [41].

UMI detected by LGE-CMR in subjects with stable CAD
Kwong et al. [2], investigated 195 individuals with no known history of MI, but with symptoms or signs suggestive of CAD, who underwent CMR for clinical purposes. Q-waves were detected in 25 subjects. LGE-CMR revealed scarring in 44, seven of whom exhibited relevant Q waves on ECG, resulting in LGE-CMR-only detected UMIs in 19% (37/195). During a follow-up period (median 16 months), 16% of the subjects (31/195) experienced a major adverse cardiac event (MACE). The mortality
rate in the 44 subjects with scarring shown by LGE-CMR was 22% per year as estimated from the reported hazard ratio. The authors argue that LGE-CMR-detected UMIs are the strongest predictor of MACEs and cardiac mortality when compared with common clinical, ECG, and left ventricular function variables. Q waves on ECG, i.e. ECG-detected UMIs were not correlated with the presence of LGE-CMR-detected UMI and did not demonstrate a significant prognostic association with MACE or cardiac mortality [2].

Kim et al. [42] conducted a prospective study of 185 subjects with suspected CAD and no history of MI who were scheduled for invasive coronary angiography. LGE-CMR was performed prior to angiography and any coronary intervention. The prevalence of LGE-CMR-detected UMI was 27%, increasing with the extent and severity of coronary disease determined on angiography. Among subjects with LGE-CMR-detected UMI, the infarct location was reported to be in the perfusion area of the LAD in 40%, of the right coronary artery (RCA) in 47% and of the left circumflex coronary artery (LCX) in 13%. Three subjects with ECG-detected UMI showed normal coronary angiograms and no evidence of infarction with LGE-CMR. The median follow-up time was 2.2 years. Overall mortality rate was 3.8% per year, while that among subjects with LGE-CMR-detected UMI was 10.8% per year, compared to 2.7% per year in those with ECG-detected UMI and 0.8% per year in subjects with no MI [42].
Troponin

The contractile apparatus of striated muscle consists of myofibrils as its basic building unit. Myofibrils are formed of myosin-based thick filaments and thin filaments that consist of two strands of actin. The contraction of the myofibrils occurs via interaction of the thick and thin filaments. The process is regulated by the troponin-tropomyosin complex, which is located on the thin filament and blocks the active sites of actin, thereby preventing myosin from binding to it.

The troponin-tropomyosin complex comprises tropomyosin and three troponin subunits: troponin T (TnT), a binding protein that attaches the troponin complex to tropomyosin; troponin C (TnC), which binds calcium; and troponin I (TnI), which binds to actin and modulates the calcium dependent interaction of actin and myosin, depending on the binding of calcium [57]. Each Tn subunit has a unique role in the response to calcium [58]. In a relaxed muscle, the attachment site of the myosin cross-bridge is blocked, preventing contraction. When the muscle cell is stimulated to contract by an action potential, calcium channels open in the sarcoplasmic reticulum membrane and release calcium into the sarcoplasm. Some of the calcium-ions bind to TnC and induce a conformational change, which increases the affinity of TnC for TnI. TnI then moves away from the actin-tropomyosin complex and exposes the binding sites for myosin on the actin filaments, myosin and actin create cross-bridge formations, and the muscle contracts. Calcium dissociation from TnC restores the original status, allowing muscle relaxation [57]. The Tn sub-units undergo extensive physiological regulation through phosphorylation [58].

The majority of Tn is bound to myofibrils, but a small fraction of structurally unbound Tn is present, dissolved in the cytosol or as part of an early-release pool [59-61] (Figure 2). When a cell dies, regardless of the cause, the cell membrane dissolves and its free pool of Tn is immediately released. As the cell decomposes, the remaining, bound, fraction of Tn leaks out. The half-life of Tn in blood is approximately 2 hours [61]. Tn levels may be elevated for as long as 4-10 days after myocyte necrosis because of the gradual degradation of myofibrils and gradual release of troponin [61].
Figure 2. Representation of the cardiac myofibrillar thin filament. Cardiac troponin exists in a bound form and in a free cytosolic pool. Cardiac troponins are released from myocytes in complexes or as free protein. With permission of the British Medical Journal [60].

Several mechanisms can potentially cause Tn elevation [61] with necrosis being the most common (Table 3).

<table>
<thead>
<tr>
<th>Type</th>
<th>Mechanism</th>
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<tbody>
<tr>
<td>Type 1</td>
<td>Necrosis</td>
</tr>
<tr>
<td>Type 2</td>
<td>Apoptosis</td>
</tr>
<tr>
<td>Type 3</td>
<td>Normal myocyte turnover</td>
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<tr>
<td>Type 4</td>
<td>Cellular release of proteolytic troponin degradation products</td>
</tr>
<tr>
<td>Type 5</td>
<td>Increased cell wall permeability</td>
</tr>
<tr>
<td>Type 6</td>
<td>Formation and release of membranous blebs</td>
</tr>
</tbody>
</table>

Adapted with permission from the Journal of the American College of Cardiology [61].
Troponin and the heart
Cardio-specific isoforms of TnT and TnI (cTnT and cTnI), but not of TnC, have been identified. Determination of cTn levels is a prerequisite for the diagnosis or exclusion of acute MI [1] and thus essential in the evaluation of individuals with chest pain. Defining what constitutes a significant cTn change, or delta troponin, has remained elusive, with a range of criteria for both relative (\%Δ) and actual numeric (Δ) changes reported. Recent guidelines describes 'rule-in' and 'rule-out' algorithms with different time frames using assay dependent Δ change values and/or cut off levels [62]. Decision levels for %Δ have ranged between 20 and 243% in different studies for cTnI and cTnT [63]. Several studies have reported a superior diagnostic performance for Δ compared to %Δ, with cut offs for cTnI between 20 to 30 ng/l and between 6.9 to 9.2 ng/L for cTnT [63].

Another approach is to compare the observed cTn difference to a pre-determined critical difference or a so called reference change value (RCV). The RCV use the analytical precision of the assay and the biological (or individual) variation of the analyte to calculate the maximum size of a difference that can occur by chance with a specified probability [64]. An observed change in cTn that exceeds this threshold RCV is considered significant.

Although cTn elevation indicates cardiac damage, it does not define the nature of the injury. Apart from thrombotic events, cTn elevation may also occur with increased oxygen demand (e.g. tachyarrhythmia, hypertensive crisis), decreased oxygen supply (e.g. hypotension), increased myocardial wall tension due to volume or pressure overload (e.g. heart failure), or disturbance of cardiomyocyte cell integrity (e.g. sepsis) [1] (Table 4).

Elevated concentrations of cTn can be observed in individuals from the general population [65, 66] and in those with chronic diseases such as stable CAD [1, 67, 68]. Chronically elevated levels of cTn are associated with an increased risk for morbidity and mortality in both the general population [65, 66, 69, 70] and in individuals with stable CAD [67, 71, 72].
Although cTn elevation indicates cardiac damage and cTn elevations with certain pattern are necessary for the diagnosis of acute MI the association with UMI is still unclear.

<table>
<thead>
<tr>
<th>Table 4. Elevation of cTn due to myocardial injury</th>
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<tr>
<td>Injury related to primary myocardial ischemia</td>
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<td>Injury related to supply/demand imbalance</td>
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<td>Injury unrelated to myocardial ischemia</td>
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<td>Multifactorial or indeterminate myocardial injury</td>
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Adapted with permission from Oxford University Press [1].

**Individual variation of cTn**

The dynamic changes of cTn associated with AMI must be discriminated from fluctuations due to analytical imprecision or normal biological variation. The degree of rise or fall in cTn necessary for a reliable diagnosis of AMI has not been specified [1].
A 20% increase from an already elevated cTn value is indicative of additional MI [73-75]. This 20% change represents a significant (>3 standard deviations of the variation associated with an elevated baseline concentration) change in cTn on the basis of a 5-7% analytical total coefficient of variation (CV) [74, 76].

High-sensitivity cTn assays permits calculation of conjoint biological and analytical variation in healthy individuals [77]. The short-term biological variation of cTn is estimated to be in the range 3-48% while estimations of long-term biological variation range from 3 to 117% [78-82].

Assessment of biological variation, by definition, can only be conducted in healthy individuals. However, data derived from healthy individuals may not be representative of patients most frequently seen in the emergency unit, i.e. those with acute chest pain in whom AMI ultimately needs to be diagnosed or ruled out. Individuals in the general population [65, 69] as well as those with stable CAD [67, 68] may show chronically elevated cTn levels. It is not known whether biological variation in individuals with chronically high cTn differs from variation in those without elevated levels. Individual variation of cTn in subjects with stable CAD has not been characterized.

**Troponin and assay considerations**

Troponin is measured with two-sided “sandwich” immunoassays using a capture antibody to bind the molecule and a detection antibody to determine the quantity of bound troponin. Measurements of cTn are influenced by multiple factors, among which phosphorylation, proteolytic degradation, complexing with other molecules (e.g., TnC, heparin, heterophile or human antimouse antibodies, and specific autoantibodies) [83]. Antibodies directed against the stable central part of the cTn molecule, which are not affected by the numerous modifications, are preferred.

To define an assay as high-sensitivity, several aspects need to be addressed. An ideal hs-cTn assay is described as one that measure ≥95% of normal values below the 99th percentile of the reference population, thereby allowing for an accurate calculation of the 99th percentile upper reference limit (URL) with a 99% confidence interval (CI) together with ≤ 10% total CV at the 99th percentile for a young, healthy reference population diversified by sex, race and ethnicity [76, 84-86]. Guidelines describe a high-
sensitivity assay as one allowing for detection of cTn in 50-90% of healthy individuals [62]. Elements with potential to interfere with the analytical precision of hs-cTn assays, include reductions in hs-cTnT concentrations due to hemolysis, increases due to heterophilic antibodies, and decreases due to auto-antibodies in hs-cTnI concentrations [76].

The cTnI assays lack standardization, and comparison of test results among different assays is difficult [87]. The prevalence of elevated hs-cTnI and hs-cTnT levels above the 99th percentile in CAD patients, in the emergency unit, with a final diagnosis other than AMI is high and differ largely among assays, ranging from 13 to 40% [88].

The lower limit of blank (LoB) is the highest signal in a test that can be expected from a sample without the analyte, and the lower limit of detection (LoD) is the lowest concentration of an analyte in a sample that can be reliably differentiated from a sample without the analyte. The LoD is always higher than the LoB [76].

**N-terminal pro-B-type natriuretic peptide**

Natriuretic peptides are hormones secreted from specific locations in the myocardium in response to pressure- and volume overload associated with the stretch of cardiomyocytes [89]. Initially, the precursor pre-pro-B-type natriuretic peptide is formed and is then cleaved into pro-B-type natriuretic peptide (proBNP), which in turn is cleaved into B-type natriuretic peptide (BNP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) [90]. BNP exhibits biological activity, but NT-proBNP appears to be biologically inactive [91]. Secretion of natriuretic peptides promotes natriuresis, diuresis, and vasodilatation and antagonizes the effects of renin-angiotensin-aldosterone system [90]. The half-life of NT-proBNP ranges from 25 to 70 minutes, and it is cleared passively by kidneys, the liver, and the musculature [90].

NT-proBNP levels may be increased in patients with stable CAD after episodes of ischemia [92]. Possible causes include increased myocardial stretch secondary to ischemia-induced left ventricular systolic and/or diastolic dysfunction, as well as ischemia and cellular hypoxia stimulated production of NT-proBNP in the absence of demonstrable hemodynamic changes [92].
The determination of NT-proBNP levels for the diagnosis of heart failure (HF) has been evaluated in numerous studies and recommended in recent guidelines [93]. The use of natriuretic peptide levels to rule out acute or chronic HF has been thoroughly discussed [91, 94]. Quantification of natriuretic peptide levels may also be useful for prognostic evaluation in various cardiovascular conditions. Elevated levels of BNP or NT-proBNP are associated with high morbidity and mortality in individuals with HF [95], left ventricular hypertrophy [96], ACS [92, 97, 98], chest pain [99], and stable CAD [72, 92, 100-102] as well as in the general population [103]. Long-term monitoring of NT-proBNP levels may be useful in medical therapy for HF [104]. High NT-proBNP is associated with clinically significant coronary disease at angiography, but is not a useful screening test for detecting the presence of significant angiographic lesions in patients with stable CAD [105].

Although the associations between levels of NT-proBNP and a variety of factors linked to CAD, ischemia, and AMI has been demonstrated, the possible association between NT-proBNP and UMI has been overlooked. Themudo et al. have however recently demonstrated an association between elevated levels of NT-proBNP and UMI [106].

**Individual variation of NT-proBNP**

NT-proBNP is known to show wide intra-individual variation in healthy individuals [107-110], in individuals with stable HF [111-117], and in subjects with hypertension [118]. Knowledge about the individual variation in NT-proBNP is essential when serial measurements are used monitoring of medical treatment or for predication of prognosis. To the best of my knowledge, the individual variation of NT-proBNP in individuals with stable CAD has not been described.

**NT-proBNP and assay considerations**

NT-proBNP is stable at room temperature for at least 2 days, and frozen samples are stable for at least 1 year at -80 °C [90]. For collection, storage, and analysis of NT-proBNP, serum or heparin/ethylene diamine tetra acetic (EDTA) plasma in glass or plastic tubes is acceptable, however EDTA plasma may give an underestimation of 8-10% compared to serum [90]. Currently available assays are not standardized, which means that results of different assays are not comparable in a given subject. NT-proBNP levels, in the present studies, were analyzed in EDTA plasma.
The capture antibody and the detection antibody used were monoclonal mouse and monoclonal sheep antibodies, respectively.

**Galectin-3**
Galectin-3 (Gal-3), a member of the family of beta-galactoside-binding lectins, is a 30 kDa glycoprotein with a carbohydrate recognition domain of 130 amino acids that plays a role in many biological processes, including fibrosis [119]. Activated macrophages may secrete Gal-3, which induces cardiac fibroblast proliferation, collagen deposition, and ventricular dysfunction [120]. Experimental studies have shown that myocardial Gal-3 expression is up regulated shortly after MI, both in the infarct area and border zone, and, at a later stage, in the spared myocardium, contributing to tissue repair and fibrosis [121].

