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CORONARY COMPUTED TOMOGRAPHY ANGIOGRAPHY IN PATIENTS WITH MYOCARDIAL INFARCTION AND NON-OBSTRUCTED CORONARY ARTERIES

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CORONARY COMPUTED TOMOGRAPHY ANGIOGRAPHY IN PATIENTS WITH MYOCARDIAL INFARCTION AND NON-OBSTRUCTED CORONARY ARTERIES

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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ABSTRACT

Cardiovascular disease (CVD) is the number one cause of death worldwide. In Sweden, almost 30,000 people suffer an acute myocardial infarction (AMI) each year and, despite the greatly improved survival after AMI, CVD remains the leading cause of death among women and men. During the last decade, there has been increasing awareness of the significant minority of patients with acute myocardial infarction, for whom invasive coronary angiography (ICA) does not show any coronary artery stenoses. This condition is called myocardial infarction and non-obstructed coronary arteries (MINOCA) and is still incompletely understood. Another condition that has gained increasing attention is Takotsubo syndrome (TS), also known as stress-induced cardiomyopathy or the broken heart syndrome. Patients with TS may be considered a sub-group of MINOCA. Important advances in coronary computed tomography angiography (CTA) technology have enabled safe and accurate non-invasive imaging of the coronary arteries. In contrast to ICA, coronary CTA allows for detection of non-obstructive as well as obstructive coronary artery disease (CAD). Coronary CTA is also useful for assessment of plaque characteristics and detection of myocardial bridging (MB). In order to improve CVD risk prediction, numerous risk markers have emerged, among them carotid artery intima-media thickness (IMT), endothelial function determined by digital reactive hyperemia peripheral arterial tonometry (RH-PAT) and different categories of circulating biomarkers. Coronary CTA plaque burden has a prognostic value in CVD risk assessment, but its association with other risk markers is incompletely studied.

There were two major aims of this thesis. The first aim was to investigate the underlying mechanisms of MINOCA (study I and III). The second aim was to examine the association between coronary CTA plaque burden and other risk markers of CVD (study II and IV).

In study I we compared coronary CTA plaque burden in MINOCA patients and controls, matched by age and gender. We found that coronary CTA plaque burden was similar in the two groups and that a large proportion of MINOCA patients (42%) had no signs of CAD at coronary CTA. Non-obstructive CAD is most likely not a frequent cause of MINOCA.

In study II, 58 volunteers, free from clinical CVD, underwent testing for IMT and RH-PAT as well as coronary CTA. More than half of the study group had evidence of subclinical CAD at coronary CTA. There was no association between IMT or RH-PAT and presence or extent of CAD. Neither evaluation of IMT nor RH-PAT can reliably be used to predict coronary CTA plaque burden in clinically healthy subjects.

In study III the prevalence of MB, determined by coronary CTA, was compared for MINOCA patients, including a subgroup with TS, and matched controls. The MB depiction rate of coronary CTA and ICA was compared. MB was frequent, with a similar prevalence in MINOCA patients, patients with TS and controls, suggesting MB is not a frequent cause of MINOCA or TS. Coronary CTA detects significantly more MB than ICA.
In study IV, 115 subjects with predominantly low-to-intermediate CVD risk and normal or mildly reduced kidney function, underwent coronary CTA and laboratory testing. The groups without and with CAD differed with regard to levels of adiponectin, lipoprotein(a) and cystatin C. However, in a multivariable logistic regression model, only male sex and levels of cystatin C were independently associated with non-obstructive CAD at coronary CTA.

In conclusion, non-obstructive CAD is not a frequent cause of MINOCA in patients with angiographically normal or near-normal coronary arteries. MINOCA should probably not be considered a definitive diagnosis, but rather a working diagnosis, warranting additional diagnostic evaluation. TS, which is one of the possible underlying causes of MINOCA, is most likely not caused by MB. For TS, future consensus on the diagnostic criteria will facilitate research on pathophysiological mechanisms, diagnosis, prognosis and patient management. Circulating cystatin C was associated with non-obstructive CAD and may thus have a potential to serve as a screening test for subclinical CAD. However, CVD risk assessment is complex and large-scale studies are necessary to investigate which combination of imaging parameters and other risk markers yields the most accurate individual risk prediction.
LIST OF SCIENTIFIC PAPERS

The thesis is based on the following four papers, referred to in the text by their roman numerals:


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<tr>
<td>AMI</td>
<td>Acute myocardial infarction</td>
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<tr>
<td>CAC</td>
<td>Coronary artery calcium</td>
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<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>CCA</td>
<td>Common carotid artery</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CM</td>
<td>Contrast medium</td>
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<tr>
<td>CMR</td>
<td>Cardiovascular magnetic resonance</td>
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<tr>
<td>CT</td>
<td>Computed tomography</td>
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<tr>
<td>CTA</td>
<td>Computed tomography angiography</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>FRS</td>
<td>Framingham risk score</td>
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<tr>
<td>GFR</td>
<td>Glomerular filtration rate</td>
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<tr>
<td>HU</td>
<td>Hounsfield units</td>
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<tr>
<td>ICA</td>
<td>Invasive coronary angiography</td>
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<tr>
<td>IMT</td>
<td>Intima-media thickness</td>
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<tr>
<td>LAD</td>
<td>Left anterior descending artery</td>
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<td>MB</td>
<td>Myocardial bridging</td>
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<td>MINCA</td>
<td>Myocardial infarction and normal coronary arteries</td>
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<td>MINOCA</td>
<td>Myocardial infarction and non-obstructed coronary arteries</td>
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<td>NSTEMI</td>
<td>Non-ST elevation myocardial infarction</td>
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<tr>
<td>OR</td>
<td>Odds ratio</td>
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<tr>
<td>RHI</td>
<td>Reactive hyperemia index</td>
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<tr>
<td>RH-PAT</td>
<td>Digital reactive hyperemia peripheral arterial tonometry</td>
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<tr>
<td>ROI</td>
<td>Region of interest</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SMINC</td>
<td>Stockholm Myocardial Infarction with Normal Coronaries</td>
</tr>
<tr>
<td>STEMI</td>
<td>ST elevation myocardial infarction</td>
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<td>TS</td>
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1 INTRODUCTION

Cardiovascular disease (CVD) is the number one cause of death globally. According to the World Health Organization (WHO) [1] more than 17 million people died from CVD in 2012, representing approximately one-third of all global deaths. An estimated 7.5 million of these deaths were due to coronary heart disease [1]. In Sweden, almost 30 000 people suffer an acute myocardial infarction (AMI) each year. Even though survival after AMI has increased significantly during the last 20 years, CVD remains the leading cause of death among women and men [2]. In a significant minority of patients with AMI, invasive coronary angiography (ICA) shows no significant stenoses of the coronary arteries. The condition is called myocardial infarction with non-obstructed coronary arteries (MINOCA) and is still incompletely understood.

The studies of this thesis have been conceived and realized in a setting of rapid technological advancements and a constantly expanding field of knowledge. Important advances in computed tomography (CT) technology have enabled safe and accurate non-invasive imaging of the heart and the coronary arteries [3,4]. There has been an increasing awareness of MINOCA, for which there are currently no therapeutic guidelines [5]. Another condition that has gained increasing attention during the last decade and for which underlying mechanisms are largely unknown is the Takotsubo syndrome (TS), also referred to as stress-induced cardiomyopathy or the “broken heart syndrome” [6-9].

The screening phase of the multicenter Stockholm Myocardial Infarction with Normal Coronaries (SMINC) study [10] started in 2007. By then, coronary computed tomography angiography (CTA) was an established method in the Radiology department in Huddinge, Karolinska University Hospital, and the idea emerged to use coronary CTA to detect and quantify coronary artery disease (CAD) in MINOCA patients and in controls (study I). It soon became clear that a large proportion of MINOCA patients fulfilled the diagnostic criteria for TS [10,11]. The frequent observation of myocardial bridging (MB) at coronary CTA, in a clinical setting, together with the publication of research suggesting a causative role of MB in ischemia and in TS [12,13] gave rise to the idea of studying MB in MINOCA patients (study III). During the last decade, different biomarkers for improving CVD risk prediction have attracted considerable attention and new knowledge has emerged regarding the prognostic value of coronary CTA [14-16]. Studies II and IV investigate the association between coronary CTA plaque burden and different biomarkers of CVD.

1.1 CORONARY COMPUTED TOMOGRAPHY ANGIOGRAPHY

Since 1998, when the first generations of multi-detector CT were introduced, remarkable progress has been made in the field of CT technology. In order to visualize the heart and the coronary arteries high spatial resolution is required as well as high temporal resolution. Most 64-detector CT scanners can provide spatial resolution of 0.4 mm and temporal resolution of 175 ms (i.e. the required time for data acquisition for one slice). Electrocardiographic (ECG) synchronization makes it possible to obtain motion-free images despite the constant motion
Coronary CTA has a very good accuracy for diagnosing stenoses of the coronary arteries, compared to ICA. In particular, an excellent negative predictive value has been demonstrated in several meta-analyses [3,19]. Coronary CTA has proven highly sensitive in detecting atherosclerotic plaques in the proximal segments of the coronary arteries, as compared to intravascular ultrasound [20]. A histopathologic study [21] showed that coronary CTA detected all advanced plaques, but missed many of the very early lesions (Stary I-II). In contrast to ICA, coronary CTA not only shows the lumen of the coronary arteries, but also the vessel wall and what lies beyond it. Coronary CTA thus allows for the detection and characterization of coronary atherosclerotic plaques even when they do not give rise to stenoses and whether they are calcified or not. Accordingly, coronary CTA makes it possible to assess atherosclerotic plaques undetected by ICA. Figures 1(A) and 5 show different plaque types, as seen on coronary CTA. The method has also proven suitable for anatomic depiction of MB, since the epicardial fat and the myocardium as well as the vessel lumen, are well visualized (Figures 3 and 6).

Recent clinical practice guidelines issued by the European Society of Cardiology (ESC) and the American College of Cardiology Foundation (ACCF)/American Heart Association (AHA) identify a number of appropriate clinical indications for coronary CTA, among them ruling out CAD in symptomatic patients with intermediate pre-test probability of CAD or before valve surgery in patients with low probability of CAD [22].

Coronary artery calcium (CAC) scoring dates back to the 1980s, when electron beam CT was used to determine the amount of calcified plaque in the coronary arteries. The CAC score developed by Agatston et al. (1990) [23] has been shown to improve CVD risk prediction beyond commonly used risk assessment algorithms [23-26]. In 2007, when we started the work eventually leading to this thesis, little was known on the prognostic value of coronary CTA. Since then, however, several studies have demonstrated a prognostic value of coronary CTA regarding CVD events, in asymptomatic and symptomatic subjects [14,15,27,28].

1.2 OTHER IMAGING MODALITIES FOR CORONARY ARTERY DISEASE

The atherosclerotic process is complex and involves a number of local and systemic factors, including local hemodynamics, endothelial dysfunction, hyperlipidemia and widespread inflammation [29]. Development of an atherosclerotic lesion is a gradual process. The earliest imaging sign is diffuse intimal thickening, which may later progress to a localized atheroma with a fibrous cap. If the lipid core enlarges and the fibrous cap thins, the lesion is called a
thin-cap fibroatheroma. This plaque phenotype is associated with an increased risk of plaque rupture. At a later stage, increasing calcification leads to the formation of a fibrocalcific plaque, which is considered to be more stable. Even though these plaques rarely cause thrombosis and acute coronary syndromes they may cause chronic ischemic symptoms due to gradual lumen narrowing [30].

For many years, ICA has been the gold standard for evaluating the coronary arteries. Spatial and temporal resolution is very high (0.2 mm, 5 ms) compared to coronary CTA. ICA allows for visualization of CAD with stenotic lesions, but often misses early CAD with preserved vessel lumen and does not show the composition of atherosclerotic lesions. An advantage of ICA is the possibility of performing physiological assessment through fractional flow reserve measurements, which is the gold standard for identification of lesions that cause ischemia [31]. During coronary artery catheterization, the use of coronary intravascular ultrasound allows detection of early atherosclerotic intimal changes, due to its high spatial resolution (100-150 µm) and is the gold standard for plaque quantification. Even higher spatial resolution (10-40 µm) can be attained by optical coherence tomography, which is the only method allowing direct visualization of the cap of the thin-cap fibroatheroma (<65 µm) and is sensitive for detecting plaque rupture. The method uses near-infrared light, which does not penetrate tissues as effectively as ultrasound and does therefore not allow visualization of the outer vessel wall [30,32].

Hence, the earliest phases of CAD can be detected by intravascular ultrasound or by optical coherence tomography. When atheromas have developed, they can be demonstrated and characterized by coronary CTA and when the degree of stenosis increases they may be visualized by ICA. If calcification ensues CAD can also be quantified by CAC scoring.

1.3 MYOCARDIAL INFARCTION AND NON-OBSTRUCTED CORONARY ARTERIES

1.3.1 Acute myocardial infarction

In pathology, myocardial infarction is defined as myocardial cell death due to prolonged ischemia (i.e. decreased blood flow and oxygen supply to the heart muscle). In an expert consensus document from 2007 [33] AMI was defined as “evidence of myocardial necrosis in a clinical setting consistent with acute myocardial ischemia”. Elevated blood levels of sensitive and specific biomarkers such as cardiac troponins indicate myocardial injury and cell death. Detection of a rise and/or fall in troponins is essential to the diagnosis of acute myocardial infarction (AMI), since chronic elevation of troponins can occur in a number of conditions, such as heart failure, renal failure or pulmonary embolism. Myocardial ischemia typically gives rise to symptoms such as chest discomfort, discomfort of arm, mandibula or epigastrium, shortness of breath or fatigue, but can also present with atypical symptoms or even no symptoms. Electrocardiographic changes can be used to classify patients with AMI into two groups: with ST elevation myocardial infarction (STEMI) or with non-ST elevation
myocardial infarction (NSTEMI), which has implications for recommended treatment strategies [34].

Typically, AMI is considered to be a manifestation of pre-existing CAD, with plaque rupture or ulceration and subsequent intraluminal thrombus formation in a coronary artery, leading to decreased myocardial blood flow. This is classified as AMI type 1, according to the universal classification of myocardial infarction. AMI type 2 corresponds to myocardial injury with necrosis, where a condition other than CAD causes an imbalance in myocardial oxygen supply and demand, e.g. tachycardia, hypertrophic cardiomyopathy or coronary spasm [33]. Early ICA studies from the 1980s [35,36] demonstrated the presence of significant obstructive CAD in >97% of patients with STEMI or NSTEMI, stressing the importance of obstructive CAD in AMI.

1.3.2 Myocardial infarction and non-obstructed coronary arteries

During the last two decades a large number of ICA studies have shown a higher prevalence of non-obstructive CAD in patients with AMI than what was previously recognized. The reported prevalence of MINOCA ranges between 3.5% and 18% of all AMIs [37-41]. The different findings of these studies may partly be attributed to different definitions of normal or non-obstructed coronary arteries. In women, the prevalence has been reported to be as high as 33% [40,41]. The increasing awareness of MINOCA is likely largely due to the frequent use of ICA [42] and the introduction of new sensitive troponin assays [43], leading to an increasing number of patients with an unclear diagnosis: elevated troponins signaling myocardial injury and cell death, but normal ICA. Figure 1 shows coronary CTA and ICA findings of a patient with MINOCA.

**Figure 1.** The right coronary artery in a patient with MINOCA. Coronary CTA (A) shows a large atherosclerotic plaque and more distally a small plaque, both with <20% stenosis. ICA (B) shows only minimal signs of atherosclerosis. Abbreviations: MINOCA, myocardial infarction and non-obstructed coronary arteries; CTA, computed tomography angiography; ICA, invasive coronary angiography.
The terminology for this condition has been inconsistent. Researchers have called it myocardial infarction with normal coronary arteries (MINCA), MINOCA, myocardial infarction with no significant CAD, acute coronary syndrome with culprit-free angiogram, etc. For paper I we chose the term MINCA to stress that we only included patients with normal or nearly-normal coronary arteries at ICA (<30% stenosis) in contrast to other studies where patients with stenosis <50% were included. However, since then the term MINOCA has become the most widely used, which is why this term was chosen for papers III and IV and for this doctoral thesis.

Due to the limited investigation of this condition, MINOCA is still incompletely understood and there are currently no therapeutic guidelines relating to the management of these patients. MINOCA patients are more likely to be female and are younger compared to patients with AMI and obstructive CAD [5,40]. Apart from that, most traditional CVD risk factors are similar in the two patient groups. Approximately one-third of MINOCA patients were categorized with STEMI and two-thirds with NSTEMI. A pooled meta-analysis of studies investigating prognosis in patients with MINOCA [5] showed an in-hospital mortality of 0.9% and a 12-month all-cause mortality of 4.7%, both of which are lower than for patients with AMI and obstructive CAD. Compared to patients with stable chest pain and non-obstructed coronary arteries, however, patients with MINOCA had a significantly poorer prognosis [5].

Studies investigating the pathophysiological mechanisms of MINOCA have provided evidence that the condition is most likely heterogeneous. Several potential underlying causes have been proposed: CAD with rupture of a non-stenotic plaque, coronary embolism, microvascular obstruction, disturbed endothelial function and vasospasm [44-46]. In a clinical setting, patients with TS might be considered as a subgroup of MINOCA, since these patients present with a similar clinical picture. A pooled meta-analysis of cardiovascular magnetic resonance (CMR) imaging studies [5] showed subendocardial infarction in 24%, myocarditis in 33%, evidence of TS in 18% and no detectable myocardial abnormalities in 26% of MINOCA patients. Inherited thrombotic disorders have been demonstrated in 14% of patients with MINOCA [5]. Previous studies using intravascular ultrasound and coronary CTA have suggested that CAD is an important underlying cause of MINOCA [45,47-49]. None of these studies, however, included a control group.

1.4 TAKOTSUBO SYNDROME

TS is an acute cardiac syndrome commonly triggered by an emotionally or physically stressful event and with a clinical presentation indistinguishable from an AMI [6-9]. It is characterized by transient left ventricular wall-motion abnormalities that are not restricted to a single coronary distribution. Typically, the apical and mid-portions of the left ventricle are hypokinetic, with hypercontraction of the basal left ventricle, leading to an unusual shape of the left ventricle during systole, as shown in Figure 2. This shape resembles the ceramic pot used by Japanese fishermen to trap octopuses, hence the term tako-tsubo (octopus pot) like
left ventricular dysfunction, which was introduced by Sato et al. (1990) [50]. It is estimated that approximately 2% of patients presenting with a clinical picture of AMI have TS [7].