High concentrations of Gal-3 in high cardiovascular risk patients referred for coronary angiography is a strong independent predictor of cardiovascular death [122]. Gal-3 is associated with increased risk for incident HF and mortality in the general population [123]. Gal-3 is also associated with left ventricular remodeling and increased mortality in individuals with both acute and chronic HF [124, 125].

The Gal-3 level is inversely related to renal function in individuals with and without clinical HF. The concentration of Gal-3 does not seem to depend on the level of decompensation or type of HF [126].

Associations between levels of Gal-3 and a variety of factors related to CAD, ischemia and fibrosis have been demonstrated, but no study has yet, to the best of my knowledge, investigated the possible association between Gal-3 and UMI.

**Galectin-3 and assay considerations**
The assay principle combines a one-step immunoassay sandwich method with a rat anti-galectin-3 monoclonal antibody and a final fluorescent detection. Hemolysis and certain sera containing antibodies directed against reagent components may interfere with measurement of Gal-3 according to the manufacturer.
Late gadolinium enhancement cardiovascular magnetic resonance

Magnetic resonance imaging is based on detection of magnetic resonance of hydrogen protons that are a major component of soft tissue of the human body, and different tissues exhibits different proton densities. The differing proton densities in combination with an external strong static magnetic field differentiate tissues in the cardiovascular magnetic resonance (CMR) image.

Gadolinium-based contrast agents disperse in the extracellular space of normal myocardium and are excluded from normal myocardial cells [52]. Both acute and chronic myocardial injury exhibit late gadolinium enhancement (LGE) upon administration of gadolinium contrast medium. The increased gadolinium concentration in infarcted tissue shortens the T1 relaxation time. Hence, on reaching a transient steady state of wash-in and washout of the interstitium, infarcts appear hyper-enhanced compared to the neighboring non-infarcted tissue [49]. The mechanism of enhancement of myocardial scar tissue in chronic infarction is likely the expansion of the extracellular space and reduced contrast medium washout due to decreased capillary density [49]. LGE indicative of MI has a subendocardial portion and may extend to a variable degree into the myocardial wall [53]. The location of the area of LGE corresponds to an affected coronary artery.

In an acute MI, the contrast medium distributes in both the extra- and intra-cellular spaces after loss of myocardial membrane integrity in the infarct region [52]. To distinguish acute from chronic infarction, assessment of morphological features such as wall thinning or combining T2-weighted imaging with LGE-CMR can be used [52]. Differentiation can also be accomplished by using two contrast agents; one with low molecular weight, which produces delayed enhancement in both acute and chronic MIs, and one of larger molecular size, which produces selective enhancement of acute MIs [52]. In animal studies, the zone of enhancement after gadolinium administration has been found to have a close relationship to the size of the MI demarcated by post-mortem histochemical staining [52].
LGE-CMR-detected UMI and methodological considerations
Animal models have proved LGE-CMR to be an accurate method for detecting MIs and other myocardial scars [53]. LGE involving the subendocardial layer may be interpreted as an MI scar [127]. Subendocardial LGE is however not specific for MI. It may also be present in myocarditis [128], sarcoidosis [127], amyloidosis [129], dilated cardiomyopathy [130] and hypertrophic cardiomyopathy [131].
AIMS

The overarching aim of the thesis was to explore the occurrence and clinical importance of two manifestations of myocardial injury, UMI and altered levels of cardiac biochemical markers, in patients with stable CAD.

Specific aims

The specific aims were to investigate:

I. the prevalence, size, and localization of UMI and its relationship to corresponding atherosclerotic stenoses in patients with stable CAD;

II. the prognostic implication of UMI and its relationship to atherosclerotic stenoses in patients with stable CAD;

III. the association between UMI, atherosclerotic stenoses and the cardiac biochemical markers troponin, NT-proBNP, and Galectin-3;

IV. the short- and long-term individual variation in troponin levels in patients with stable CAD, and

V. the short- and long-term individual variation in NT-proBNP levels in patients with stable CAD.
MATERIALS AND METHODS

Ethics
All studies were approved by the Ethical Review Board in Uppsala, Sweden (2007/214) and conformed to the principles of the Helsinki Declaration of Human Rights. Signed informed consent was obtained from all participants.

Overview of methods
An overview of the study designs, number of subjects, primary outcome, outcome measures, and primary statistical methods is presented in Table 5.
Table 5. Overview of the study designs.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
<th>Study V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study design</td>
<td>Prospective multicenter cohort study</td>
<td>Prospective multicenter cohort study</td>
<td>Prospective multicenter cohort study</td>
<td>Study of individual variation in cardiac troponin</td>
<td>Study of individual variation in NT-proBNP</td>
</tr>
<tr>
<td>Subjects</td>
<td>235</td>
<td>235</td>
<td>235</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Primary outcome</td>
<td>Prevalence, size, and localization of UMI and the association with corresponding coronary artery stenosis</td>
<td>Composite endpoint of death, resuscitated cardiac arrest, MI, and hospitalization for congestive heart failure or unstable angina within 24-26 months</td>
<td>cTnl, NT-proBNP and Gal-3 levels at study inclusion in relation to the presence of UMI and coronary artery stenosis</td>
<td>Short- and long-term individual variation in cTnl and cTnT</td>
<td>Short- and long-term individual variation in NT-proBNP</td>
</tr>
<tr>
<td>Outcome measure</td>
<td>LGE-CMR and coronary angiography</td>
<td>Telephone interview, review of hospital records and death certificates</td>
<td>Analysis of cTnl, NT-proBNP and Gal-3, LGE-CMR, and coronary angiography</td>
<td>Assessment of cTn blood levels</td>
<td>Assessment of NT-proBNP blood levels</td>
</tr>
<tr>
<td>Primary statistical method</td>
<td>Fishers exact test, Mann-Whitney U-test and GEE-model</td>
<td>Logistic regression</td>
<td>Linear regression</td>
<td>Equations for variation</td>
<td>Equations for variation</td>
</tr>
</tbody>
</table>

cTnl; cardiac troponin I, cTnT, cardiac troponin T, Gal-3; galectin-3, GEE; generalized estimating equation, LGE-CMR; late gadolinium enhancement cardiovascular magnetic resonance, MI; myocardial infarction, UMI, unrecognized myocardial infarction.
Study design
This thesis was based on material from the prospective multicenter cohort study “Prevalence and prognostic value of unrecognized myocardial injury in stable coronary artery disease” (PUMI) and substudies of individual variation in cardiac biomarkers. The PUMI study is registered at ClinicalTrials.gov (NCT01257282).

The PUMI study
Two-hundred-sixty-five subjects scheduled for elective coronary angiography and with symptoms of stable CAD, according to the treating physician, were enrolled at six Swedish hospitals from January 2008 to March 2011: Danderyd University Hospital (n = 13), Falun County Hospital (n = 68), Gävle County Hospital (n = 22), Linköping University Hospital (n = 32), Uppsala University Hospital (n = 87) and Örebro University Hospital (n = 43). Admission for coronary angiography was by discretion by a cardiologist prior to study enrollment.

Inclusion criteria:
- stable CAD
- scheduled for elective coronary angiography

Exclusion criteria:
- pathological Q-wave on 12-lead ECG
- previously diagnosed MI
- previous PCI or CABG
- history of congestive heart failure
- estimated glomerular filtration rate below 30 mL/min/1.73 m²
- conditions contraindicating CMR (e.g. pacemaker, claustrophobia, intracranial clips)
- lack of suitability for participation for any reason judged by the investigator.

After study inclusion, blood samples were drawn and LGE-CMR investigation was conducted prior to coronary angiography. During the study, subjects received treatment at the discretion of the responsible physicians. Subjects requiring revascularization underwent PCI or CABG. All subjects were followed up by telephone call, review of hospital records and, when necessary, death certificates, 24-26 months after inclusion (Figure 3). The first occurrence of MI, death, resuscitated cardiac arrest, subsequent re-
vascularization or hospitalization for unstable angina pectoris, HF, or other heart disease was recorded.

Figure 3. Timeline for investigation and treatment in the PUMI study.

Of the 265 initial subjects, 235 that underwent both a coronary angiography and CMR investigation of adequate quality allowing analysis and constituted the cohort used in studies I, II, and III. Five subjects did not undergo LGE-CMR; 19 subjects were excluded because of poor CMR quality; and six subjects either did not undergo coronary angiography or the angiography could not be evaluated (Figure 4). No subjects were lost to follow up.
Figure 4. Number of patients and reasons for drop-outs in the PUMI study. MRI; Magnetic resonance imaging.
The sample size for the PUMI study was calculated based on the following assumptions:

(i) At least 6% of the study population would reach the primary composite endpoint at 24 months [39]
(ii) The relative risk ratio of reaching the primary composite endpoint was approximately 5:1 in those with UMI compared to those without UMI [2]
(iii) An UMI prevalence of 25% [51].

These assumptions resulted in an estimated event rate of 15% in the group with UMI and 3% in those without UMI. To demonstrate a statistically significant difference at the 5% level with 80% power, a total of 244 subjects (61 with UMI and 183 without UMI) was required. Due to the uncertainty in the assumptions, and since some CMR results may not be interpretable due to technical errors, we aimed to enroll 350 subjects in the study. As inclusion rate was slow, we closed enrollment at 265 subjects.

The substudy, individual variation in cardiac biomarkers

Of the subjects included in the PUMI study, 24 were enrolled in a substudy at two centers, Uppsala and Örebro hospitals, from October 2009 to April 2010. There were no additional inclusion or exclusion criteria. The subjects were admitted to hospital the day before scheduled coronary angiography. On average, 23 days (4-58) passed between enrollment and admission to the hospital. At admission, an ECG was obtained, and continuous multi-lead ST-monitoring was conducted for 24 hours. Blood samples and blood pressure measurements were taken every four hours, on six occasions prior to coronary angiography with the first sample taken between 08.00 and 10.00 am. The subjects were not fasting and had low physical activity, but were not confined to bed. Except for the additional day of hospitalization, the substudy subjects followed the same protocol as other the PUMI study subjects.

The six blood samples taken during the day and night of admission were used to study the short-term study variation in cTn and NT-proBNP. A study of the long-term variation in cTn and NT-proBNP compared the blood
samples taken at the time of enrollment with the first blood sample at the
time of admission (Figure 5).

Figure 5. Timeline for the short- and long-term blood samplings. A mean interval
of 23 (4-58) days separated the first and second blood sampling in the long-term
study. In the short-term study, the first blood sample was taken between 08.00
and 10.00 am and every 4 hours thereafter for 24 hours.

The sample size for the substudy was estimated based on sample sizes
previously used in similar studies; 12-24 individuals for cTn investigation
[78, 79, 81, 82, 132, 133] and 15-45 individuals for NT-proBNP [107,

**Late gadolinium enhancement cardiovascular magnetic resonance**
Clinical 1.5-T scanners (Philips Intera, Best, Netherlands; Philips Achieva,
Best, the Netherlands; or Siemens Symphony, Erlangen, Germany) were
used to conduct CMR-examinations with a general scanning protocol
consisting of cine short axis images and a viability sequence in short axis,
long axis 2-chamber, 3-chamber and 4-chamber views using ECG-
triggering and breath-holding. Viability imaging was performed with a
minimum delay of 15 minutes after intravenous administration of 0.15
ml/kg bodyweight (maximum dose 15 ml) of gadobutrol (Gadovist®,
Bayer, Leverkusen, Germany). The viability sequence was a 3D inversion
recovery gradient echo sequence with the following parameters: repetition time set to shortest (typically 4.0-4.2 ms), echo time set to shortest (typically 1.18-1.28 ms), inversion time chosen by the operator to null normal myocardium, flip angle 15°, image matrix 256x100, field-of-view 375 x 281 mm, and reconstructed voxel size 0.73x0.73x5 mm. Eleven slices were acquired per breath hold for the long axis slices and 22 slices divided into two breath holds for the short axis. Each breath hold was 16 seconds at heart rate 60 beats per minute.

Presence of LGE in each subject was recorded. Two radiologists unaware of the clinical history, in consensus, localized areas of LGE visible in at least two imaging planes, using the AHA 17-segment model [134] (Figure 6). Subjects exhibiting LGE with a subendocardial component were categorized as having UMI. Subjects without LGE or with an LGE area lacking a subendocardial component were categorized as no MI. The attending physician had no access to CMR examination results, with the exception of the calculated left ventricle ejection fraction (LVEF) and any observed wall motion abnormalities.

Figure 6. The AHA 17-segment model.
Coronary angiography

In accordance with Swedish Coronary Angiography and Angioplasty Registry (SCAAR) practice, the coronary tree was divided into 19 segments, derived from the 16-segment model proposed by Austen [135] (Figure 7). The degree of reduction in diameter of each coronary artery segment was categorized as 0-29%, 30-49%, 50-69%, 70-99%, or 100% (occlusion) based on visual examination. If the obstruction was ≥30%, we visually assessed, taking the individual coronary anatomy into consideration, which myocardial segments in the AHA 17-segment model were affected downstream of the lesion. This was done by two radiologists in consensus, blinded to the subject’s clinical history and the results of LGE-CMR.

A stenosis with a diameter narrowing of ≥70% was considered hemodynamically significant. The extent of atherosclerosis was defined as the number of vessels affected by a ≥70% stenosis and the severity of atherosclerosis as the degree of stenosis.

Figure 7. The coronary vessels divided into 19 segments, derived from the 16-segment model proposed by Austen [135].
Correlation between coronary artery stenosis and LGE

The correlation between coronary artery stenosis and presence of LGE was analyzed under two protocols. In the primary analysis, a perfect match was required between the myocardial segments deemed to be supplied by a coronary artery with stenosis and the myocardial segment(s) showing LGE. As it was not always possible to determine which myocardial segments were supplied by a given coronary artery, we performed a secondary, less conservative, “near match” analysis. Near match was considered to exist when LGE was present in any segment adjacent to segments that were deemed to be supplied by a coronary artery with stenosis. The border between the septum and the free wall was, in this study considered, angiographically clear, therefore the segments separated by this border were not considered adjacent.