![Figure 2](image.jpg)

**Figure 2.** Illustration of a normal heart in systole (left) and the heart of a patient with TS (middle) in systole, with the characteristic left ventricular apical hypokinesia, giving the left ventricle the shape of an octopus pot (right). Abbreviations: TS, Takotsubo syndrome.

During the last decade, a large number of case reports and small case series have been published with an inconsistent terminology: TS, broken heart syndrome, apical ballooning syndrome, stress-induced cardiomyopathy, etc. The typical patient is a postmenopausal woman, presenting with chest pain or dyspnea after an event of acute emotional or physical stress, with positive cardiac biomarkers or an abnormal electrocardiogram. The predominant type of TS involves apical hypokinesia, but other types involving mid-ventricular, basal or focal hypokinesia have also been described [6,51]. Right ventricular involvement has been demonstrated for a significant proportion of patients with TS, in particular for those with more severe impairment of left ventricular systolic function [52].

There is no consensus on the diagnostic criteria for TS. However, the Mayo clinic diagnostic criteria (modified in 2008) [8] are widely used. These four criteria should be fulfilled:

1. Transient hypokinesis, akinesis, or dyskinesis in the left ventricular mid segments with or without apical involvement; the regional wall motion abnormalities extend
beyond a single epicardial vascular distribution; a stressful trigger is often, but not always present.

(2) Absence of obstructive CAD or angiographic evidence of acute plaque rupture.

(3) New electrocardiographic abnormalities (either ST-segment elevation and/or T-wave inversion) or modest elevation in cardiac troponin.

(4) Absence of pheochromocytoma and myocarditis.

These criteria have been questioned for several reasons:

(1) There are forms of TS that do not involve the mid segments of the left ventricle [51].
(2) TS may develop in patients with obstructive CAD [6,8,53]. In some cases, the stress of an AMI may even be the trigger of TS [54].
(4) TS may coexist (or be triggered by) pheochromocytoma or myocarditis [53,55].

In a majority of patients with TS the condition is preceded by an emotionally or physically stressful event, but in a significant minority there is no evident trigger [6,9]. Potential physical stressors include non-cardiac surgery, critical illness with or without sepsis and subarachnoid hemorrhage. TS is probably still underrecognized and often misdiagnosed, due to the wide spectrum of the condition, its varied presentation and the diagnostic difficulties.

Left ventricular systolic function usually improves within weeks and total recovery eventually occurs in most patients. However, there are complications in the acute phase of the disease, such as fulminant heart failure and cardiogenic shock, arrhythmias and cardiac arrest [6,56,57]. Conflicting results have been reported regarding short- and long-term prognosis. Early studies reporting a favorable prognosis, similar to that of the general population, may have underestimated the risk of early and late complications [6,8]. More recent studies have reported short- and long-term mortality rates similar to those of patients suffering from AMI and a recurrence rate of approximately 10% [6,7,58,59].

Causes of TS are still incompletely understood, even though a number of pathophysiologic mechanisms have been proposed. A potential role of catecholamine excess has been suggested, as well as an involvement of the “brain-heart connection” [6,7,60]. Other proposed mechanisms (not mutually exclusive) include coronary vasospasm, microvascular dysfunction, left ventricular outflow tract obstruction, effects of epinephrine on the β2-adrenoreceptor and catecholamine-mediated myocardial stunning [7,8,53,60,61]. A recent publication [13] suggested MB of the left anterior descending artery (LAD) might be one underlying cause of TS, after demonstrating an elevated prevalence of MB in patients with TS compared to controls.

1.4.1 Imaging in Takotsubo syndrome

ICA with left ventricular angiography is central in the diagnosis of TS, to visualize the coronary arteries (and exclude an acute coronary syndrome) as well as the function and shape of the left ventricle. Early CMR imaging may be valuable for assessing patients with TS,
since functional analysis (long and short axis cine) allows for evaluation of wall-motion abnormalities and assessment of cardiac function. In addition, T2-weighted sequences allow for the determination of presence and extent of myocardial edema. Late gadolinium enhancement sequences allow for detection of myocardial necrosis and/or fibrosis, as well as differentiation between myocardial infarction and myocarditis [62,63]. Typical CMR findings in TS are mid-apical myocardial edema with corresponding regional wall-motion abnormalities and apical ballooning, but with no or only subtle late gadolinium enhancement [63]. Echocardiography allows for detection of wall-motion abnormalities, assessment of systolic function and evaluation of left ventricular outflow tract obstruction, which is common in patients with TS [61].

1.5 MYOCARDIAL BRIDGING

Myocardial bridging (MB) refers to a condition where a segment of an epicardial coronary artery (the “tunneled segment”) is covered by cardiac muscle fibres (the “myocardial bridge”), thus coursing through the myocardium for a variable distance (Figure 3).

![Figure 3. MB visualized by coronary CTA. Curved multiplanar reformation of the LAD of a healthy volunteer with MB with full encasement (A). In (B) MB with partial encasement is shown, in a patient with TS. Abbreviations: MB, myocardial bridging; CTA, computed tomography angiography; LAD, left anterior descending artery; TS, Takotsubo syndrome.](image)

MB is mostly found in the LAD, even though it has been reported in other coronary vessels [64,65]. MB can be classified as MB with full encasement or MB with partial encasement, which is further discussed in the methods section of study III. The different types of MB are illustrated in detail in Figure 6. The reported prevalence of MB in the general population varies greatly and its significance has been widely debated. Autopsy studies have demonstrated a prevalence of MB ranging between 15% and 85% [66]. The detection rate at ICA is much lower: in general between 0.5% and 4.5% [65]. Detection of MB at ICA mainly
relies on the presence of systolic compression of the tunneled arterial segment, also referred to as “milking effect”, for indirect visualization of the MB. However, a short or superficial MB might not cause any detectable systolic compression, which probably accounts for the low prevalence reported at ICA compared to autopsy. A number of research groups have examined the feasibility of coronary CTA for detecting MB and reported a prevalence between 26% and 58% [13,65,67,68], which is similar to numbers reported from autopsy studies. A very good correlation has been reported between coronary CTA and coronary intravascular ultrasound for detecting MB of the LAD [69]. For the majority of people with MB, the condition can be considered to be a benign congenital variant, unlikely to ever lead to any complications [70,71]. However, serious adverse events such as myocardial ischemia, AMI, arrhythmia and sudden death have been reported [12,66].

There are two main mechanisms by which MB is believed to cause ischemia: the first is acceleration of the development of atherosclerosis proximal to the MB and the second is dynamic compression of the intramural segment, resulting in hemodynamic alterations that might affect both systolic and diastolic blood flow. Several conditions might facilitate or trigger ischemia related to MB, among them tachycardia and increased myocardial contractility during stress or exercise, increasing left ventricular diastolic dysfunction associated with ageing, development of left ventricular hypertrophy or endothelial dysfunction stimulating coronary vasospasm or thrombus formation [12,72,73].

1.6 CARDIOVASCULAR DISEASE RISK ASSESSMENT

Considering CVD worldwide, different levels of risk factors can be identified. At a fundamental level we find determinants such as globalization, urbanization, poverty and an ageing population [1]. These determinants have an impact on behavioural risk factors, among them physical inactivity, an unhealthy diet and use of tobacco and alcohol. The behavioural risk factors, in turn, manifest themselves in individuals through for instance obesity, hypertension, hyperlipidemia or diabetes mellitus, all of which are measurable risk markers for CVD or cerebrovascular disease events. Interventions aiming to decrease the prevalence of CVD may thus operate at a population level (strategies to reduce the harmful use of tobacco and alcohol, healthy school meals for children, etc.) or at an individual level [1]. In primary prevention at the individual level, modifying behavioural risk factors (smoking cessation, increased physical activity, etc.) and for some individuals drug treatment of for instance hypertension or hyperlipidemia has been shown to reduce the risk of CVD.

To guide primary prevention interventions it is essential to identify individuals at increased risk of future CVD events. CVD risk assessment is traditionally based on risk factors such as smoking, hypertension, hyperlipidemia and diabetes mellitus or risk assessment algorithms, such as the Framingham, the SCORE or the Reynolds risk scores [74-78]. Although these risk factors and risk scores have proven useful on a population level, they do not identify individuals who will eventually develop CVD [79,80]. As many as half of individuals who suffer a CVD event have only one or none of the traditional risk factors [79]. A number of additional risk markers that might improve the predictive power of risk scores have been
proposed, among them the CAC score, carotid artery ultrasound measurement of intima-media thickness (IMT) or plaque detection, measures of peripheral endothelial function and different categories of circulating biomarkers [25,26,79,81,82].

1.6.1 Carotid artery ultrasound

The carotid arteries are easily accessible for non-invasive ultrasound imaging that allows for detection of plaque and measurement of IMT. IMT is measured between the luminal-intimal and the medial-adventitial interfaces of the carotid artery wall. A good accuracy has been demonstrated for IMT measurements of the far wall of the common carotid artery, when compared to histologic specimens [83]. The IMT measure reflects intimal thickening, but also medial smooth muscle hyperplasia and/or hyperthrophy. Carotid artery ultrasound findings have implications for the risk of cerebrovascular disease. Carotid artery IMT has also proven to be associated with prevalent CVD and to predict CVD events [81,84], motivating its use as a surrogate marker for CAD. According to the American College of Cardiology (ACC)/American Heart Association (AHA) guidelines from 2010 [25], measurement of IMT can be useful for risk assessment for intermediate-risk individuals, but according to updated guidelines from 2013 [85], routine measurement of IMT is no longer recommended for prediction of CVD events. Recently, it has been shown that carotid plaque predicts CAD events more accurately than IMT [82,83].

1.6.2 Endothelial function

A functioning endothelium is a fundamental element of vascular health. The endothelium is a highly selective barrier between the circulating blood and the vascular wall and it is also metabolically active [86]. Nitric oxide is central in the normal functioning of the endothelium, primarily because it promotes vasodilation, but also due to other effects, such as inhibition of platelet aggregation and leucocyte adherence [87]. In endothelial dysfunction, there is impaired production of vasoprotective endothelial mediators and excessive production of pro-oxidant, pro-inflammatory and pro-thrombotic mediators, thus promoting inflammation and atherothrombosis [88]. Endothelial dysfunction is considered to be an early marker and a precursor of atherothrombotic disease. It is closely related to CVD risk factors and predicts CVD events in patients with or without established CVD [87,89,90]. Several classes of CVD drugs have been shown to lead to improved endothelial function and serial measurements of endothelial function have been proposed in order to monitor therapeutic effects [87,88]. There are several methods for evaluating endothelial function. Quantitative ICA with intracoronary acetylcholine is a direct method for assessing endothelium-dependent vasodilation of the coronary arteries [88]. Since endothelial dysfunction is a systemic disorder, measurement of peripheral endothelial function can be used as a surrogate for coronary endothelial function [91-93]. Flow-mediated dilation of the brachial artery is the most established method for non-invasive assessment of endothelial function. Digital reactive hyperemia peripheral arterial tonometry (RH-PAT) is a recently introduced method for assessing peripheral endothelial function that has the advantage of being easily accessible,
largely operator-independent and highly reproducible [94]. An association between RH-PAT and several CVD risk factors has been demonstrated [95].

### 1.6.3 Circulating biomarkers

A large number of circulating biomarkers, representing different pathophysiological pathways, have been proposed to predict CAD. Markers of inflammation include C-reactive protein and several interleukins. Lipoprotein(a) and adiponectin are markers of lipid metabolism. Cystatin C is an accurate marker of kidney function. There are numerous other circulating biomarkers, reflecting for instance oxidative stress, thrombosis and endothelial dysfunction [79,96-98].

Even though circulating biomarkers have received considerable attention during the last decade, their potential role in CVD risk assessment is not yet established [79,96]. An advantage of circulating biomarkers is that some may provide information early and some late in the CVD process, whereas imaging biomarkers may detect subclinical disease, but not earlier stages of CVD. Genetic markers, on the other hand, can be evaluated long before development of CVD and provide information on an individual’s susceptibility, but fail to tell us whether subclinical disease has developed or not. To date, studies investigating the performance of risk prediction based on combinations of multiple circulating biomarkers, have shown, at best, modest improvement in risk prediction [79].

### 1.6.4 Coronary computed tomography angiography plaque burden versus other risk markers

A prognostic value regarding CVD events has been demonstrated for coronary CTA, as previously discussed. IMT and RH-PAT are supposed to reflect the presence and extent of CAD, but only a few studies have compared these measures with coronary CTA plaque burden [99]. Even fewer have focused on clinically healthy subjects, with predominantly low-to-intermediate risk profiles [100]. An association between circulating biomarkers and CAD determined by coronary CTA has only been studied by a small number of research groups, with varying results [101-104]. Imai et al. [103] reported that cystatin C was associated with the extent of non-obstructive CAD at coronary CTA. Studies by Bamberg et al. [101] and Matsuda et al. [104] showed an association between levels of adiponectin and coronary CTA plaque burden, whereas Caselli et al. [102] did not find an association between circulating adiponectin and coronary CTA findings. Lipoprotein(a) was not associated with CAD determined by coronary CTA [101,102].
2 AIMS OF THE THESIS

There were two major aims of this thesis. The first aim was to investigate the underlying mechanisms of MINOCA by studying coronary CTA plaque burden (study I) and myocardial bridging (study III). The second aim was to examine the association between coronary CTA plaque burden and other risk markers of CVD: carotid artery IMT and peripheral endothelial function (study II) and circulating biomarkers (study IV).

The specific objectives of each study were:

I. To investigate whether patients with MINOCA had a greater coronary plaque burden determined by coronary CTA than a control group matched by age and gender.

II. To examine the ability of carotid artery IMT and endothelial function determined by RH-PAT to predict coronary CTA plaque burden in clinically healthy subjects.

III. To investigate the prevalence of MB in patients with MINOCA and TS compared to healthy volunteers and to compare the MB depiction rates of coronary CTA and ICA.

IV. To investigate whether circulating markers of lipid metabolism, inflammation and kidney function could predict non-obstructive CAD determined by coronary CTA.

By pursuing these aims we hope to increase the understanding of the underlying pathophysiological mechanisms of MINOCA and to some degree TS, thereby contributing to the development of diagnostic strategies, that may guide therapeutic management and hopefully ultimately improve prognosis for these patients. We also hope to provide new information in the important field of CVD risk assessment in low-to-intermediate risk groups.
3 MATERIALS AND METHODS

The studies of this thesis are a subset of the multicentre Stockholm Myocardial Infarction with Normal Coronaries (SMINC) study [10,11]. The studies conform to the principles of the Declaration of Helsinki and were approved by the Regional Ethical Review Board in Stockholm and by the Radiation Protection Committee of the Karolinska University Hospital. Written informed consent was obtained from all patients and healthy volunteers. Study participants were assigned study identification numbers and records were kept confidential at the Cardiology Unit at Karolinska University Hospital in Solna, Sweden.

3.1 STUDY GROUP (I-IV)

The study participants (MINOCA patients and controls) of the four studies of this thesis form a subgroup of the study participants of the SMINC study [10,11].

Study I, III and IV: In the first step, patients with MINOCA were screened for the SMINC study at five different coronary care units in the Stockholm metropolitan area between June 2007 and May 2011, as previously described [10]. Patients were eligible to take part in the study if they were between 35 and 70 years old, fulfilled the criteria for acute myocardial infarction (AMI) according to the universal definition of AMI [33] and underwent ICA showing no or minimal signs of atherosclerosis (defined as the presence of plaque discernible on ICA, but no stenosis exceeding 30% by visual estimation). All patients also underwent cardiovascular magnetic resonance (CMR) imaging at a median of 12 days after hospital admission. Exclusion criteria were myocarditis (based on CMR findings or a clinical diagnosis), a clinical diagnosis of pulmonary embolism, non-sinus rhythm on admission, pacemaker use, and a patient history of structural or coronary heart disease, chronic obstructive lung disease or renal disease [10]. After patient inclusion, the coronary angiograms as well as the clinical AMI diagnoses were re-evaluated by an additional independent investigator. Patients with TS according to the Mayo clinic diagnostic criteria [8] were considered part of the MINOCA group and thus not excluded.

A control group, matched by age and gender, was recruited using a registry comprising all Stockholm residents. Randomly selected persons of matching age and gender to MINOCA patients received a letter of invitation followed by a telephone call. Subjects who were willing to participate and who had no known CVD underwent an exercise stress test. If the test was normal they were invited to take part in the study.

In the second step, MINOCA patients and controls of the SMINC study were recruited to the coronary CTA substudy. Additional exclusion criteria for the coronary CTA study were age under 45, an estimated glomerular filtration rate (GFR) < 50ml/min/1.73m² (based on serum creatinine), previous adverse reaction to an iodine-based contrast agent and an irregular heart rate (jeopardizing the diagnostic quality of the CT scan). Patient selection and study flow are shown in Figure 4.
Study II: The study group of study II was composed of the 58 clinically healthy controls of the group described above.

3.2 RISK FACTOR ASSESSMENT (I-IV)

Current smoking was defined as active regular use and previous smoking as a history of regular use. Hypertension and hyperlipidemia were defined as previously diagnosed and
medically treated. A positive family history of CAD was defined as CVD event in a parent or sibling before 65 years of age. Risk assessment was performed according to the Framingham risk algorithm [74].

**Study I-III:** Diabetes mellitus was defined as previously diagnosed.

**Study IV:** An oral glucose tolerance test was performed, with measurement of the plasma glucose concentration 2 hours after ingestion of 75 g glucose dissolved in 150 ml of water. Impaired glucose tolerance was defined as a plasma glucose concentration $\geq$7.8 mmol/L and diabetes mellitus as a plasma glucose concentration $\geq$11.1 mmol/L.

### 3.3 INVASIVE CORONARY ANGIOGRAPHY (I, III-IV)

**Study I, III and IV:** For patients with MINOCA, ICA was performed at the time of initial hospital admission, according to clinical routines and using standard techniques, including a transfemoral or transradial approach and a minimum of two different projections of each coronary artery. ICAs were evaluated using the modified American Heart Association 17 segment classification [106]. Clinical reassessments of all ICAs were performed at a later time by an additional independent investigator.

**Study III:** Additional joint readings of ICAs were performed by two experienced interventional cardiologists focusing specifically on the presence of MB. They assessed indirect signs of MB: systolic compression of the tunneled segment (by visual estimation) or a localized change in direction of the vessel course towards the ventricle (the “step down-step up” phenomenon).

### 3.4 CORONARY COMPUTED TOMOGRAPHY ANGIOGRAPHY (I-IV)

All study participants underwent coronary CTA at Karolinska University Hospital in Huddinge, Sweden. For MINOCA patients, the CTA examinations were performed between 3 and 6 months after the acute event.