Biochemical analysis

Blood was collected into EDTA-containing tubes and immediately centrifuged, and plasma was stored at −70°C until analysis. cTnI was analyzed on an ARCHITECT i2000SR platform using the ARCHITECT STAT hs-cTnI assay (Abbott Laboratories, Abbott Park, IL). According to the manufacturer, the LoB and LoD of the assay used range from 0.7 to 1.3 ng/L and from 1.1 to 1.9 ng/L, respectively, and the lowest measurable concentration with 10% CV is 4.7 ng/L. The 99th percentile among healthy subjects is 23 ng/L [87]. A recent study validating the analytical performance of the assay verified the manufacturers LoB and LoD ranges and demonstrated the lowest concentration with 10% CV to be 5.6ng/L [136]. The 99th percentile concentration obtained for the reference population was 19.3ng/L and was higher in men than in women [136].

The cTnT was analyzed twice using the Elecsys®_hs-cTnT assay on a Cobas instrument (Roche Diagnostics, Basel, Switzerland). Samples were reassessed with lot no. 167 345 having an expiration date of 2013-07 with the recently reformulated calibration curve, given previous problems with lot no. 160 197 (expiration date 2012-03) and the former calibration [137]. All cTnT results presented were analyzed with the new batch of reagents unless otherwise stated. The LoB for this assay is 3 ng/L, the LoD 5 ng/L and the 99th percentile in apparently healthy individuals is 13.5 ng/L: 14.5 ng/L for men and 10.0 ng/L for women [86]. The 10% CV...
concentration is 13 ng/L [86]. Significant differences between the 99th percentiles for healthy men and women have been reported [138].

The analyses were performed strictly according to the manufacturer’s instructions using a single lot of reagents for cTnI and cTnT. The within-run analytical coefficient of variation (CVa) was determined internally for cTnI on 230 duplicate samples and found to be 8% at a concentration of 12 ng/L, which corresponded to the mean value of cTnI found in the present study. Based on 45 duplicate samples the CVa for cTnT was 4% at the mean concentration of 13 ng/L.

NT-proBNP was analyzed in EDTA plasma with the Elecsys proBNP sandwich immunoassay, using two monoclonal antibodies, on an Elecsys 2010 instrument (Roche Diagnostics, Basel, Switzerland). The capture antibody and the detection antibody used were monoclonal mouse and monoclonal sheep antibodies, respectively. According to the manufacturer, the analytic range extends from 5 to 35,000 ng/L and the recommended cut-off value to indicate cardiac dysfunction is 125 ng/L. The mean total CV value is <10% at 20 ng/L [139]. The analyses were performed strictly according to the manufacturer’s instructions using a single lot of reagents. The within-run CVa was determined internally on duplicate samples and found to be 3% at both 125 ng/L and 4440 ng/L.

Galetin-3 was analyzed using a VIDAS® assay (bioMérieux, Marcy-l’Etoile, France). According to the manufacturer the LoD for this assay is 2.4 ng/mL and the URL is 18.6 ng/mL.

Cystatin-C was analyzed using the Tina-quant® Cystatin C assay (Roche Diagnostics GmbH, Mannheim, Germany) on a Cobas c501 with an analytical CV of 1% at 1.0 mg/L and 2% at 4.26 mg/L. The total CV is <5 % for values from 0.6 -4.6 mg/mL [140].

**Electrocardiography**

Resting 12-lead ECGs were analyzed by a single cardiologist, and continuous multi-lead ST-segment monitoring were analyzed by two cardiologists, blinded to all other results. ECG changes were classified according to the Minnesota Code Classification System for Electrocardiographic Findings [141]. Continuous multi-lead ST-segment monitoring was performed for 24 hours using the Telegard 6.6 (GE Marquette Medical Systems,
Milwaukee, Wisconsin, USA) or CoroNet system (Ortivus Medical AB, Täby, Sweden). An ST vector magnitude or ST vector-magnitude increase or decrease of at least 50 μV from the baseline for at least one minute was considered indicative of ischemia. A ventricular rate exceeding 120 beats per minute for one minute or longer was considered to be an episode of tachycardia.

**Statistical analysis**
The primary statistical methods used in the studies are presented in Table 5.

**Parametric and non-parametric tests**
Continuous data are presented as mean ± standard deviation (SD) or, if data were not normally distributed, as median and inter-quartile range (IQR). All statistical tests were two-tailed, with \( p < 0.05 \) regarded as statistically significant.

Between-group differences in categorical variables were examined using the Chi-square test, or, in cases of small numbers of data, Fisher’s exact test (studies I-III). For continuous variables not normally distributed, the Mann-Whitney U-test was used (studies I-III). The biochemical markers were not non-normally distributed and were logarithmically transformed before used in multivariate linear regression models (study III). To illuminate the non-normally distribution both the normal and log-transformed reference change value (RCV) were used in study IV-V.

The Kruskal-Wallis test was used to describe the association of the extent of CAD (more than two groups) with the non-normally distributed values of the biochemical markers (study III) and to examine the circadian variation of NT-proBNP (study V).

The Kolmogorov-Smirnov test and the Shapiro-Wilk test were used to assess normality of the distribution of clinical characteristics and cardiac biochemical markers compared with a standard normal distribution (studies I-V).

**Logistic regression**
Logistic regression was used to identify the clinical characteristics associated with UMI and their relationship to the primary composite endpoint. Both univariable and multivariable logistic regression analyses were performed.
Given the limited number of events, four multivariable models with a maximum of three covariates in each model were created. All models were adjusted for age. Model 1 also included sex, and models 2, 3, and 4 included coronary stenosis ≥70%, extent of CAD and matched UMI, respectively. The point-estimates of the odds ratio (OR) are shown with 95% confidence interval (CI).

**Linear regression**

Linear regression was used to identify factors associated with the level of cardiac biomarkers. Both univariable and multivariable linear regression analyses were conducted (study III). The biochemical markers were not normally distributed and were therefore logarithmically transformed before use in multivariable linear regression models. In the multivariable linear regression models, all univariable factors associated with the level of biomarkers were used.

**Other statistical analyses**

A generalized estimating equation (GEE) model was used to analyze data of each coronary artery segment in study I to fully account for any between-segment dependency in an individual [142]. This approach addresses error estimates in the context of correlated observations.

The Pearson correlation coefficient was used to determine the linear relationship/correlation among logarithmic transformed values of the biochemical markers (study III).

Spearman’s rank correlation coefficient was used to assess the association of UMI size and with blood levels of cTnI and NT-proBNP (study III).

Equations used for calculation of the intra-individual coefficient of variation (CVi), inter-individual coefficient of variation (CVg), the RCV, the positive and negative log-normal RCV and the index of individuality (II) in studies IV and V are given in Table 6. The CVi was calculated from the total coefficient of variation (CVt) at all time-points. The analytical coefficient of variation (CVa) was determined from our internal validation of within-run CV.
### Table 6. Equations used in paper IV-V.

<table>
<thead>
<tr>
<th>Equation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVi = (CVt^2 - CVa^2)^0.5</td>
<td>Intra-individual CV (CVi)</td>
</tr>
<tr>
<td>RCV = z * 2^0.5 * (CVa^2 + CVi^2)</td>
<td>Reference change value (RCV)</td>
</tr>
<tr>
<td>σ = [\ln(CVt^2 + 1)]^0.5</td>
<td>Median normal deviation of the log-normal distribution (σ)</td>
</tr>
<tr>
<td>Positive log-normal RCV = [\exp(1.96 * 2^0.5 * σ) - 1] * 100.</td>
<td>Positive log-normal RCV</td>
</tr>
<tr>
<td>Negative log-normal RCV = [\exp(-1.96 * 2^0.5 * σ) - 1] * 100.</td>
<td>Negative log-normal RCV</td>
</tr>
<tr>
<td>II = ((CVi^2 + CVa^2)^0.5) / CVg</td>
<td>Index of individuality (II)</td>
</tr>
</tbody>
</table>

CV = coefficient of variation; CVa = analytical CV; CVi = intra-individual CV; CVg = inter-individual CV; CVt = total CV, RCV = reference change value, II = index of individuality. σ = median normal deviation of the log-normal distribution. z = 1.96 (z score for 95% confidence).
RESULTS

Baseline characteristics, studies I-III
The characteristics of the population and of UMI findings are shown in Table 7.
Table 7. Baseline characteristics, studies I-III. Statistically significant p-values are in bold.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All subjects</th>
<th>No UMI</th>
<th>UMI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>233</td>
<td>177</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td><strong>Age in years, median (IQR)</strong></td>
<td>65 (60-71)</td>
<td>65 (60-70)</td>
<td>66 (64-72)</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>Women (%)</strong></td>
<td>80 (34)</td>
<td>66 (37)</td>
<td>14 (24)</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>CAD risk factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist, cm, median (IQR)</td>
<td>100 (93-107)</td>
<td>99 (92-106)</td>
<td>103 (95-109)</td>
<td>0.04</td>
</tr>
<tr>
<td>BMI (kg/m²), median (IQR)</td>
<td>27 (25-30)</td>
<td>27 (25-29)</td>
<td>28 (25-30)</td>
<td>0.29</td>
</tr>
<tr>
<td>Family history of CAD (%)</td>
<td>117 (50)</td>
<td>87 (49)</td>
<td>30 (52)</td>
<td>0.76</td>
</tr>
<tr>
<td>Previous/current smoking (%)</td>
<td>143 (61)</td>
<td>105 (59)</td>
<td>38 (66)</td>
<td>0.40</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>132 (56)</td>
<td>94 (53)</td>
<td>38 (66)</td>
<td>0.13</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>49 (21)</td>
<td>32 (18)</td>
<td>17 (29)</td>
<td>0.09</td>
</tr>
<tr>
<td>Previous stroke/TIA (%)</td>
<td>13 (6)</td>
<td>7 (4)</td>
<td>6 (10)</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Symptoms of angina pectoris</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2 months (%)</td>
<td>7 (3)</td>
<td>6 (3)</td>
<td>1 (2)</td>
<td>0.85</td>
</tr>
<tr>
<td>2-12 months (%)</td>
<td>105 (45)</td>
<td>80 (45)</td>
<td>25 (43)</td>
<td></td>
</tr>
<tr>
<td>&gt; 12 months (%)</td>
<td>123 (52)</td>
<td>91 (51)</td>
<td>32 (55)</td>
<td></td>
</tr>
<tr>
<td>CCS class 0 (%)</td>
<td>9 (4)</td>
<td>5 (3)</td>
<td>4 (7)</td>
<td>0.59</td>
</tr>
<tr>
<td>CCS class 1 (%)</td>
<td>70 (30)</td>
<td>51 (29)</td>
<td>19 (33)</td>
<td></td>
</tr>
<tr>
<td>CCS class 2 (%)</td>
<td>110 (47)</td>
<td>85 (48)</td>
<td>25 (43)</td>
<td></td>
</tr>
<tr>
<td>CCS class 3 (%)</td>
<td>45 (19)</td>
<td>35 (20)</td>
<td>10 (17)</td>
<td></td>
</tr>
<tr>
<td><strong>Medications</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin (%)</td>
<td>211 (90)</td>
<td>158 (89)</td>
<td>53 (91)</td>
<td>0.81</td>
</tr>
<tr>
<td>Clopidogrel (%)</td>
<td>7 (3)</td>
<td>4 (2)</td>
<td>3 (5)</td>
<td>0.37</td>
</tr>
<tr>
<td>Warfarin (%)</td>
<td>7 (3)</td>
<td>6 (3)</td>
<td>1 (2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Beta blocker (%)</td>
<td>161 (69)</td>
<td>117 (66)</td>
<td>44 (76)</td>
<td>0.19</td>
</tr>
<tr>
<td>ACE-I or AT-II (%)</td>
<td>84 (36)</td>
<td>58 (33)</td>
<td>26 (45)</td>
<td>0.12</td>
</tr>
<tr>
<td>Calcium channel blocker (%)</td>
<td>56 (24)</td>
<td>37 (21)</td>
<td>19 (33)</td>
<td>0.08</td>
</tr>
<tr>
<td>Long-acting nitrate (%)</td>
<td>59 (25)</td>
<td>37 (21)</td>
<td>22 (38)</td>
<td>0.01</td>
</tr>
<tr>
<td>Statin/other lipid lowering agent (%)</td>
<td>167 (71)</td>
<td>121 (68)</td>
<td>46 (79)</td>
<td>0.13</td>
</tr>
</tbody>
</table>
Table 7. Baseline characteristics study I-III. Statistically significant p-values as bold.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All subjects</th>
<th>No UMI</th>
<th>UMI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ejection fraction, median % (IQR)</td>
<td>66 (62-72)</td>
<td>66 (61-71)</td>
<td>67 (62-72)</td>
<td>0.43</td>
</tr>
<tr>
<td>Coronary angiography</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stenosis ≥70% (%)</td>
<td>135 (57)</td>
<td>88 (50)</td>
<td>47 (81)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Three vessel disease (%)</td>
<td>23 (10)</td>
<td>9 (5)</td>
<td>14 (24)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stenosis &lt;50% (%)</td>
<td>92 (39)</td>
<td>82 (46)</td>
<td>10 (17)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Revascularization after angiography</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCI (%)</td>
<td>98 (42)</td>
<td>61 (34)</td>
<td>37 (64)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CABG (%)</td>
<td>23 (10)</td>
<td>15 (8)</td>
<td>8 (14)</td>
<td>0.42</td>
</tr>
<tr>
<td>Biochemistry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cTnI ng/L, median (IQR)</td>
<td>4.1 (2.8-6.0)</td>
<td>3.7 (2.6-5.4)</td>
<td>5.4 (3.7-9.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cystatin C mg/L, median (IQR)</td>
<td>0.9 (0.8-1.0)</td>
<td>0.9 (0.8-1.0)</td>
<td>1.0 (0.8-1.1)</td>
<td>0.11</td>
</tr>
<tr>
<td>Galectin-3 ng/L, median (IQR)</td>
<td>10.2 (8.6-12.4)</td>
<td>10.1 (8.3-12.0)</td>
<td>11.1 (9.0-13.3)</td>
<td>0.033</td>
</tr>
<tr>
<td>NT-proBNP ng/L, median (IQR)</td>
<td>102 (52-204)</td>
<td>94 (48-156)</td>
<td>173 (63-263)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

ACE-I = Angiotensin-Converting-Enzyme Inhibitor; AT-II = Angiotensin II receptor antagonist; BMI = Body Mass Index; CABG = Coronary Artery Bypass Grafting; CAD = Coronary Artery Disease; CCS = Canadian Cardiovascular Society; IQR = inter quartile range, PCI = Percutaneous Coronary intervention; TIA = Transient Ischemic Attack; UMI = Unrecognized Myocardial Infarction.
STUDY I

Prevalence
Unrecognized MIs were found in 58 subjects (24.7%), three of whom showed two distinctly separate areas of UMI. Areas of LGE without a subendocardial component, i.e. not fulfilling the criteria for UMI, were present in 25 subjects (11%).