#### 3.4.1 Acquisition

Examinations were performed on a 64-slice computed tomography scanner (LightSpeed VCT XT; GE Healthcare, Milwaukee, WI, USA). A prospectively ECG-triggered scan protocol was used: detector configuration 64 x 0.625 mm, rotation time 350 ms (temporal resolution 175 ms), tube potential 120 kVp, tube current 450-650 mA (according to patient size). The scans were performed in diastole, in general at 70-75% of the RR interval, with 0-200 ms padding (depending on heart rate and variability). Two patients, however, were examined using a retrospectively ECG-gated scan protocol (dose modulation, 100 kVp, 200-450 mA), as a slightly irregular heart rate might otherwise have compromised image quality. The intravenous contrast medium (CM) used was the iso-osmolar ioxixanol 320 mg I/mL (Visipaque, GE Healthcare, Stockholm, Sweden), which was individually dosed based on bodyweight (400 mg I/kg, 75-100 mL ioxixanol). The CM was administered using a dual-head injector (Medrad, Stellant Dual Head Injector, Pittsburgh, PA, USA) and a triple-phase
protocol (1, CM bolus injection; 2, mixture of saline and CM; 3, saline bolus injection). Bolus tracking software was used (SmartPrep, GE Healthcare, Milwaukee, WI, USA) in order to synchronize image acquisition with optimal CM opacification of the coronary arteries. In the absence of contraindications and depending on the initial heart rate, patients received metoprolol (25-100 mg) per os prior to the examination. Patients also received sublingual nitroglycerine (0.4 mg) 4 minutes before the scan.

**Figure 5.** Different plaque types, as seen by coronary CTA. A non-calcified plaque is shown in longitudinal and cross section (A and B). The degree of stenosis was 20-50%. A large mixed plaque is shown in longitudinal section (C) and in cross section at the level of non-calcified (D) and calcified (E) components. A large calcified plaque is shown to the right. (F and G). The mixed and calcified plaques (C to G) were both eccentric in location and the degree of stenosis was <20%. Abbreviations: CTA, computed tomography angiography.

### 3.4.2 Analysis

**Study I-IV:** The coronary CTA examinations were analyzed independently by two experienced readers (level 2 according to ACCF/AHA levels of competence [107]) who were blinded to all clinical information. Subsequent joint readings were performed in order to reach a consensus. Coronary CTA analysis was performed using the CardIQ Xpress software on the Advantage Workstation 4.4 (GE Healthcare, Milwaukee, WI, USA). Axial source images, multiplanar and curved multiplanar reformations as well as thin-slab maximum intensity projections were used. The optimal image display setting was chosen on an individual basis (in general at a window width of 800-1000 Hounsfield units (HU) and a level of 100-200 HU). Coronary arteries were subdivided into 17 segments, according to the modified American Heart Association classification [106]. Initially, each segment was assessed regarding image quality and evaluability. Segments were considered non-evaluable if artifacts prevented reliable assessment of the lumen or the vessel wall (e.g. due to motion, image noise or heavy calcification). Secondly, each segment was visually evaluated with regard to the presence of atherosclerotic plaque (with or without stenosis) (a), plaque size (b)
and composition (c). A plaque was defined as any structure, discernible in at least two planes, within or adjacent to the vessel lumen, which could be clearly separated from the lumen and from adjacent soft tissue.

a. Lesions were quantified for stenosis by visual estimation, comparing the minimal lumen of the stenotic segment with the lumen of the adjacent proximal unaffected segment, and expressed in terms of diameter stenosis: <20%, 20-50% or ≥50%. Although arbitrary, the 50% stenosis limit is the most widely used to differentiate between non-obstructive and obstructive lesions.

b. The size of the atherosclerotic plaque was determined by measuring the length of the plaque on longitudinal sections and arbitrarily classified as: small (<4mm), medium (4-8 mm) or large (≥ 8mm).

c. Plaque composition was visually assessed based on the presence or absence of calcified elements: non-calcified coronary artery plaque, mixed coronary artery plaque or calcified coronary artery plaque, the latter with ≥ 50% calcified tissue [21].

In the case of more than one atherosclerotic plaque in a single segment, the greatest degree of stenosis, the largest plaque size and the most pronounced calcification was considered. Figure 5 shows examples of different plaque types, as seen by coronary CTA.

Study III: In addition to the coronary CTA evaluations described above, an additional joint reading was performed, with focus on MB. The presence of MB was noted and described as MB with partial encasement or MB with full encasement [108]. Hence, MB was defined as a coronary artery segment in direct contact with the myocardium (partial encasement) or completely surrounded by myocardium (full encasement), instead of being surrounded by epicardial fat as it normally would. Figure 6 illustrates the types of MB. In the case of one MB involving two adjacent coronary artery segments, the MB was attributed to the more proximal segment. The length of the MB was measured on multiplanar reformations and for full encasement, the thickness of the overlying bridge was measured in a short axis view perpendicular to the tunneled segment.

3.4.3 Coronary artery calcium score

For the CAC score, a non-enhanced scan was performed, using a prospectively ECG-triggered scan protocol: detector configuration 64 x 0.625 mm, rotation time 350 ms, tube potential 120 kVp, tube current 200 mA. The CAC score was calculated using semi-automatic software (SmartScore 4.0, GE Healthcare, Milwaukee, WI, USA) on the Advantage Workstation 4.4 (GE Healthcare, Milwaukee, WI, USA). The total calcium burden of the coronary arteries was reported in terms of AJ-130 score, based on the scoring algorithm of Agatston et al. (1990) [23].
Figure 6. Classification of MB, as seen on coronary CTA. Bottom images show short axis views of the LAD, and its relation to surrounding structures. Top images show the corresponding short axis views with annotations. Usually, the LAD is located above the interventricular gorge, completely surrounded by epicardial fat (A). In MB with partial encasement a vessel segment is in direct contact with left ventricular myocardium (B). In MB with full encasement the vessel segment is completely surrounded by myocardium, either with an unmeasurable overlying muscle bridge (C) or with measurable overlying muscle (D). The LAD is marked with a pink circle and the myocardium-epicardium interface is marked with a blue line. RV denotes the right ventricle; LV, the left ventricle; S, the interventricular septum; M, left ventricular myocardium; E, epicardial fat and L, lung tissue. Abbreviations: MB, myocardial bridging; CTA, computed tomography angiography; LAD, left anterior descending artery.

3.5 ENDOTHELIAL FUNCTION ASSESSMENT (II)

Study II: Peripheral endothelial function was assessed using the digital RH-PAT device EndoPAT (Itamar Medical Ltd., Caesarea, Israel), according to the method described by Hamburg et al. [109]. The method involves measuring pulse wave amplitude of the fingertip (namely the PAT signal), using a fingertip probe, at rest and after a 5-minute occlusion of the brachial artery, using a standard blood pressure cuff inflated to supra-systolic pressure. When the cuff is deflated, the surge of blood causes an endothelium-dependent flow mediated dilatation leading to reactive hyperemia and an increase in the PAT signal amplitude. A fingertip probe is also placed on the index finger of the contralateral side for internal control. As a measure of reactive hyperemia the reactive hyperemia index (RHI) was calculated, using dedicated software, as the ratio between the post-occlusion and the pre-occlusion PAT signal amplitude divided by the corresponding ratio of the contralateral side, to adjust for any spontaneous or systemic alterations of vascular tone. The test was performed in a thermo-neutral and quiet surrounding, after fasting and avoiding pre-test smoking or consumption of caffeine.

3.6 CAROTID ARTERY ULTRASOUND (II)

Study II: Two-dimensional images of the left and right common carotid artery (CCA) were acquired using an ultrasound scanner equipped with a 12 MHz transducer (Vivid 7, General Electric Company, Horten, Norway). From each CCA a long-axis cine loop of three beats and
three diastolic images at the time of the electrocardiographic R-wave were digitally stored for off-line analysis. The IMT of the CCA far wall was measured in three diastolic images using semi-automatic IMT analysis software (General Electric Company). A 10 mm region of interest (ROI) was manually placed starting 1 cm proximal to the carotid bulb. The luminal-intimal and the medial-adventitial interfaces were identified automatically. In case of suboptimal tracking, the ROI was adjusted or another diastolic frame was chosen. Manual correction was not performed. The left and right IMT results were calculated as the mean of three semi-automatic measurements, and finally the average of these two results was obtained. All examinations were performed and interpreted by the same experienced ultrasonographer, who did not have access to the coronary CTA findings.

3.7 LABORATORY ANALYSES (IV)

Study IV: For MINOCA patients, data from routine clinical chemistry analyses performed at admission were acquired from medical records: fasting plasma triglycerides, fasting plasma LDL cholesterol, fasting plasma HDL cholesterol [11]. Additional blood samples were acquired at a 3-month follow-up visit. For the control group all blood sampling was performed at a single study visit. Plasma from all study participants was stored at −80°C for subsequent analyses. Plasma high-sensitivity C-reactive protein, lipoprotein(a) and cystatin C were analyzed using immuno-turbidimetric assays and adiponectin using enzyme-linked immunosorbent assay. Analyses were performed using accredited methods at an accredited laboratory (the Karolinska University Laboratory). Estimated GFR was calculated based on serum creatinine.

3.8 STATISTICAL ANALYSES (I-IV)

Data were characterized using descriptive statistics and assessed for normal distribution either graphically (study II) or using the Shapiro-Wilk test (studies I, III, IV). Categorical variables are presented as absolute value and percentage and were compared using the chi-square test or Fisher’s exact two-sided test. Continuous variables following a normal distribution are presented as mean ± standard deviation (SD) and non-normally distributed continuous variables are presented as median (range or interquartile range), as specified. Student’s t-test (for normally distributed variables) and the Mann-Whitney U-test (in the case of non-normal distribution) were used in order to test differences between two independent groups. The Pearson test (for normally distributed variables) or the Spearman test (in the case of non-normal distribution) were used to test correlation between continuous variables. The 5% level of significance was considered (two-tailed p<0.05).

Study I and II: All analyses were carried out using the SAS system (the SAS system for Windows 9.3, SAS Institute Inc., Cary, NC, USA).

Study III: Analyses were carried out using IBM SPSS Statistics version 22.0.

Study IV: In addition to analyses described above, univariable logistic regression analyses were performed and variables with p≤0.1 (sex, body mass index, systolic blood pressure,
HDL cholesterol, adiponectin, lipoprotein(a) and cystatin C) were incorporated into a multivariable logistic regression analysis with a forward stepwise method for variable selection (criterion for entering variable: \( p < 0.05 \)). Additional variables incorporated into the multivariable regression model were: smoking, subgroup (MINOCA, control) and estimated GFR. Levels of plasma biomarkers were dichotomized according to the median. Results of logistic regression analyses are presented as odds ratio (OR) with a 95% confidence interval (CI). All analyses were carried out using IBM SPSS Statistics version 22.0.
4 MAIN RESULTS

The principal findings were:

Study I: MINOCA patients did not have more CAD than healthy controls, matched by age and gender. A large proportion of MINOCA patients had no signs of CAD at coronary CTA.

Study II: Neither assessment of endothelial function by the RH-PAT method nor ultrasound measurement of carotid artery IMT predicts coronary CTA plaque burden in clinically healthy subjects with low-to-intermediate CVD risk.

Study III: MB was frequent, with a similar prevalence in MINOCA patients, patients with TS and controls. Coronary CTA detects MB at a significantly higher rate than ICA.

Study IV: Plasma levels of cystatin C were associated with non-obstructive CAD at coronary CTA, independently of GFR, in subjects with low-to-intermediate CVD risk.
5 RESULTS AND METHODOLOGICAL DISCUSSION

Clinical characteristics of the study group (MINOCA and controls) are shown in Table 1.

Table 1. Clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>MINOCA patients</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>60 ± 5</td>
<td>61 ± 6</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>42 (74%)</td>
<td>39 (67%)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Smoking habits</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>29 (52%)</td>
<td>33 (57%)</td>
<td></td>
</tr>
<tr>
<td>Previous smoker</td>
<td>17 (30%)</td>
<td>23 (40%)</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>10 (18%)</td>
<td>2 (3%)</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Family history of CAD</strong></td>
<td>16 (28%)</td>
<td>14 (24%)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>25.8 ± 3</td>
<td>25.8 ± 3</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Diabetes mellitus</strong></td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Treated hypertension</strong></td>
<td>19 (33%)</td>
<td>6 (10%)</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Treated hyperlipidemia</strong></td>
<td>8 (14%)</td>
<td>3 (5%)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>SBP (mm Hg)</strong></td>
<td>144 (129-161)</td>
<td>129 (115-140)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>GFR (ml/min/1.73m²)</strong></td>
<td>77.9 ± 12</td>
<td>78.1 ± 13</td>
<td>ns</td>
</tr>
<tr>
<td><strong>FRS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low</td>
<td>22 (39%)</td>
<td>31 (53%)</td>
<td></td>
</tr>
<tr>
<td>intermediate</td>
<td>22 (39%)</td>
<td>18 (31%)</td>
<td></td>
</tr>
<tr>
<td>high</td>
<td>13 (23%)</td>
<td>9 (16%)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: MINOCA, myocardial infarction and non-obstructed coronary arteries; CAD, coronary artery disease; BMI, body mass index; SBP, systolic blood pressure; GFR, estimated glomerular filtration rate based on serum creatinine; FRS, Framingham Risk Score (low risk ≤10%, intermediate risk 10-20%, high risk >20% 10-year risk). Data are presented as mean ± standard deviation, absolute value (percentage) or median (interquartile range). P-values <0.05 are given, obtained through Student’s t-test, the Mann-Whitney U-test or the chi-square test, as appropriate. A Diabetes mellitus was defined as previously diagnosed.

Baseline characteristics were similar in MINOCA patients and controls, apart from current smoking and treated hypertension, which were more common in the MINOCA group. At presentation, 56 MINOCA patients had no signs of heart failure (Killip class 1) and only one had heart failure (Killip class 2). Signs of acute ischemia (ST-T changes or left bundle branch block) on admission ECG were present in 31 (54%) out of whom 10 had ST elevations. The median (interquartile range) peak troponin level was 18 (7-43) times the upper limit of normal. Myocardial infarction was detected by CMR in 11 (19%) patients. The criteria for TS were fulfilled in 15 (26%).
5.1 STUDY I

5.1.1 Results

Coronary CTA examinations of 57 MINOCA patients and 58 controls were analyzed. Fifteen (1.9%) individual segments in the MINOCA group and 11 (1.4%) in the control group were non-evaluable and excluded from analyses. There were 765 evaluable segments in the MINOCA group and 781 in the control group.

Coronary CTA plaque burden analyses are presented in Tables 2 and 3, on per patient and per segment basis, comparing the MINOCA group with the control group.

Table 2. Coronary CTA plaque burden per patient

<table>
<thead>
<tr>
<th>Severity of CAD(d)</th>
<th>MINOCA patients</th>
<th>Controls</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CAD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stenosis &lt;20%</td>
<td>24 (42%)</td>
<td>25 (43%)</td>
<td>ns</td>
</tr>
<tr>
<td>Stenosis 20-50%</td>
<td>11 (19%)</td>
<td>9 (16%)</td>
<td></td>
</tr>
<tr>
<td>Stenosis ≥50%</td>
<td>0 (0%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>All CAD(\d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 segments</td>
<td>24 (42%)</td>
<td>25 (43%)</td>
<td>ns</td>
</tr>
<tr>
<td>1 segments</td>
<td>14 (25%)</td>
<td>10 (17%)</td>
<td></td>
</tr>
<tr>
<td>2 segments</td>
<td>8 (14%)</td>
<td>12 (21%)</td>
<td></td>
</tr>
<tr>
<td>3 segments</td>
<td>4 (7%)</td>
<td>6 (10%)</td>
<td></td>
</tr>
<tr>
<td>4 segments</td>
<td>2 (4%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>5 segments</td>
<td>3 (5%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>6 segments</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>7 segments</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>8 segments</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>9 segments</td>
<td>0 (0%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>10 segments</td>
<td>0 (0%)</td>
<td>2 (3%)</td>
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</tr>
<tr>
<td>Calcium score (AJ-130)</td>
<td>6 (0-778)</td>
<td>8 (0-1882)</td>
<td>ns</td>
</tr>
</tbody>
</table>

Abbreviations: CTA, computed tomography angiography; MINOCA, myocardial infarction and non-obstructed coronary arteries; CAD, coronary artery disease; ns, non-significant. Values are presented as absolute value (percentage) or median (range). *refers to the maximum diameter stenosis; †refers to obstructive and non-obstructive CAD.

On a per patient level there were no statistically significant differences in severity or extent of CAD. Twenty-four (42%) MINOCA patients and 25 (43%) controls had no signs of CAD.

When analyzing the data on a per segment level, however, there were statistically significant differences regarding degree of stenosis, plaque size and plaque composition. Compared to controls, MINOCA patients had fewer segments with stenosis ≥20%. They also had fewer large and mixed type plaques. The CAC scores within each group were diverse, but no significant differences were found between the groups.
There were no differences regarding coronary CTA plaque burden when subgroups of MINOCA patients were compared with controls (MINOCA patients with AMI detected by CMR, with ST elevations or with TS).

Table 3. Coronary CTA plaque burden per segment

<table>
<thead>
<tr>
<th>Severity of CAD</th>
<th>MINOCA patients 765 segments</th>
<th>Controls 781 segments</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CAD</td>
<td>684 (89%)</td>
<td>687 (88%)</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Stenosis &lt;20%</td>
<td>68 (9%)</td>
<td>58 (7%)</td>
<td></td>
</tr>
<tr>
<td>Stenosis 20-50%</td>
<td>13 (2%)</td>
<td>35 (4%)</td>
<td></td>
</tr>
<tr>
<td>Stenosis ≥50%</td>
<td>0 (0%)</td>
<td>1 (0.1%)</td>
<td></td>
</tr>
<tr>
<td>Plaque size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No CAD</td>
<td>684 (89%)</td>
<td>687 (88%)</td>
<td>0.04*</td>
</tr>
<tr>
<td>Small</td>
<td>41 (5%)</td>
<td>31 (4%)</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>24 (3%)</td>
<td>29 (4%)</td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>16 (2%)</td>
<td>34 (4%)</td>
<td></td>
</tr>
<tr>
<td>Plaque composition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No CAD</td>
<td>684 (89%)</td>
<td>687 (88%)</td>
<td>0.04*</td>
</tr>
<tr>
<td>Non-calcified plaque</td>
<td>10 (1%)</td>
<td>10 (1%)</td>
<td></td>
</tr>
<tr>
<td>Mixed plaque</td>
<td>10 (1%)</td>
<td>28 (4%)</td>
<td></td>
</tr>
<tr>
<td>Calcified plaque</td>
<td>61 (8%)</td>
<td>56 (7%)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CTA, computed tomography angiography; MINOCA, myocardial infarction and non-obstructed coronary arteries; CAD, coronary artery disease; Values are presented as absolute value (percentage). *P-values apply to the comparison of the four categories in the two columns to the left of the value, using the chi-square test.