UMI size and localization
The LGE was transmural in 21 subjects, and subendocardial in 37 subjects. The median size of the UMI was 2.1 g (IQR 0.7–4.5 g). The maximum size was 27.8 g. A total of 110 myocardial segments were affected in the 58 subjects with UMI. The sites of the segments with UMI are shown in Figure 8. Fifty-six percent of UMIs were located in the inferior and inferior-lateral myocardial segments (AHA segments 4, 5, 10, 11, 15, 16).

Figure 8. Localization of UMI identified by LGE-CMR. The number in each of the 17 myocardial segments shows number of UMIs found in that segment.
Results of coronary angiography
At coronary angiography, 32 subjects (13.6%) exhibited total occlusion of at least one coronary branch: 103 (43.8%) had maximum stenosis (narrowing of the vessel lumen) ≥70-99%; 8 (3.4%) maximum stenosis ≥50-69%; 63 (30.2%) showed maximum stenosis of ≥30-49%; and 29 (12.3%) had no stenosis or maximum stenosis lower than 30%. Sixty-four percent of males had a maximum stenosis ≥70%, while this was present in only 45% of females (p = 0.008). In subjects with diabetes, 80% exhibited a maximum stenosis ≥70%, compared with 52% of subjects without diabetes (p < 0.0001). The lesions resulting in stenosis ≥70% were predominately located in the LAD (Figure 9), with the exception of those subjects with diabetes, in whom there was an equal occurrence in the LAD and LCX.

Figure 9. The number of lesions causing a >70 % stenosis and the proportion of UMI downstream a >70 % stenosis, in left anterior descending artery (LAD), left circumflex artery (LCX) and right coronary artery (RCX). LM = left main artery.

60  ANNA NORDENSKJÖLD  Unrecognized myocardial infarction and biochemical markers.
Relationship between coronary stenosis and UMI

Unrecognized MIs were more prevalent in the 135 subjects with at least one coronary artery showing stenosis ≥ 70% as compared to the 100 subjects with coronary artery stenosis < 70%, 47 (34.8%) vs. 11 (11.0%) (p < 0.0001). When assessing the prevalence of UMI in myocardial segments downstream of coronary stenosis, taking the individual coronary anatomy into consideration and requiring a perfect match, a significant relationship between the stenosis degree in the supplying artery and the prevalence of UMI was found (Table 8). The prevalence of UMIs in myocardial segments supplied by an artery with < 30% stenosis was 1.3% compared to 20.1% in those segments supplied by an artery with total occlusion. UMIs were significantly more likely to be observed downstream of a stenosis ≥ 70% compared to < 70% (OR 5.1, CI 3.1-8.3, p < 0.0001). This strong association remained almost unchanged after adjustment for age, sex, diabetes and hypertension (OR 4.7, CI 2.8-7.7, p < 0.0001). If the segments supplied by an artery with total occlusion were excluded from analysis, it remained significantly more likely to detect a UMI downstream of stenosis ≥ 70-90% as compared to < 70% (adjusted OR 2.88, CI 1.6-5.1, p = 0.0002).
Table 8. Myocardial segments with and without UMI grouped by severity of coronary artery stenosis. Percentages are based on total examined segments.

<table>
<thead>
<tr>
<th>Stenosis</th>
<th>Segments without UMI</th>
<th>Segments with UMI</th>
<th>Total segments</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30%</td>
<td>1331</td>
<td>18</td>
<td>1349</td>
</tr>
<tr>
<td></td>
<td>98.7%</td>
<td>1.3%</td>
<td></td>
</tr>
<tr>
<td>≥30-&lt;50%</td>
<td>1194</td>
<td>14</td>
<td>1208</td>
</tr>
<tr>
<td></td>
<td>98.8%</td>
<td>1.2%</td>
<td></td>
</tr>
<tr>
<td>≥50-&lt;70%</td>
<td>310</td>
<td>3</td>
<td>313</td>
</tr>
<tr>
<td></td>
<td>99.0%</td>
<td>1.0%</td>
<td></td>
</tr>
<tr>
<td>≥70-99%</td>
<td>927</td>
<td>44</td>
<td>971</td>
</tr>
<tr>
<td></td>
<td>95.5%</td>
<td>4.5%</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>123</td>
<td>31</td>
<td>154</td>
</tr>
<tr>
<td></td>
<td>79.9%</td>
<td>20.1%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3885</td>
<td>110</td>
<td>3995</td>
</tr>
</tbody>
</table>

The proportion of UMI downstream a 70% stenosis tended to be unevenly distributed between RCA, LCX and LAD, 25.0%, 18.7% and 10.8%, respectively (p = 0.08). However, the size of the UMIs downstream of stenosis ≥70% did not significantly differ among areas supplied by the three coronary arteries (median 3.9 g for RCA, 3.9 g for LCX and 2.9 g for LAD).

Sixty-eight percent of the myocardial segments with UMI were downstream of a stenosis ≥70%. This proportion did not differ significantly between men and women or between subjects with and without diabetes.

In a sensitivity analysis, the correlation between coronary artery stenosis and UMI in cases of near match, but not a perfect match, of the involved artery and the myocardial segment with UMI was assessed. In this case the proportion of myocardial segments with UMI downstream of stenosis ≥70% rose from 68% to 88%. In contrast to the UMIs, areas of LGE without a subendocardial component had no clear correlation with the angiogram. Representative images of a large UMI with subendocardial component with corresponding coronary artery occlusion are shown in Figure 10.
Figure 10. Representative images of UMI with corresponding coronary artery stenosis/occlusion. a = transmural UMI in AHA segment 4, partly in segments 3 and 5, with corresponding RCA occlusion seen on coronary angiography b = subendocardial UMI in AHA segments 8 and 9 with corresponding severe stenosis in proximal LAD, c = subendocardial UMI in AHA segment 11 with corresponding severe stenosis in proximal LCX.
STUDY II

Follow-up time and subjects reaching the primary endpoint
During the follow-up period of 24-26 months (median 754 days, IQR 736-790), 18 subjects (7.7%) reached the primary composite endpoint: five subjects died (2.1%), four developed acute MI (1.7%), seven developed episodes of unstable angina pectoris (3.0%), and two were hospitalized for heart failure (0.9%). One subject experienced an acute MI 21 months after an episode of unstable angina pectoris that was not included as primary endpoint event. No subject was lost to follow-up. Of subjects with UMI, 15.5% (9/58) reached the primary endpoint compared to 5.1% (9/177) of subjects with no UMI (OR 3.4, 95% CI 1.3-9.1, p = 0.014) (Figure 11).

Figure 11. Kaplan-Meier plot of cumulative probability of remaining event free for subjects with UMI versus subjects without UMI. Green line = no UMI. Red line = UMI. p = 0.014.
Subjects with an anatomic match between the UMI site and coronary artery stenosis reached the primary endpoint in 15.4% (6/39) of cases compared to 15.8% (3/19) of subjects with UMI without a matched artery (p = 0.97). The five patient deaths resulted in a mean annual mortality rate of 1.1%. Among subjects with UMI, there was one death, producing mean mortality of 0.9% per year.

**Univariate and multivariate predictors of prognosis**

In addition to UMI, the presence of ≥70% coronary artery stenosis (OR 6.6, 95% CI 1.5-29.4, p = 0.013) and the extent of CAD (one diseased vessel, OR 2.3, 95% CI 0.4-14.1, p = 0.37, two vessels, OR 12.3, 95% CI 2.5-59.4, p = 0.002; and three vessels, OR 10.3, 95% CI 1.8-60.4, p = 0.010, compared to subjects without stenosis ≥70%) were found to be univariate predictors of prognosis (Table 9).

The prognostic value of UMI was assessed by multivariable logistic regression in several models, adjusting for clinical and angiographic factors (Table 10). Unrecognized MI was an independent predictor of the primary endpoint after adjustment for age and sex. However, after adjustment for age and the presence of a ≥70% coronary stenosis or extent of CAD, UMI was no longer significant associated with the outcome.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Endpoint (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; Median age of 65.4 years</td>
<td>6.0</td>
<td>0.34</td>
</tr>
<tr>
<td>(n=117)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ Median age of 65.4 years</td>
<td>9.3</td>
<td></td>
</tr>
<tr>
<td>(n = 118)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (n = 155)</td>
<td>9.7</td>
<td>0.12</td>
</tr>
<tr>
<td>Female (n = 80)</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td><strong>CMR image</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With UMI (n = 58)</td>
<td>15.5</td>
<td>0.014</td>
</tr>
<tr>
<td>Without UMI (n = 177)</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td><strong>Coronary angiography</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stenosis ≥70% (n = 135)</td>
<td>11.9</td>
<td>0.013</td>
</tr>
<tr>
<td>No stenosis ≥70% (n = 100)</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td><strong>Extent of CAD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No stenosis ≥70% (n = 100)</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>One diseased vessel (n = 67)</td>
<td>4.5</td>
<td>0.37*</td>
</tr>
<tr>
<td>Two diseased vessels (n=45)</td>
<td>20.0</td>
<td>0.002*</td>
</tr>
<tr>
<td>Three diseased vessels (n=23)</td>
<td>17.4</td>
<td>0.010*</td>
</tr>
<tr>
<td><strong>UMI and stenosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UMI and matched artery stenosis</td>
<td>15.4</td>
<td>0.97</td>
</tr>
<tr>
<td>UMI and unmatched artery stenosis</td>
<td>15.8</td>
<td></td>
</tr>
</tbody>
</table>

*Compared to subjects without stenosis ≥70%.
Table 10. Multivariable logistic regression analyses of the primary endpoint as outcome. Presented as OR and 95% CI. Statistically significant p-values in bold.

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.92</td>
<td>2.42</td>
<td>2.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.08-7.89)</td>
<td>(0.88-6.61)</td>
<td>(0.73-6.13)</td>
<td></td>
</tr>
<tr>
<td>UMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.06</td>
<td>1.04</td>
<td>1.04</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td>(0.99-1.13)</td>
<td>(0.98-1.11)</td>
<td>(0.97-1.11)</td>
<td>(0.99-1.13)</td>
</tr>
<tr>
<td>Female</td>
<td>0.40</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(0.11-1.45)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stenosis ≥70%</td>
<td>-</td>
<td>4.46</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.96-20.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extent of CAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No stenosis ≥70%</td>
<td>-</td>
<td>-</td>
<td>Reference</td>
<td>-</td>
</tr>
<tr>
<td>One vessel disease</td>
<td>-</td>
<td>-</td>
<td>1.76</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.28-11.1)</td>
<td></td>
</tr>
<tr>
<td>Two vessel disease</td>
<td>-</td>
<td>-</td>
<td>8.72</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1.72-44.2)</td>
<td></td>
</tr>
<tr>
<td>Three vessel disease</td>
<td>-</td>
<td>-</td>
<td>5.89</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.91-38.2)</td>
<td></td>
</tr>
<tr>
<td>Matched UMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No UMI</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UMI with match</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(1.04-9.48)</td>
</tr>
<tr>
<td>UMI without match</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.73-12.8)</td>
</tr>
</tbody>
</table>
STUDY III

Biochemical markers and UMI
The levels of cTnI were significantly higher in subjects with UMI (median 5.4 ng/L, IQR 3.7-9.5) than in subjects without UMI (median 3.7 ng/L, IQR 2.6-5.4), p < 0.001. The levels of Gal-3 and NT-proBNP were also significantly higher in subjects with UMI compared to those without UMI, p = 0.033 and p = 0.006 respectively. No difference in levels of Cystatin C between the groups was found (Table 7).