5.1.2 Methodological discussion

For MINOCA patients, stenosis >30% at ICA was an exclusion criterion. For ethical reasons, healthy volunteers did not undergo ICA and individuals with stenoses were thus not excluded, which might explain the fact that there were more segments with stenosis ≥20% in the control group than in the MINOCA patient group. Still, on per patient basis there were roughly equal numbers of MINOCA patients (19%) and controls (18%) with stenosis ≥20%.

5.2 STUDY II

5.2.1 Results

All 58 volunteers, free from clinical CVD, underwent coronary CTA with subsequent plaque burden analysis, as well as testing for peripheral endothelial function by the RH-PAT method. Ultrasound of the carotid arteries and measurement of IMT were successfully performed in 57 study participants. A non-enhanced CT scan for CAC scoring was obtained in 51 subjects.

According to the Framingham risk algorithm [74] 31 study participants (53%) had a 10-year risk of 10% or less, whilst 18 (31%) had a 10-20% risk and 9 (16%) had an elevated risk exceeding 20%. Only three subjects were on lipid-lowering medication.
Coronary CTA demonstrated normal coronary arteries in 25 (43%) study participants. Thirty-two (55%) subjects had non-obstructive CAD and only one (2%) had obstructive CAD. The median (range) of the RHI was 2.2 (1.4-4.9) and of the IMT 0.70 mm (0.49 mm-0.99 mm). The CAC score ranged from 0 to 1882, with a median of 4.

When comparing the groups with and without evidence of CAD at coronary CTA, no statistically significant differences were found concerning RHI or IMT (Table 4). Nor was there any correlation between the number of diseased segments on coronary CTA and RHI ($r_s=0.13$) or IMT ($r_s=0.098$) (Figure 7). Not surprisingly, there was a statistically significant difference in CAC score when comparing the groups with and without CAD demonstrated by coronary CTA ($p<0.001$) (Table 4). Similarly, there was a strong correlation between the CAC score and the number of diseased coronary segments ($r_s=0.86$, $p<0.001$) (Figure 7).

### Table 4. Comparison between groups with and without CAD at coronary CTA (clinically healthy subjects)

<table>
<thead>
<tr>
<th></th>
<th>No CAD</th>
<th>CAD</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=25</td>
<td>n=33</td>
<td></td>
</tr>
<tr>
<td>RHI</td>
<td>2.2 (1.4-4.9)</td>
<td>2.1 (1.4-3.6)</td>
<td>ns</td>
</tr>
<tr>
<td>IMT (mm)</td>
<td>0.70 (0.53-0.99)</td>
<td>0.71 (0.49-0.99)</td>
<td>ns</td>
</tr>
<tr>
<td>CAC</td>
<td>0 (0-4)</td>
<td>36 (0-1882)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: CAD, coronary artery disease; CTA, computed tomography angiography; RHI, reactive hyperemia index; IMT, carotid artery intima-media thickness; CAC, coronary artery calcium score; ns, non significant. Values are presented as median (range).

### 5.2.2 Unpublished results

Study I showed that MINOCA patients and controls were similar with respect to coronary plaque burden and a previous SMINC study found no difference in RHI or IMT between the two groups [11]. We therefore found it reasonable to conduct additional analyses with the two groups put together into one (n=115). The median (range) of the RHI was 2.2 (1.0-4.9) and of the IMT 0.70 mm (0.49 mm-1.0 mm). The CAC ranged from 0 to 1882, with a median of 1.5. All results were similar to those obtained when studying only the healthy volunteers (Table 5). Hence, there was no association between the presence or extent of CAD and RHI or IMT, but a strong correlation between the number of diseased segments and the CAC score ($r_s=0.80$, $p<0.001$).

### 5.2.3 Methodological discussion

Ultrasound examinations of the carotid arteries focused only on IMT measurement of the CCA, while additional analyses such as plaque detection might have yielded different results. Several studies published after the initiation of this study have shown that plaque assessment of the carotid arteries, including the carotid bulb and the internal carotid artery, predicted CAD and outcome better than IMT alone [82,83]. IMT does not only reflect subclinical
atherosclerosis, but may be influenced by for instance hypertension. In addition, atherosclerotic plaques tend to form in the carotid bulb and in the internal carotid artery, which may also explain why IMT of the CCA has less predictive value [83]. In our study, even though the focus of the ultrasound examination was the CCA, plaques in the carotid bulb were evident for three subjects, all with CAD at coronary CTA.

**Figure 7.** Correlation between number of diseased segments, as determined by coronary CTA, and RHI (A), IMT (B) and CAC (C). A significant correlation was found only for CAC ($r_s=0.86$, $p<0.001$) Abbreviations: CTA, computed tomography angiography; RHI, reactive hyperemia index; IMT, intima-media thickness; CAC, coronary artery calcium.
The choice of RH-PAT, in order to ensure a highly reproducible method, instead of for instance brachial artery flow mediated dilation might also have affected the results. It has been shown that different risk factors affect endothelial function of the microvasculature (measured with RH-PAT) and the conduit vessels (measured with brachial artery flow mediated dilation) differently [110]. Another factor that may have influenced our results is the fact that endothelial function was determined with one single test. Repeated measurement has been proposed in order to augment accuracy, since endothelial function is a dynamic process, which can be transiently affected by a variety of factors [111].

### 5.3 STUDY III

#### 5.3.1 Results

All study participants underwent coronary CTA and all MINOCA patients also underwent ICA, as previously described.

In total, 54 (47%) study participants had evidence of MB. MB with full encasement was found in 33 (29%) and MB with partial encasement in 21 (18%). A majority of MBs were located in segment 7 (mid LAD). Only three study participants had MB (detected by coronary CTA) in vessels other than the LAD. Due to the vast majority of MBs being located in the LAD, only these were included in further analyses.

Table 6 shows the prevalence, type and location of MB in the different study groups. There were no statistically significant differences between MINOCA patients or the TS subgroup and the control group regarding the prevalence or type of MB. Nor were there any differences between the groups regarding the location, the length or the thickness of MB. Nine MINOCA patients had no signs of CAD or MB. Out of these 9 patients, 3 were diagnosed with TS.

In the MINOCA patient group, the depiction rate of MB was compared for coronary CTA and ICA. Coronary CTA demonstrated MB in 28 (49%) patients, while ICA showed indirect signs of MB in 13 (23%). The difference in depiction rate between the two methods was statistically significant (p<0.01). In four patients MB was suspected at ICA, but not confirmed by coronary CTA. ICA thus “correctly” detected MB in 9 (16%) patients. When comparing the cases of MB detected by both modalities with those detected by coronary CTA
but missed by ICA, there were no statistically significant differences regarding localization, type, length or thickness of MB (Table 7).

Table 6. Myocardial bridging in MINOCA and TS patients and in matched controls.

<table>
<thead>
<tr>
<th></th>
<th>MINOCA patients n=57</th>
<th>TS subgroup n=15</th>
<th>Control group n=58</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB (coronary CTA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of MB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial encasement</td>
<td>11 (19%)</td>
<td>2 (13%)</td>
<td>10 (17%)</td>
<td>ns</td>
</tr>
<tr>
<td>Full encasement</td>
<td>17 (30%)</td>
<td>6 (40%)</td>
<td>16 (28%)</td>
<td></td>
</tr>
<tr>
<td>Location of MB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal LAD</td>
<td>1 (2%)</td>
<td>1 (7%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Mid LAD</td>
<td>23 (40%)</td>
<td>7 (47%)</td>
<td>20 (34%)</td>
<td></td>
</tr>
<tr>
<td>Distal LAD</td>
<td>4 (7%)</td>
<td>0 (0%)</td>
<td>6 (10%)</td>
<td></td>
</tr>
<tr>
<td>Length of MB (mm)</td>
<td>14.5 (2.0-51.0)</td>
<td>7.3 (2.0-10.9)</td>
<td>13.5 (3.8-45.0)</td>
<td>ns</td>
</tr>
<tr>
<td>Thickness of MB (mm)*</td>
<td>0 (0-4.8)</td>
<td>0 (0-2.0)</td>
<td>0 (0-5.0)</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as absolute value (percentage) or median (range). Abbreviations: MINOCA, myocardial infarction and non-obstructed coronary arteries; TS, takotsubo syndrome; MB, myocardial bridging; CTA, computed tomography angiography; LAD, left anterior descending artery; ns, non-significant. The chi-square test was used for comparing the MINOCA group and the TS subgroup respectively with the control group. Neither test demonstrated any statistically significant differences. * in the case of full encasement.

5.3.2 Methodological discussion

The prevalence of MB according to ICA was higher than in most previous studies, but similar to that demonstrated by Leschka et al [65]. The high depiction rate at ICA can probably be attributed to the thorough review of the angiogram, with specific focus on detecting MB. In the present study MB was suspected at ICA in 4 cases, where no MB was demonstrated by coronary CTA, which occurred only once in the previous study, where automated quantitative coronary angiography software was used to measure systolic diameter narrowing. The use of quantitative analysis at ICA might thus pose an advantage in order to quantify systolic compression as well as to avoid over-diagnosing MB [65].