The correlation among biomarkers was weak to moderate (Table 11). In univariate analyses, UMI, the extent of CAD, diabetes, age, male sex, and low left ventricular ejection fraction (LVEF) were significantly associated with higher cTnI levels. Unrecognized MI, low LVEF, age, and smoking were significantly associated with higher NT-proBNP levels. Unrecognized MI, age, and diabetes were significantly associated with higher Gal-3 levels (Table 12).

| Table 11. Correlation among biochemical markers after logarithmic transformation. |
|---------------------------------|----------------|----------------|----------------|----------------|
|                                | Cystatin C   | Gal-3          | NT-proBNP      | Troponin I     |
| Cystatin C                     | R             |                |                |                |
| p-value                         | -1            | 0.497          | 0.352          | 0.173          |
| Gal-3                           | R             | -              | 0.239          | 0.152          |
| p-value                         | -1            | <0.001         | <0.001         | 0.020          |
| NT-proBNP                       | R             | --             | -1             | 0.341          |
| p-value                         | --            |                | <0.001         |                |
| Troponin I                      | R             | -              | -              | -1             |
| p-value                         | -             |                |                |                |

R, Pearson’s Correlation Coefficient
Table 12. Factors associated with the level of Cystatin C, Galectin-3, NT-proBNP and Troponin I, in univariate analysis. Statistically significant p-values in bold.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Cystatin C (mg/L)</th>
<th>Galectin-3 (ng/L)</th>
<th>NT-proBNP (ng/L)</th>
<th>Troponin I (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, median 65.4 years (IQR)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; Median (n=117)</td>
<td>0.8 (0.7-1.0)</td>
<td>9.0 (7.6-11.2)</td>
<td>67 (34-138)</td>
<td>3.6 (2.3-5.1)</td>
</tr>
<tr>
<td>≥ Median (n=118)</td>
<td>1.0 (0.8-1.1)</td>
<td>11.3 (9.9-13.6)</td>
<td>147 (91-301)</td>
<td>4.7 (3.3-8.1)</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n=49)</td>
<td>0.9 (0.8-1.0)</td>
<td>11.1 (8.9-13.9)</td>
<td>215 (67-242)</td>
<td>5.3 (3.4-6.2)</td>
</tr>
<tr>
<td>No (n=186)</td>
<td>0.9 (0.8-1.0)</td>
<td>10.1 (8.5-11.8)</td>
<td>173 (50-184)</td>
<td>3.9 (2.6-5.9)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.03</td>
<td>0.02</td>
<td>0.12</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>LVEF, Median 66%</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; Median (n=110)</td>
<td>0.9 (0.8-1.0)</td>
<td>10.3 (8.7-12.3)</td>
<td>132 (57-236)</td>
<td>4.4 (2.9-7.7)</td>
</tr>
<tr>
<td>≥ Median (n=123)</td>
<td>0.9 (0.8-1.0)</td>
<td>10.2 (8.4-12.2)</td>
<td>93 (48-156)</td>
<td>3.8 (2.6-5.3)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.89</td>
<td>0.86</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Extent of CAD</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0 vessels (n=100)</td>
<td>0.9 (0.8-1.0)</td>
<td>10.0 (8.0-11.7)</td>
<td>89 (39-164)</td>
<td>3.3 (2.3-5.3)</td>
</tr>
<tr>
<td>1 vessel (n=67)</td>
<td>0.9 (0.8-1.1)</td>
<td>11.0 (9.0-12.0)</td>
<td>107 (57-215)</td>
<td>4.1 (3.0-5.8)</td>
</tr>
<tr>
<td>2 vessels (n=45)</td>
<td>0.9 (0.8-1.1)</td>
<td>11.4 (8.8-11.4)</td>
<td>105 (54-235)</td>
<td>5.1 (3.5-8.8)</td>
</tr>
<tr>
<td>3 vessels (n=23)</td>
<td>1.0 (0.8-1.1)</td>
<td>10.0 (9.4-11.1)</td>
<td>145 (93-221)</td>
<td>5.3 (3.8-8.1)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.19</td>
<td>0.10</td>
<td>0.06</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female (n=80)</td>
<td>0.9 (0.7-1.0)</td>
<td>10.2 (8.1-11.8)</td>
<td>114 (61-219)</td>
<td>3.2 (2.1-4.5)</td>
</tr>
<tr>
<td>Male (n=155)</td>
<td>0.9 (0.8-1.0)</td>
<td>10.3 (8.7-12.8)</td>
<td>95 (48-182)</td>
<td>4.6 (3.3-6.6)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.20</td>
<td>0.28</td>
<td>0.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n=143)</td>
<td>0.9 (0.8-1.1)</td>
<td>10.5 (8.7-12.5)</td>
<td>112 (62-220)</td>
<td>4.1 (2.7-5.7)</td>
</tr>
<tr>
<td>Never (n=92)</td>
<td>0.9 (0.8-1.0)</td>
<td>10.1 (8.3-12.2)</td>
<td>83 (45-161)</td>
<td>4.1 (2.9-7.4)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.31</td>
<td>0.48</td>
<td>0.04</td>
<td>0.33</td>
</tr>
<tr>
<td><strong>UMI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n=58)</td>
<td>1.0 (0.8-1.1)</td>
<td>11.1 (9.0-13.3)</td>
<td>173 (65-263)</td>
<td>5.4 (7.7-9.5)</td>
</tr>
<tr>
<td>No (n=177)</td>
<td>0.9 (0.8-1.0)</td>
<td>10.1 (8.3-12.0)</td>
<td>94 (48-156)</td>
<td>3.7 (2.6-5.4)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.11</td>
<td>0.033</td>
<td>0.006</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

CAD=coronary artery disease, IQR = inter quartile range, LVEF = left ventricular ejection fraction, UMI = unrecognized myocardial infarction.
Multivariable linear regression analysis showed UMI, extent of CAD, sex, and NT-proBNP to be independent associated with cTnI level (Table 13), while neither UMI nor extent of CAD was independently associated with NT-proBNP or Gal-3 level.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Beta</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.03</td>
<td>0.72</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>-0.006</td>
<td>0.94</td>
</tr>
<tr>
<td>Diabetes</td>
<td>-0.001</td>
<td>0.98</td>
</tr>
<tr>
<td>Extent CAD</td>
<td>0.13</td>
<td>0.047</td>
</tr>
<tr>
<td>Galectin-3</td>
<td>0.03</td>
<td>0.68</td>
</tr>
<tr>
<td>LVEF</td>
<td>-0.06</td>
<td>0.36</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>0.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.21</td>
<td>0.002</td>
</tr>
<tr>
<td>UMI</td>
<td>0.14</td>
<td>0.031</td>
</tr>
</tbody>
</table>

Baseline characteristics in studies IV and V

The baseline characteristics of the 24 subjects included in studies IV and V are shown in Table 14. Coronary angiography was performed in all 24 subjects. Ten subjects (42%) underwent PCI, and 2 (8%) had CABG. The mean LVEF was 68% (range 51-78%). None of the subjects exhibited ECG signs of left ventricular hypertrophy, Q-waves, ST-segment elevation, or ST-segment depression at the time of blood sampling. Two subjects showed pathological T-waves at inclusion but not at admission to hospital. Continuous multi-lead ST-monitoring did not reveal signs of acute ischemia or persistent tachycardia in any patient during the 24 hour period preceding coronary angiography.
Unrecognized myocardial infarction and biochemical markers.

Table 14. Baseline characteristics, study IV and V

<table>
<thead>
<tr>
<th>Subject (number)</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, median, IQR)</td>
<td>68 (64-72)</td>
</tr>
<tr>
<td>Female</td>
<td>11 (46%)</td>
</tr>
<tr>
<td>Smoker/former smoker</td>
<td>16 (67%)</td>
</tr>
<tr>
<td>Family history of premature cardiovascular disease</td>
<td>11 (46%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>15 (63%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>6 (25%)</td>
</tr>
<tr>
<td>Angina symptoms 2-12 months</td>
<td>10 (42%)</td>
</tr>
<tr>
<td>Angina symptoms &gt;12 months</td>
<td>14 (58%)</td>
</tr>
<tr>
<td>CCS class I</td>
<td>4 (17%)</td>
</tr>
<tr>
<td>CCS class II</td>
<td>11 (46%)</td>
</tr>
<tr>
<td>CCS class III</td>
<td>9 (38%)</td>
</tr>
<tr>
<td>Chest pain less than 72 hours before admission</td>
<td>15 (63%)</td>
</tr>
<tr>
<td>Chest pain more than 15 minutes in past 72 hours</td>
<td>4 (17%)</td>
</tr>
</tbody>
</table>

CCS = Canadian Cardiovascular Society.

Baseline cTn and NT-proBNP values at study inclusion and hospital admission are shown in Table 15. One subject had only three blood samples collected and was excluded from further analyses. In the remaining 23 subjects, samples were obtained at six time points.

Table 15. Baseline cTn and NTpro-BNP at inclusion and at admission.

<table>
<thead>
<tr>
<th>Marker (ng/L)</th>
<th>Inclusion</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>cTnI</td>
<td>1.2-42.7</td>
<td>7.8 ±10.5</td>
</tr>
<tr>
<td>cTnT</td>
<td>5.5-32.1</td>
<td>12.7±2.0</td>
</tr>
<tr>
<td>NTproBNP</td>
<td>20.0-1472.0</td>
<td>220.5±344.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Admission</th>
<th>Range</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>cTnI</td>
<td>1.2-104.6</td>
<td>11.7±22.4</td>
</tr>
<tr>
<td>cTnT</td>
<td>6.0-32.7</td>
<td>12.5±8.2</td>
</tr>
<tr>
<td>NTproBNP</td>
<td>16.0-1281.0</td>
<td>205.8±292.0</td>
</tr>
</tbody>
</table>
STUDY IV

The cTnI concentrations in all samples were above the LoD. The cTnT concentrations in 21 subjects were above the LoB, and in 16 subjects the cTnT concentrations in all samples were above the LoD. Two subjects had cTnI concentration values above the 99th percentile for a healthy reference population at inclusion, as did two additional subjects at the time of admission to the hospital. Six subjects had cTnT values above the 99th percentile at inclusion, as did five subjects when admitted for the short-term study.

Short- and long-term variation in cTn

The mean short-term and long-term CVt, CVa, CVi, CVg, RCV, and II of cTnI and cTnT in the total study population are summarized in Table 16. The long-term variation was calculated based on the blood samples taken at inclusion and admission mean interval of 21 days (range 4-58). The individual RCV-lognormal rise values ranged from 13 to 160% for cTnI, and from 7 to 58% for cTnT (Figure 12).

Figure 12. Distribution of the individual RCV-lognormal rise values of cTnI and cTnT.
### Table 16. Analytical and individual variation of cTnl and cTnT.

<table>
<thead>
<tr>
<th>Variable</th>
<th>cTnl</th>
<th>cTnI</th>
<th>cTnT</th>
<th>cTnT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Short-term</td>
<td>Long-term</td>
<td>Short-term</td>
<td>Long-term</td>
</tr>
<tr>
<td>No. of values¹ /subjects</td>
<td>138/23</td>
<td>46/23</td>
<td>96/16</td>
<td>32/16</td>
</tr>
<tr>
<td>CVt% (range)</td>
<td>16 (4-35)</td>
<td>25 (1-87)</td>
<td>8 (3-17)</td>
<td>11 (1-47)</td>
</tr>
<tr>
<td><strong>Analytical variation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVa%</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><strong>Individual variation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVi%</td>
<td>14</td>
<td>24</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>CVg%</td>
<td>187</td>
<td>163</td>
<td>70</td>
<td>65</td>
</tr>
<tr>
<td>RCV%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>49</td>
<td>69</td>
<td>23</td>
<td>32</td>
</tr>
<tr>
<td>Lognormal % (rise, fall)</td>
<td>54, -35</td>
<td>97, -49</td>
<td>26, -21</td>
<td>37, -27</td>
</tr>
<tr>
<td>II</td>
<td>0.08</td>
<td>0.15</td>
<td>0.12</td>
<td>0.18</td>
</tr>
</tbody>
</table>

¹Total number of values after omitting values below the LoD. CV = coefficient of variation; CVa = analytical CV; CVi = intra-individual CV; CVg = inter-individual CV; CVt = total CV, RCV = reference change value, II = index of individuality.
STUDY V

The NT-proBNP levels were higher than age-dependent reference levels in at least one sample in 5 of the 24 subjects.

Short- and long-term variation in NT-proBNP

The mean variation of NT-proBNP at 4 hour intervals (6 measurements) and at a 20 hour interval (2 measurements) as well as at long-term (average 21 days) are presented in Table 17.

<table>
<thead>
<tr>
<th>Variable</th>
<th>4 hour</th>
<th>20 hour</th>
<th>21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>23</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>CVt %</td>
<td>12.2</td>
<td>12.7</td>
<td>20.6</td>
</tr>
<tr>
<td>CVa %</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>CVi %</td>
<td>11.8</td>
<td>12.4</td>
<td>20.4</td>
</tr>
<tr>
<td>CVg %</td>
<td>141</td>
<td>143</td>
<td>149</td>
</tr>
<tr>
<td>II</td>
<td>0.087</td>
<td>0.089</td>
<td>0.138</td>
</tr>
<tr>
<td>RCV %</td>
<td>34</td>
<td>35</td>
<td>57</td>
</tr>
<tr>
<td>Lognormal RCV %</td>
<td>41, -29</td>
<td>42, -30</td>
<td>76, -43</td>
</tr>
</tbody>
</table>

CV = coefficient of variation, CVa = analytical CV; CVi = intra-individual CV; CVg = inter-individual CV; CVt = total CV, RCV = reference change value, II = index of individuality.

The short-term individual positive and negative RCV lognormal values for NT-proBNP were essentially normally distributed and ranged from 9 to 80% and –8 to –44 %, respectively, in the short-term study. The long-term RCV lognormal positive and negative values ranged from 1.3 to 352% and –1.3 to –78%, respectively. The long-term individual positive RCV lognormal values showed a positive (right) skewed distribution.

The NT-proBNP results in two male subjects were high and considered statistical outliers. The first subject exhibited ≥70% coronary artery stenosis, LVEF of 51%, and blood pressure of 150-185/70-80 mm Hg and underwent PCI. The other outlier showed ≥70% coronary artery stenosis; LVEF of 65% and blood pressure of 160-215/75-90 mm Hg. This patient
underwent CABG after angiography. The CVi and lognormal RCV did not change significantly when these subjects were excluded. There was also no significant difference in mean CVt for NT-proBNP in males and females or between subjects with CAD requiring revascularization and those who did not.

No significant circadian variation was revealed by the Kruskal-Wallis test either with or without outliers (p = 0.99).
DISCUSSION

The main findings of these studies were

(i) a UMI prevalence of 25% in subjects with stable CAD without previously known MI;

(ii) a statistically significant association between ≥70% stenosis and the presence of UMI in the myocardial segment supplied by the stenotic artery as well as a significant association between the severity and extent of CAD and UMI at an individual level;

(iii) a statistically significant association between UMI and the prognosis;

(iv) a statistically significant association between UMI and the level of the cardiac biochemical markers troponin, NT-proBNP and Gal-3, and

(v) the individual variations of cTnI, cTnT, and NT-proBNP in subjects with stable CAD were similar to the biological variation in healthy individual.

Prevalence of UMI

In study I, the prevalence of UMI was 25% in subjects with stable CAD and no previously diagnosed MI. The prevalence was in line with the 27% prevalence found in a previous study in a similar population [42] and may be compared to the 30%[56], 20% [51], and 17% [41] prevalence reported in the general population. The high prevalence in the present study compared to the prevalence of 17-20% in the general population is not surprising given the higher probability of ischemic heart disease in our study population. The study that found a 30% prevalence [56] included subjects 10 years older than our cohort, which may explain the deviant result.

Comparison of our prevalence of LGE-CMR-detected UMIs with the prevalence reported in studies of ECG-detected UMIs in subjects with CAD (8-36%) [2, 42, 43] is difficult, since we measured different factors. In our cohort, all subjects with Q-waves on ECG were excluded; and the
obtained prevalence of UMI in the cited ECG studies would likely have been different if LGE-CMR had been used.

**Extent and localization of UMI**

In study I, UMIs were predominantly small subendocardial infarctions, which is in accordance with previous findings [51]. Nevertheless, a significant proportion of UMIs consisted of transmural infarctions (21/58) that were not associated with the presence of Q-waves. In contrast to what is seen in recognized MIs [143], the UMIs were located predominantly in the inferior and inferior-lateral areas of the myocardium, in agreement with the findings of Barbier [51]. This distribution was present despite stenoses ≥70% being more common in the LAD than in the RCA and LCX. The reason for this paradox is unclear, but it is likely that ischemia in the inferior and inferior-lateral areas of the myocardium leading to small infarctions may cause less severe, and more often atypical, symptoms and therefore not prompt the individual to seek medical attention. In addition, ECGs have a low sensitivity for MI in the inferior-lateral area of the myocardium [144, 145]: hence, these infarctions may go undetected.

**Coronary stenosis and UMI**

Study I revealed UMIs significantly more frequently in subjects with ≥70% coronary artery stenosis than in subjects without stenosis, which is in accordance with previous findings [42]. A unique feature of the present study was the attempt to evaluate the relationship between the presence of UMI and the occurrence and severity of atherosclerotic lesions in the coronary artery supplying the affected myocardial segment, which, to the best of my knowledge, has not been previously studied. A strong association was observed between stenosis grade ≥70% in a coronary artery and the presence of UMI in the myocardial segments supplied by that artery, as well as a dose/response effect: UMI was more common in segments downstream of a total occlusion compared to a 70-99% grade stenosis.

Since, due to individual differences in coronary anatomy, it is difficult to precisely match a coronary artery to its corresponding myocardial segment, our finding that 68% of the UMIs were supplied by an artery with ≥70% stenosis is probably an underestimate. Therefore a secondary sensitivity analysis requiring only near match was conducted, which may
have led to an overestimation. In the analysis, 88% of the segments with UMIs were supplied by a coronary artery with ≥70% stenosis.

**Prognosis and UMI**

In study II, we found subjects with UMI have a poorer cardiovascular prognosis compared to those without UMI, in agreement with results in previous studies [2, 42]. In multivariable analyses, the severity and extent of CAD was the most important predictor of new cardiac events and death, while the presence of UMI was not a statistically significant predictor, although the point estimate of the odds ratio showed a two-fold higher risk.

We hypothesized that a UMI located in an area supplied by a coronary artery with ≥70% stenosis would indicate aggressive disease and be associated with worse prognosis. However, there was no difference in event rates of subjects with UMI in a myocardial segment supplied by an artery with ≥70% stenosis and those with a less severe stenosis in the supplying artery.

The average annual mortality rate in our cohort was approximately 1% in subjects both with and without UMI, which is in accordance with estimates of 1.2-2.4% annual mortality in individuals with stable CAD derived from global clinical trials of anti-anginal and preventive therapy [23]. In two previous studies of the prognostic impact of UMI, the mortality in the UMI groups was about 11% [42] and 22% annually [2] as estimated from the reported hazard ratios. The discrepancy in mortality between our study and previously reported results may be due to differences in subject characteristics. In the study by Kwong et al. [2], all subjects were referred for CMR on clinical grounds, and patients with clinically indicated CMR may differ from those undergoing standard evaluation for suspected CAD. The former population may include a greater number of individuals with atypical clinical presentation and/or multiple cardiac issues. In contrast to our study, the cohort of Kwong et al. [2] included subjects who had undergone coronary revascularization with PCI and/or CABG prior to CMR. The revascularization procedures may have resulted in procedure-related myocardial injuries [1, 146] reported as UMIs, resulting in higher UMI prevalence.
In the study by Kim et al. [42], the selection of subjects was similar to that of our study. No subjects had undergone previous coronary interventions, and CMR was conducted for research purposes only. Despite this, there were considerable differences in clinical characteristics between the subjects in the study by Kim et al. [42] and our subjects. A higher proportion of our subjects reported a family history of CAD (52% vs. 30%), were more frequently current or previously smokers (66% vs. 32%), and fewer suffered from diabetes mellitus (29% vs. 44%). At inclusion, our patients reported a more exhaustive anti-anginal medical treatment. The use of aspirin, beta blockers and cholesterol lowering drugs was 91% vs. 64%, 76% vs. 42% 79% vs. 38%, respectively. A high proportion of the UMI subjects in our study underwent revascularization shortly after angiography, while a smaller proportion of those with UMI in the study by Kim et al. [42] did. The impact of revascularization on the survival of individuals with stable CAD is debated [33]. The differences in medical treatment are remarkable and the subjects in our cohort received treatment more in accordance with guideline recommendations [23], which may contribute to explain the deviant outcomes.

Kim et al. [42] demonstrated an association between UMI and extent and severity of CAD, as did we. However, we elaborated further and adjusted the data of UMI and prognosis for the extent and severity of CAD and investigated the relationship between the location of significant coronary artery stenosis and the site of UMI.

**Cardiac biochemical markers and UMI**

Study III demonstrated an association of cTnI levels with the presence of UMI, which remained statistically significant after adjustment for age, sex, LVEF, extent of CAD, and other biomarkers. We also found univariate associations between levels of NT-proBNP and Gal-3 and the presence of UMI.

The difference in median cTnI levels between subjects without UMI (3.7 ng/L) and those with UMI (5.4 ng/L) was statistically significant but numerically small, well within the short-term individual variation of cTnI observed in study IV, and with overlapping IQRs. Thus, despite a difference in mean cTnI levels between groups, the levels cannot be used to reliably predict the presence of UMI in individual subjects.
Studies have shown an association between levels of NT-proBNP and the severity of CAD as documented by angiography [147, 148]. A previous study demonstrated an association between increased levels of NT-proBNP and UMI [106]. In contrast, study III demonstrated an association between NT-proBNP and UMI in univariate analyses that disappeared in multivariable analysis, indicating that NT-proBNP is neither a clinically useful predictor of UMI nor CAD in patients with stable CAD.

Both NT-proBNP and cTnI have been shown to be associated with left ventricular function and infarct size one year after clinically recognized MI in patients with stable CAD [149]. Study III confirms a statistically significant association between NT-proBNP, low LVEF, cTnI and high age remains even in multivariate analysis, indicating that NT-proBNP is a useful predictor for left ventricular function but not for small scars in the myocardium.

The Gal-3 levels were significantly higher in subjects with UMI than in those with no UMI. Levels of cTnT and NT-proBNP have been shown to be associated with biomarkers of collagen metabolism relevant to myocardial fibrosis, other than Gal-3 [150]. The level of Gal-3 correlated with levels of Cystatin-C, NT-proBNP, and cTnI in the present study. In multivariable analyses only high age and Cystatin-C remained significantly associated with Gal-3 concentration, indicating that aging process and renal function are more important for the Gal-3 concentration than small processes in the myocardium.

The pathogenesis of UMI

Several pathophysiological mechanisms may cause UMI. In study I, an association of UMI with the extent and severity of CAD was observed, which is supported by the results of another study [42]. The progression of atherosclerosis may include repeated silent plaque ruptures and thrombosis [21, 151], which may occasionally lead to UMI. These subclinical episodes of plaque disruption are followed by wound healing, with an increase in plaque burden and narrowing of arteries [20]. Thus, the presence of UMI may indicate the progression of atherosclerosis into more severe and widespread CAD.

An alternative scenario for the origin of UMI is the combination of severe stenosis and episodes of tachy- or bradyarrhythmia causing myocardial
ischemia due to an imbalance of oxygen supply and demand [1]. A third possible mechanism may be ischemia and necrosis associated with episodes of coronary spasm [152].

Study II demonstrated that subjects with UMI are more likely to experience additional cardiac events and death than are those without UMI. The absence of a significant difference in event rates between subjects with or without stenosis ≥70% in the artery supplying the UMI-affected myocardial segment may be explained by the fact that plaques, obstructive or non-obstructive, generating new events may be located anywhere in the coronary tree. This somewhat contradictory finding is supported by other studies suggesting that most MIs are not necessarily a consequence of a severe stenosis, but rather due to the development of new plaque ruptures in distant coronary artery segments without flow-limiting stenoses [18, 19, 33]. The total coronary stenosis burden has been shown to predict the incidence of subsequent cardiac events better than the number of high-grade stenoses [153], and severe stenoses are more often associated with protective collateral circulation [18]. However, the lack of difference in event rates might also be a consequence of insufficient statistical power due to the small number of events in the present study, i.e. type II error.

Unrecognized MI was statistically significant associated with the level of cTnI, as reported in study III. Several pathophysiological processes may cause cTnI release [1, 61]. Elevated levels of cTnI in subjects with UMI might reflect on-going low-grade, reversible or irreversible, damage of myocytes and/or slow clearance of cTnI from the circulation. In the present study, we attempted to minimize interference from other known causes of chronically elevated cTnI, such as heart failure, cardiomyopathy, and severe renal failure, by applying stringent exclusion criteria. As all patients suffered symptoms of stable angina pectoris, strenuous exercise as a cause of cTnI elevation seemed unlikely. In addition, cTnI was measured when subjects were clinically stable and experiencing no acute chest pain or exhibiting other obvious symptoms of myocardial ischemia. LGE-CMR was performed at a median of four days later under similarly stable conditions, with no indication of an ongoing acute myocardial event. The association of higher levels of cTnI with UMI existed despite the fact that the levels of cTnI in most cases were well below the 99th percentile value seen in a healthy reference population. Thus, an acute coronary syndrome as explanation of the association between cTnI levels and the presence of
UMI is highly unlikely. Furthermore, renal function, inferred from cystatin C levels, was within the normal range and did not differ statistically significantly between subjects with and without UMI. Hence, impaired renal elimination of cTnI is not likely as an explanation of the difference in cTnI levels between groups.

A slower, possibly chronic, process is more likely. The free cytosolic pool of cTn may be released from viable cardiomyocytes via several mechanisms [154] including transport across a compromised cell membrane [61] and development of cytoplasmatic membranous blebs during ischemia [155]. Previous studies of hs-cTnI [156, 157] in patients with reversible ischemia have shown conflicting results, and in one study no increase in hs-cTnT [158] concentrations were reported. The reason for the difference may be molecule size, as transport across the cell membrane is required for the release of cTn from viable cardiomyocytes, and the smaller size of cTnI (approximately 26 kDa) may facilitate passage less probable with the larger sized cTnT (37-39 kDa).

Study III also demonstrated an association between angiographical severity of CAD and cTnI level, in line with previous studies [159-161]. After adjusting for the presence of CAD, the association between UMI and cTnI remained statistically significant. Obviously, factors other than epicardial obstructive CAD may influence cTn levels. Interestingly, Hochholzer et al. [162] recently demonstrated that elevation of cTn in individuals without obstructive CAD is predictive of a poor prognosis.

Clinically silent ruptures of non-obstructive plaques resulting in microscopic MIs, conditions affecting the coronary microvascular circulation (e.g. coronary microvascular disease or dysfunction) resulting in episodes of silent ischemia [155] or the myocardium itself may give rise to increased release of cTn. Studies of elevated levels of cTn in apparently healthy individuals have indicated the possibility of physiological renewal and remodeling of myocytes [163].

The differing sensitivity for detection of myocardial necrosis of the two techniques is noteworthy. The smallest LGE-CMR detected UMI in the present study weighed approximately 0.2 g, whereas the smallest amount of infarcted myocardial tissue able to detect by increasing circulating levels of cTn corresponds to a few milligrams [163, 164]. Thus, episodes of minimal
myocardial necrosis, manifested as temporary minor elevations of cTnI, might go undetected by LGE-CMR imaging.

A cross-sectional study like ours is unable to establish a temporal association of UMI with cTnI. However, Barbier et al. [165] have recently shown that cTnI levels in the general population may predict LGE-CMR-detected UMIs during the ensuing 5 years, indicating that increased cTnI levels precede development of MI.

**Individual variation in cardiac troponin**

Study IV is, to the best of my knowledge, the first to evaluate individual variation of cTn in subjects with stable CAD. The short-term individual variation, CVi, in subjects with stable CAD was 13.5% and 7.3% for cTnI and cTnT, respectively. The individual variation of cTn concentrations in subjects with CAD was similar to, or lower than, the biological variation reported in healthy individuals (Table 18). This is in agreement with a recent study of patients presenting with chest pain in an emergency department, excluding subjects with known stable angina pectoris [166].
Table 18. Short and long-term biological and individual variation in cTnI and cTnT.

<table>
<thead>
<tr>
<th>Assay and manufacturer</th>
<th>CVa</th>
<th>CVi</th>
<th>CVg</th>
<th>II</th>
<th>RCV</th>
<th>RCV+</th>
<th>RCV-</th>
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</thead>
<tbody>
<tr>
<td><strong>Short-term cTnI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abbott ARCHITECT i2000SR [132]</td>
<td>13.8</td>
<td>15.2</td>
<td>70.5</td>
<td>0.22</td>
<td>50.1</td>
<td>69.3</td>
<td>-40.9</td>
</tr>
<tr>
<td>Beckman Coulter Access 2 [132]</td>
<td>14.5</td>
<td>6.1</td>
<td>34.8</td>
<td>0.46</td>
<td>44.5</td>
<td>63.8</td>
<td>-38.9</td>
</tr>
<tr>
<td>Siemens Dimension Vista [132]</td>
<td>13</td>
<td>12.9</td>
<td>12.3</td>
<td>0.11</td>
<td>47</td>
<td>57.5</td>
<td>-36.5</td>
</tr>
<tr>
<td>Abbott ARCHITECT STAT, serum [82]</td>
<td>16.8</td>
<td>24.4</td>
<td>124</td>
<td>0.24</td>
<td>82</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Abbott ARCHITECT STAT, plasma [82]</td>
<td>16.9</td>
<td>37.1</td>
<td>129.2</td>
<td>0.23</td>
<td>113</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Erenna Immunoassay System Singulex [82]</td>
<td>8.3</td>
<td>9.7</td>
<td>57</td>
<td>0.21</td>
<td>NA</td>
<td>46</td>
<td>-32</td>
</tr>
<tr>
<td>Beckman coulter hs-cTnI [80]</td>
<td>3.5</td>
<td>3.4</td>
<td>45.3</td>
<td>0.1</td>
<td>NA</td>
<td>45.2</td>
<td>-15.8</td>
</tr>
<tr>
<td>Abbott ARCHITECT STAT hs-cTnI (study IV)</td>
<td>8</td>
<td>13.5</td>
<td>187</td>
<td>0.08</td>
<td>49</td>
<td>54</td>
<td>-35</td>
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<tr>
<td><strong>Long-term cTnI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roche E170 [81]</td>
<td>7.8</td>
<td>15</td>
<td>NA</td>
<td>NA</td>
<td>47</td>
<td>64</td>
<td>-39</td>
</tr>
<tr>
<td>Roche Elecsys 2010 [81]</td>
<td>9.7</td>
<td>21</td>
<td>NA</td>
<td>NA</td>
<td>62</td>
<td>90</td>
<td>-47</td>
</tr>
<tr>
<td>Roche Modular E170[78]</td>
<td>53.5</td>
<td>48.2</td>
<td>85.9</td>
<td>0.84</td>
<td>NA</td>
<td>84.6</td>
<td>NA</td>
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<tr>
<td>Elecsys hs-cTnI (study IV)</td>
<td>4</td>
<td>7.3</td>
<td>70</td>
<td>0.12</td>
<td>23</td>
<td>26</td>
<td>-21</td>
</tr>
<tr>
<td><strong>Short-term cTnT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Roche E170 [81]</td>
<td>7.8</td>
<td>15</td>
<td>NA</td>
<td>NA</td>
<td>47</td>
<td>64</td>
<td>-39</td>
</tr>
<tr>
<td>Roche Elecsys 2010 [81]</td>
<td>9.7</td>
<td>21</td>
<td>NA</td>
<td>NA</td>
<td>62</td>
<td>90</td>
<td>-47</td>
</tr>
<tr>
<td>Roche Modular E170[78]</td>
<td>53.5</td>
<td>48.2</td>
<td>85.9</td>
<td>0.84</td>
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<td>84.6</td>
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<tr>
<td>Elecsys hs-cTnT (study IV)</td>
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<td>7.3</td>
<td>70</td>
<td>0.12</td>
<td>23</td>
<td>26</td>
<td>-21</td>
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<td><strong>Long-term cTnT</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roche E170 [81]</td>
<td>7.8</td>
<td>31</td>
<td>NA</td>
<td>NA</td>
<td>87</td>
<td>138</td>
<td>-58</td>
</tr>
<tr>
<td>Roche Elecsys 2010 [81]</td>
<td>9.7</td>
<td>30</td>
<td>NA</td>
<td>NA</td>
<td>86</td>
<td>135</td>
<td>-58</td>
</tr>
<tr>
<td>Roche Modular E170[78]</td>
<td>98</td>
<td>94</td>
<td>94</td>
<td>1.4</td>
<td>NA</td>
<td>315</td>
<td>NA</td>
</tr>
<tr>
<td>Elecsys hs-cTnT (study IV)</td>
<td>4</td>
<td>10.7</td>
<td>65</td>
<td>0.18</td>
<td>32</td>
<td>37</td>
<td>-27</td>
</tr>
</tbody>
</table>

CVa= Coefficient of analytical variation, CVi= Coefficient of inter-individual variation, CVg= coefficient of intra-individual variation, II=index of individuality, RCV=reference change value, RCV+=positive log-normal RCV, RCV-=negative log-normal RCV. NA = not available.
Current guidelines recommend analytical precision to be ≤ 10% CV at the 99th percentile value in a healthy reference population [1]. In study IV the CVa was well within this recommendation for both assays. When individual variation is included, the change required to detect AMI will obviously increase. Based on our log-normal RCV results, a mean rising pattern of 54% or mean falling pattern of -35% for cTnI and 26% and -21% for cTnT would be required to indicate a significant short-term change.

The distribution of individual log-normal RCV for rising values indicates wide inter-individual variation. Thus, in some individuals a larger change in rising concentrations than the mean log-normal RCV would be needed to diagnose an AMI with certainty. In 22 of 23 patients (96%), the rising log-normal RCV for cTnI was lower than 100%, and, in the 16 subjects with measurable cTnT, the rising log-normal RCV was below 65%. Thus, applying a conservative approach, the diagnostic cut-off level for rising log-normal RCV would be 100% for cTnI and 65% for cTnT. However, this may lead to a substantial number of patients in whom AMI would be falsely ruled out based on a small observed increase in cTn.

Our results suggest, as an appropriate clinically compromise, that a change in cTn concentrations of 50% can be used in attempting to diagnose AMI. However, when using any selected cut-off level the trade-offs between sensitivity and specificity must be taken into consideration. Moreover, the results of this thesis are applicable on values below or close to the 99th percentile of a healthy reference population. The individual variation in patients with chronically elevated levels of cTn above the 99th percentile has not been investigated in this thesis and may differ.

Subjects suspected of stable CAD represent a heterogeneous group. We therefore analyzed the individual variation of cTn both with and without the inclusion of outliers. In addition, we probed for possible cTn differences among subjects with and without symptoms during the 72 hours preceding blood sampling and compared subjects requiring revascularization to those not requiring revascularization. There were only minor differences in CVi among these subgroups, similar to data reported for healthy individuals. Thus, stable coronary arteriosclerosis per se does not seem to affect individual variation in cTn concentrations to a great extent.
The long-term individual variation in cTn was almost two-fold the short-term individual variation. The long-term log-normal RCV for cTnI was 97% and -49%. Thus, when measurements are done weeks apart, cTn should increase almost 100% to reflect a change that reliably exceeds fluctuations due to long-term variation alone. In individuals suffering recurring chest pain, a change of 100% or less in cTn measured over a two-week interval may not indicate an AMI, but represent individual variation, requiring further testing for an accurate diagnosis.

The cTn levels show low within-subject variation but a large between-subject variation, which results in a low II. An II below 0.6 indicates that a population-based reference interval with a fixed high limit, i.e. the 99th percentile for cTn, is of limited value for diagnostic purposes [80]. In study IV the short-term II for cTnI and cTnT of 0.08 and 0.12, respectively, was at the low end of what has been reported in comparable studies [67, 79, 81, 82, 132] and considerably lower than the 0.6 limit. I found only a single previous study that reported an II above 0.6 [78]. These results indicate that serial testing showing a rising or falling pattern is essential to establish a reliable diagnosis of AMI using cTn.

**Individual variation in NT-proBNP**

Individual variation in NT-proBNP in subjects with stable CAD was found to be at levels similar to the variation in subjects with HF and hypertension as well as the biological variation in healthy individuals [107-113, 115-118, 167] (Table 19).

Variation of NT-proBNP in subjects with a stenosis grade ≥70% did not differ statistically significantly from those without. The variation in itself is thus not an indication of morbidity, but rather a consequence of the vulnerability of the myocardium to stress factors such as the level of exercise [168], heart rhythm [169], volume status, and diuretic therapy [170]. The actual level of NT-proBNP, on the other hand, has been shown to be linked to conditions such as HF and CAD and indicates a worse prognosis for the affected individuals [95, 100].
Table 19. Short-term biological and individual variation in NT-pro BNP.

<table>
<thead>
<tr>
<th>Author</th>
<th>Time period</th>
<th>CVa</th>
<th>CVt</th>
<th>CVi</th>
<th>CVg</th>
<th>II</th>
<th>RCV</th>
<th>Log RCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wu [107]</td>
<td>week-to-week</td>
<td>1.6</td>
<td>NA</td>
<td>33.3</td>
<td>36.5</td>
<td>0.9</td>
<td>92</td>
<td>NA</td>
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<tr>
<td>Pagani [110]</td>
<td>week-to-week</td>
<td>4.0</td>
<td>NA</td>
<td>35.0</td>
<td>56.4</td>
<td>NA</td>
<td>98*</td>
<td>NA</td>
</tr>
<tr>
<td>Melzi d’Erli [108]</td>
<td>day-to-day</td>
<td>2.7</td>
<td>NA</td>
<td>9.1</td>
<td>14.0</td>
<td>0.64</td>
<td>26</td>
<td>NA</td>
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<tr>
<td>Fradley [109]</td>
<td>week-to-week</td>
<td>2.7</td>
<td>NA</td>
<td>36</td>
<td>NA</td>
<td>NA</td>
<td>100</td>
<td>NA</td>
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<td>Heart failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Bruins [111]</td>
<td>within-day</td>
<td>3.0</td>
<td>9.1</td>
<td>8.6</td>
<td>NA</td>
<td>NA</td>
<td>25</td>
<td>NA</td>
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<tr>
<td>day-to-day</td>
<td>3.0</td>
<td>20</td>
<td>20</td>
<td>NA</td>
<td>NA</td>
<td>55</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>week-to-week</td>
<td>3.0</td>
<td>35</td>
<td>35</td>
<td>NA</td>
<td>NA</td>
<td>98</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>week-to-week</td>
<td>1.0</td>
<td>15</td>
<td>15</td>
<td>102</td>
<td>0.03</td>
<td>42</td>
<td>NA</td>
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<tr>
<td>Schou [112]</td>
<td>year-to-year</td>
<td>NA</td>
<td>35</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
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<tr>
<td>Schou [113]</td>
<td>week-to-week</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>98</td>
<td>157/-61</td>
<td></td>
</tr>
<tr>
<td>Cortés [115]</td>
<td>week-to-week</td>
<td>2.8</td>
<td>21.1</td>
<td>20.9</td>
<td>NA</td>
<td>NA</td>
<td>49.2</td>
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</tr>
<tr>
<td>week-to-week</td>
<td>2.8</td>
<td>6.9</td>
<td>6.3</td>
<td>NA</td>
<td>NA</td>
<td>16.1</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Frankenstein [117]</td>
<td>week-to-week</td>
<td>1.4</td>
<td>18.5</td>
<td>18.4</td>
<td>NA</td>
<td>0.11</td>
<td>51.1</td>
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<td>week-to-week</td>
<td>1.4</td>
<td>11.4</td>
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<td>0.07</td>
<td>31.4</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>week-to-week</td>
<td>1.4</td>
<td>17.7</td>
<td>17.6</td>
<td>NA</td>
<td>0.11</td>
<td>34.6</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>month-month</td>
<td>1.4</td>
<td>19</td>
<td>18.9</td>
<td>NA</td>
<td>0.12</td>
<td>52.5</td>
<td>NA</td>
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</tr>
<tr>
<td>2 months</td>
<td>1.4</td>
<td>15.6</td>
<td>15.5</td>
<td>NA</td>
<td>0.09</td>
<td>43.1</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>1.4</td>
<td>16.3</td>
<td>16.2</td>
<td>NA</td>
<td>0.10</td>
<td>45</td>
<td>NA</td>
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</tr>
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</table>
### Table 19. Short-term biological and individual variation in NT-pro BNP.

<table>
<thead>
<tr>
<th>Author</th>
<th>Time period</th>
<th>CVa</th>
<th>CVt</th>
<th>CVi</th>
<th>CVg</th>
<th>II</th>
<th>RCV</th>
<th>Log RCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>Roselló-Lleti [118]</td>
<td>Year 1</td>
<td>NA</td>
<td>12.3</td>
<td>12.1</td>
<td>NA</td>
<td>NA</td>
<td>34</td>
</tr>
<tr>
<td>Hypertrophy</td>
<td>Year 2</td>
<td>NA</td>
<td>12.5</td>
<td>12.3</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>35</td>
</tr>
<tr>
<td>Hypertrophy</td>
<td>2 Years</td>
<td>NA</td>
<td>14.7</td>
<td>14.5</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>41</td>
</tr>
<tr>
<td>Hypertrophy</td>
<td>Year 1</td>
<td>NA</td>
<td>21.1</td>
<td>20.9</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>58</td>
</tr>
<tr>
<td>Hypertrophy</td>
<td>Year 2</td>
<td>NA</td>
<td>28.5</td>
<td>28.4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>79</td>
</tr>
<tr>
<td>Hypertrophy</td>
<td>2 Years</td>
<td>NA</td>
<td>29.1</td>
<td>28.9</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>81</td>
</tr>
<tr>
<td>Stable CAD</td>
<td>Nordenskjöld, study V</td>
<td>Within-day</td>
<td>3.0</td>
<td>12.2</td>
<td>11.8</td>
<td>141</td>
<td>0.087</td>
<td>33.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>day-to-day</td>
<td>3.0</td>
<td>12.7</td>
<td>12.4</td>
<td>143</td>
<td>0.089</td>
<td>35.3</td>
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<tr>
<td></td>
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<td>3 weeks</td>
<td>3.0</td>
<td>20.6</td>
<td>20.4</td>
<td>149</td>
<td>0.138</td>
<td>57.1</td>
</tr>
</tbody>
</table>

CVa = Coefficient of analytical variation, CVg = Coefficient of inter-individual variation, CVi = coefficient of intra-individual variation, CVt = coefficient of total variation, II = index of individuality, LogRCV = lognormal reference change value, RCV = reference change value. NA = not available.
Both the short- and long-term variations are wide. A rise of >42% or a fall of 30% relative to baseline values are required to indicate a reliable within-day change, and this should be considered if measurements are used for prognostic evaluation in patients with chest pain, ACS, or stable CAD. In the long-term, a rise of >76% or a fall >43% is needed to indicate reliable change. This should be considered if repeat measurements are used for monitoring medical therapy in outpatients.

The II values of NT-proBNP levels in stable CAD are lower than in healthy individuals, but observed levels in both groups confirms that all patients should be assessed with respect to her or his individual hormone levels. The difference between healthy individuals and those with stable CAD might be explained by the wider variation among individuals in the latter group.

**Study design and methodological considerations**

Studies I-IV were based on subjects included in the PUMI study, a prospective multicenter cohort study. The subjects were recruited, examined, treated, and followed up by physicians at seven hospitals in Sweden. This design may have been associated with some confounding related to local treatment practices. However, analyses showed no statistically significant differences among investigating hospitals in subject characteristics or prognosis. All ECGs, coronary angiograms, and LGE-CMRs were analyzed centrally, in a blinded fashion, by one (ECG) or two physicians in consensus (coronary angiography and LGE-CMR), to minimize the possibility of local deviation. Blood samples were collected in EDTA-containing tubes and immediately centrifuged. The plasma was stored at –70°C until analysis. The samples were analyzed at a central laboratory. The risk that the results were biased by the inclusion and investigations at different sites must therefore be considered small.

**Sample size**

The sample size for the PUMI-study was calculated based on three assumptions (methods section), resulting in a predicted event rate of 15% for subjects with UMI and 3% for subjects with no UMI. To show a significant difference at the 5% level with 80% power, a total of 244 subjects (61 with UMI and 183 subjects without UMI) were needed. A margin of approximately 100 subjects was proposed, to minimize impact
of technical errors. Due to slow inclusion rate, enrollment was closed when 265 subjects were included. The assumptions used in the power calculation proved to be correct: the prevalence of UMI in the 235 subjects with satisfactory coronary angiography and LGE-CMR was 25%. Fifteen percent of the 58 subjects with UMI and 5% of the 177 subjects without UMI reached the primary composite endpoint. Although we did not enroll the minimum desired number of subjects, we were able to show a statistically significant difference in prognosis between subjects with and without UMI. The present research included more subjects than previous studies on this topic [2, 42], but the event rate was lower in our study. The low number of events precluded use of complex multivariable models that allow adjustment for a greater number of potentially relevant co-variates. Adjustment for a greater number of co-variates may facilitate understanding of the associations between variables.

The substudy included 24 individuals, and the sample size was based on sample sizes previously used in similar studies, 12-24 individuals for cTn [78, 79, 81, 82, 132, 133] and 15-45 individuals for NT-proBNP [107, 108, 110-113, 116, 117]. The distribution of the individual log-normal RCV values in the present thesis revealed one subject (1/24) with a much larger variation than the others. A larger cohort could more accurately have accounted for individuals with large variations.

The PUMI study was primarily designed to determine the association between UMI and prognosis. To investigate an association between cardiac biomarker levels and prognosis, a larger cohort and a longer follow-up would be required. Previous studies of cTn and prognosis in subjects with stable CAD have included 1469-3679 subjects and had 5.2-7.5 years of follow-up [67, 71, 72]. Studies of NT-proBNP and prognosis in subjects with stable CAD have enrolled 507-4372 subjects with 2-9.2 year follow-up periods [72, 100-102].

**Representativeness**

Individuals with stable CAD can be categorized as patients with effort angina pectoris, patients with previous MIs or patients with heart failure of ischemic origin. Our study cohort was not chosen to be representative of all individuals with stable CAD, but to find true MIs not previously detected. However, our baseline data describes a common population seen in Swedish coronary angiography laboratories [39]. According to the
SWEDEHEART registry, stable CAD is the most common indication for coronary angiography in Sweden, at approximately 21.3% of all coronary angiographies [39]. The mean age of individuals undergoing coronary angiography is 67 years, 69 for females and 66 for males and the proportion of normal angiographies ranges between 20% and 40% for all indications [39]. In the PUMI study, the average age was 65 years, and 43% showed no significant coronary artery stenosis. Despite the selective process, the subjects in our cohort resemble a common population at Swedish coronary angiography laboratories.

The characterization of the studied population would be more complete if the presence of myocardial ischemia had been assessed, either by non-invasive testing or as pre-test probability. However, the subjects had been evaluated by a cardiologist and referred to coronary angiography prior to enrollment, and thus, most likely the selection process for coronary angiography was conducted according to guidelines [73].

**Coronary angiography**

The presence and severity of coronary artery stenoses were visually assessed by two experienced radiologists in consensus. More accurate descriptions of the coronary anatomy might have been obtained via quantitative coronary angiography [171] or function assessment through determination of fractional flow reserve (FFR) [172] and documentation by SYNTAX score [173]. In this thesis a luminal stenosis grade of ≥70% was regarded as hemodynamically significant, in accordance with the traditional understanding of stable CAD as narrowing ≥50% in the left main coronary artery or ≥70% in at least one other major coronary artery [23]. More recent guidelines suggest indication for revascularization of stable CAD with a stenosis grade >50% in combination with documented ischemia or FFR <0.80 [35].

However, the physician in charge of the coronary angiography laboratory made independent decisions with respect to additional diagnostic studies to be conducted and treatment to be administered. Data regarding FFR and/or SYNTAX score may have been available for some subjects in the local medical records but was not considered in this study.

In 135 subjects, our central assessment of the coronary angiography results identified at least one >70% coronary artery stenosis. The attending
A physician adopted a conservative approach in 14 subjects as only 121 subjects were referred for coronary artery revascularization after the initial angiography. Normal FFR assessments or stenoses in vessels unsuitable for PCI are the most likely explanations for not performing PCI in these subjects. Of the 121 subjects receiving revascularization, only four were not judged in our assessment. Hence our visual assessment of ≥70% stenosis did not differ markedly from that made by attending physicians at the local sites, possibly since many PCI operators in daily practice still base decisions on visual evaluation of the coronary angiogram. The overall proportion of FFR use in angiographies for stable CAD was 27.3% during 2013 according to the SWEDEHEART registry [39].

**Individual variation**

Studies IV and V were based on the 24 subjects included in both the PUMI study and the substudy. The substudy subjects were recruited, examined and treated at two hospitals in Sweden. Continuous multi-lead ST-segment monitoring was performed using two systems, both common in routine clinical practice. A cardiologist familiar with the local system read and interpreted the recordings. To minimize analytical variation, blood samples in the substudy and the PUMI study were frozen and transferred to a central laboratory in which all analyses were performed at the same time, strictly following assay manufacturer’s instructions using single lots of reagents. The risk that the results were biased by the inclusion and investigations at different sites must therefore be considered small.
CONCLUSIONS

(i) Unrecognized MI is common in individuals with stable CAD without previously known MI and the results indicate that the majority of the UMIs are of ischemic origin.

(ii) The presence of UMI was associated with a statistically significant threefold risk of adverse events during follow-up. After adjustment for severity and extent of CAD, UMI was associated with a statistically non-significant but numerically doubled risk. Prognosis seems mainly influenced by the extent and severity of CAD but I cannot exclude that the presence of UMI (even in cases without significant epicardial CAD) may infer independent prognostic information.

(iii) The levels of cTnI, NT-proBNP, and Gal-3 were associated with presence of an UMI. Only the association of cTnI levels with presence of UMI remained statistically significant after adjustment for clinical factors and the severity and extent of CAD. The independent association between levels of cTnI and UMI may indicate a common pathophysiological pathway for the cTnI elevation and development of UMI.

(iv) The individual variation in cTn and NT-proBNP levels in subjects with stable CAD appeared similar to the biological variation in healthy individuals.

(v) For both cTn and NT-proBNP, a large change in levels was required to represent a significant short-term and long-term change. Based on the observed log-normal RCVs, a mean rising pattern of ≥54% or mean falling pattern of ≥35% for cTnI and ≥26% and ≥21% for cTnT is required to indicate significant short-term change. A rise of ≥76% or a fall of ≥43% is required to reliably confirm a clinically relevant long-term change in NT-proBNP.
(vi) Both cardiac troponins and NT-proBNP showed a low index of individuality, indicating that all subjects should be assessed with respect to her or his own individual level of the markers.
Clinical implications

(i) The prevalence of UMIs was high in individuals without Q-waves on ECG. Therefore, relying on the presence of Q-waves for diagnosis of previous MI will substantially underestimate UMI prevalence.

(ii) Individuals with UMIs incidentally detected by LGE-CMR may be considered for a coronary angiography if they exhibit cardiac symptoms, given the close relationship of UMI with hemodynamically significant CAD.

(iii) Measurement of cTn is a prerequisite for the diagnosis or exclusion of AMI. Knowledge of the individual variation in cTn in patients with stable CAD may improve evaluation in the emergency room. Changes in cTn concentration of >50% are highly indicative of AMI in patients with ischemic symptoms. Given the differences in results produced by various cTn assays, the values indicating a reliable clinically relevant change should be determined individually for each assay.

(iv) Patients suffering recurring chest pain may seek the emergency unit repeatedly and cTn measurements may be conducted at several occasions. The difference between measured levels of cTn at intervals of days or weeks may be large. For cTn levels below or close to the 99th percentile for a healthy reference population, a change of 100% may not indicate an AMI, but be due to intra-individual variation. Further serial testing and additional methods are required for accurate diagnosis.

(v) Repeat measurements of NT-proBNP may be used for guidance of medical management of HF in outpatients. A rise of >80% or a fall of >50% can be considered a change not attributable to individual or analytical variation.

(vi) Both cTn and NT-proBNP show low intra-individual variation but wide inter-individual variation, resulting in low index of individuality. This indicates that a population-based reference value with a fixed higher limit, i.e. the 99th percentile for cTn and the recommended alternative age-corrected 97.5th percentile for NT-proBNP, are of limited value for diagnostic purposes. Ideally, all patients should be assessed with respect to change.
from her or his individual baseline level, not on fixed cutoff values.
**Future research**

Many important questions remain, and the PUMI material may be used for additional studies. For example:

(i) Examination of the material using a cTnT assay with higher sensitivity may reveal diurnal variation.

(ii) Additional cardiac biochemical markers such as microRNAs, interleukins, midregional proadrenomedullin, growth-differentiation factor-15, soluble ST2 and others, may be associated with UMI and may provide more information on the mechanisms resulting in UMI and the prognosis for the patients.

(iii) A longer follow-up period might result in a larger number of events and provide power to show statistically significant associations using models that can incorporate multiple potentially relevant covariates. A five years follow-up of the subjects in the PUMI study is planned.

(iv) The PUMI material could be merged with other Swedish research materials containing information on DE-CMR-detected UMI and cTn to gain a larger cohort. To investigate the prognostic value of UMI, a large patient group may be linked to the Swedish patient register and causes of death register.

(v) In the PUMI study, only 19% of the subjects with UMI showed no stenosis at coronary angiography. One patient with UMI and without coronary artery stenosis (9%) reached the composite endpoint, death by a non-cardiac cause. Long-term follow-up of a larger group of UMI subjects without CAD could contribute to further understanding of the mechanisms, other than CAD, causing MI and aid in predicting prognosis. A related issue of importance is whether the outcome of patients with UMI without CAD is different from that of clinically recognized AMI without CAD.
(vi) It is important to investigate the consequences of the wide individual variation in cTn to rule-in and rule-out of MI. Which is the appropriate cut-off value? Which delta value (change in cTn) may be used?

(vii) A comparative study of different imaging techniques, such as LGE-CMR, echocardiography, myocardial perfusion scintigraphy using single photon emission computed tomography, positron emission tomography and X-ray computed tomography, may reveal the optimal method for detecting UMI. Several factors should be included such as sensitivity, specificity, prognostic value, risks, accessibility and costs for various groups of individuals.
Bakgrund

Flera biomarkörer kan mätas i blodet och ge information vid hjärt sjuk domar. De kardiella troponinerna är biomarkörer helt specifika för hjärtmuskelskada och kan användas för diagnos av akut hjärtinfarkt [1]. NT-proBNP kan användas vid diagnostik av hjärtsvikt [93] och Galectin-3 är associerad med flera biologiska processer, bland annat fibrosbildning [119]. Samtliga biokemiska markörer har potential att användas för att ge prognostisk information hos både friska individer och hos patienter med stabil kranskärlssjukdom [71, 72, 122].


Tidigare oupptäckta hjärtinfarkt (UMI), eller tyst infarkt, definieras som en hjärtinfarkt som inte upptäcks i samband med den akuta fasen, utan
upptäcks senare med hjälp av Q-vågor på EKG, avbildning av hjärtmuskeln med olika tekniker, däribland magnetkamera undersökning eller obduktion. I den här avhandlingen är UMI synonymt med UMI upptäckt med magnetkamera om inget annat anges.

**Metod**


**Biomarkör studien:** 24 personer ingick dessutom i en biomarkör studie där de lades in på kardiologisk vårdavdelning ett dygn innan kranskärlsröntgen. Under det dygnet togs blodprover var 4:e timme för att under表面上 biomarkörerna troponin och NT-proBNP varierar naturligt i blodet.

**Sammanfattning av resultat**

**PUMI-studien:** Hos 25 % av personerna med stabil kärlkramp upptäcktes en eller flera UMI. UMI var oftast små och lokaliserade subendokardiellt i hjärtats nedre delar. Patienter med täta förträngningar i hjärtats kranskärl hade oftare UMI än de som inte hade några täta förträngningar. Det fanns ett starkt samband mellan graden av förträngning och UMI.

Patienter med UMI hade en trefaldigt ökad risk för sjukdom och död under uppföljningstiden. Efter justeringar för andra, för prognosen,
betydelsefulla faktorer kvarstod en numerisk två gånger ökad risk. Patien-
ter med UMI hade högre nivåer av biomärkena troponin, NT-proBNP och Galectin-3.

Biomärke studen: Den individuella variationen av troponin och NT-
proBNP hos personer med stabil kärlnapp likande den hos friska individer. 
Både troponin och NT-proBNP varierar mycket naturligt i blodet och en 
stor förändring krävs för en förändring ska tolkas som säkert orsakad av 
sjukdom.

För att med säkerhet ställa diagnosen akut hjärtinfarkt krävs en förändring 
>50 % i troponin koncentration. En ökning på >76% eller en sänkning 
på >43% behövs för att på ett säkert sätt kunna använda NT-proBNP 
som hjälpmedel vid långtids-uppföljning av läkemedelsbehandling av 
hjärtsvikt.

Både troponin och NT-proBNP har lågt individuell index (II), vilket innebär 
att personers sjukdom bör bedömas med hjälp av personernas egna 
markörnivåer istället för att nivåerna jämförs med förutbestämda total-
nivåer baserade på ett referensmaterial.
ACKNOWLEDGEMENTS

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