Assessment of dynamic compression was not performed at coronary CTA, since images were acquired only in diastole, in order to minimize radiation exposure. To adequately assess dynamic compression it would have been necessary to perform a retrospectively gated examination with full radiation dose throughout the cardiac cycle, resulting in significantly higher radiation exposure [65].

Only MB of the LAD was considered, due to the facts that MB in other vessels is rare and that dynamic compression mostly occurs in MB of the LAD [67]. The degree of dynamic compression has been shown to correlate with the risk of ischemia [66]. Thus it seems very unlikely that MBs in other vessels would be clinically significant.
Table 7. MB detected by coronary CTA and unidentified or identified by ICA.

<table>
<thead>
<tr>
<th>Type of MB</th>
<th>MB unidentified by ICA n=19</th>
<th>MB identified by ICA n=9</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial encasement</td>
<td>7 (37%)</td>
<td>4 (44%)</td>
<td>ns</td>
</tr>
<tr>
<td>Full encasement</td>
<td>12 (63%)</td>
<td>5 (56%)</td>
<td></td>
</tr>
<tr>
<td>Location of MB</td>
<td></td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>Proximal LAD</td>
<td>1 (5%)</td>
<td>1 (11%)</td>
<td></td>
</tr>
<tr>
<td>Mid LAD</td>
<td>17 (89%)</td>
<td>7 (78%)</td>
<td></td>
</tr>
<tr>
<td>Distal LAD</td>
<td>1 (5%)</td>
<td>1 (11%)</td>
<td></td>
</tr>
<tr>
<td>Length of MB (mm)</td>
<td>15.1 ± 13</td>
<td>18.5 ± 8</td>
<td>ns</td>
</tr>
<tr>
<td>Thickness of MB (mm)*</td>
<td>0 (0-4.8)</td>
<td>0 (0-1.5)</td>
<td>ns</td>
</tr>
</tbody>
</table>

Data are presented as absolute value (percentage), mean ± SD or median (range). Abbreviations: MB, myocardial bridging; CTA, computed tomography angiography; ICA, invasive coronary angiography; LAD, left anterior descending artery; ns, non-significant. * in the case of full encasement

5.4 STUDY IV

5.4.1 Results

Clinical characteristics, including laboratory results, of study participants without and with CAD are shown in Table 8. Approximately half of the study group had low CVD risk according to the Framingham risk algorithm, and only one-fifth had high CVD risk. Kidney function was in general normal or only mildly reduced.

Coronary CTA plaque burden is described in Tables 2 and 3. Briefly, there were no signs of CAD in 49 (43%) study participants and CAD in at least one coronary segment in 66 (57%). Among study participants with CAD, 44 (67%) had a maximum degree of stenosis <20%, 21 (32%) had a maximum degree of stenosis 20-50% and only one had a stenosis ≥50%. On a per segment level, out of all plaques, 20 (11%) were non-calcified, 38 (22%) were mixed and 117 (67%) were calcified. Among subjects with CAD, only 12 (18%) had exclusively non-calcified plaques.

As previously described, coronary CTA plaque burden was similar in MINOCA patients and controls (results study I) as were most baseline characteristics (Table 1). For the purpose of the current study it was crucial to find out whether the two subsets of the study group were also similar regarding the plasma biomarkers to be analyzed. LDL cholesterol was the only parameter showing a statistically significant difference in concentration between MINOCA patients and controls. The mean concentration of LDL cholesterol (±SD) was 3.2 (±0.9) mmol/L in MINOCA patients and 3.7 (±0.9) mmol/L in controls (p=0.008).

Compared to the group without CAD at coronary CTA, the study participants with CAD had lower plasma concentrations of adiponectin and higher plasma concentrations of lipoprotein(a) and cystatin C. They were also more likely to be male and had higher body mass index (Table 8). Results of univariable logistic regression analyses are shown in
Table 9. Concentrations of circulating biomarkers adiponectin, lipoprotein(a) and cystatin C were dichotomized, according to the median. The median was chosen as a cut-point, since concentrations were largely within normal ranges and there were no predefined cut-off levels.

Table 8. Clinical characteristics of subjects without and with CAD, determined by coronary CTA.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No CAD (n=49)</th>
<th>CAD (n=66)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60 ± 6</td>
<td>61 ± 7</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>40 (82%)</td>
<td>41 (62%)</td>
<td>0.023</td>
</tr>
<tr>
<td>Smoking habits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>29 (59%)</td>
<td>33 (53%)</td>
<td></td>
</tr>
<tr>
<td>Previous smoker</td>
<td>14 (29%)</td>
<td>26 (40%)</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>6 (12%)</td>
<td>6 (9%)</td>
<td></td>
</tr>
<tr>
<td>Family history of CAD</td>
<td>10 (20%)</td>
<td>20 (30%)</td>
<td></td>
</tr>
<tr>
<td>Treated hypertension</td>
<td>10 (20%)</td>
<td>15 (23%)</td>
<td></td>
</tr>
<tr>
<td>Treated hyperlipidemia</td>
<td>4 (8%)</td>
<td>7 (11%)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.9 ± 3</td>
<td>26.5 ± 4</td>
<td>0.026</td>
</tr>
<tr>
<td>OGTT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NGT</td>
<td>32 (67%)</td>
<td>44 (72%)</td>
<td></td>
</tr>
<tr>
<td>IGT</td>
<td>12 (25%)</td>
<td>13 (21%)</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>4 (8%)</td>
<td>4 (7%)</td>
<td></td>
</tr>
<tr>
<td>FRS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low</td>
<td>24 (49%)</td>
<td>29 (44%)</td>
<td></td>
</tr>
<tr>
<td>intermediate</td>
<td>17 (35%)</td>
<td>23 (35%)</td>
<td></td>
</tr>
<tr>
<td>high</td>
<td>8 (16%)</td>
<td>14 (21%)</td>
<td></td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>131 (118-149)</td>
<td>140 (125-155)</td>
<td>ns</td>
</tr>
<tr>
<td>GFR (ml/min/1.73m²)</td>
<td>80.0 ± 12</td>
<td>76.5 ± 13</td>
<td></td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>0.82 (0.39-1.85)</td>
<td>1.1 (0.56-2.20)</td>
<td>ns</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.6 (1.2-2.1)</td>
<td>1.3 (1.1-1.9)</td>
<td>ns</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.4 ± 1.0</td>
<td>3.5 ± 0.87</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.94 (0.73-1.35)</td>
<td>1.00 (0.88-1.30)</td>
<td>ns</td>
</tr>
<tr>
<td>Adiponectin (mg/L)</td>
<td>10.5 (7.8-18.3)</td>
<td>7.9 (6.0-13.0)</td>
<td>0.048</td>
</tr>
<tr>
<td>Lipoprotein(a) (nmol/L)</td>
<td>16.5 (5.0-37.0)</td>
<td>24.0 (14.0-76.0)</td>
<td>0.027</td>
</tr>
<tr>
<td>Cystatin C (mg/L)</td>
<td>0.79 (0.72-0.88)</td>
<td>0.86 (0.77-0.95)</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Abbreviations: CAD, coronary artery disease; CTA, computed tomography angiography; BMI, body mass index; OGTT, oral glucose tolerance test; NGT, normal glucose tolerance; IGT, impaired glucose tolerance; FRS, Framingham Risk Score (low risk ≤10%, intermediate risk 10-20%, high risk >20% 10-year risk); SBP, systolic blood pressure; GFR, estimated glomerular filtration rate based on serum creatinine; hs-CRP, high-sensitivity C-reactive protein; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; ns, non significant. Data are presented as mean ± standard deviation, absolute value (percentage) or median (interquartile range). P-values <0.05 are given, obtained through Student´s t-test, the Mann-Whitney U-test or the chi-square test, as appropriate.

The final adjusted multivariable model incorporated all risk markers with p≤0.1 in univariable analysis as well as smoking habits, subgroup (MINOCA, control) and estimated GFR (Table 9). The remaining independent variables were male sex (OR 2.77, 95% CI 1.11, 6.90) and cystatin C (OR 2.50, 95% CI 1.12, 5.59).
When examining the correlation between the number of diseased segments at coronary CTA and plasma biomarker levels, a statistically significant correlation was found only for cystatin C ($r_s=0.25, p<0.01$).

**Table 9.** Univariable and multivariable logistic regression models for the outcome variable CAD, determined by coronary CTA.

<table>
<thead>
<tr>
<th>Logistic regression model</th>
<th>Risk marker</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariable analyses$^a$</td>
<td>Male sex</td>
<td>2.71</td>
<td>1.13, 6.52</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>1.13</td>
<td>1.01, 1.25</td>
</tr>
<tr>
<td></td>
<td>SBP</td>
<td>1.02</td>
<td>1.00, 1.03</td>
</tr>
<tr>
<td></td>
<td>HDL cholesterol</td>
<td>0.51</td>
<td>0.25, 1.06</td>
</tr>
<tr>
<td></td>
<td>Adiponectin</td>
<td>0.48</td>
<td>0.22, 1.03</td>
</tr>
<tr>
<td></td>
<td>Lipoprotein(a)</td>
<td>1.87</td>
<td>0.87, 4.00</td>
</tr>
<tr>
<td></td>
<td>Cystatin C</td>
<td>2.56</td>
<td>1.19, 5.49</td>
</tr>
<tr>
<td>Multivariable adjusted model$^b$</td>
<td>Male sex</td>
<td>2.77</td>
<td>1.11, 6.90</td>
</tr>
<tr>
<td></td>
<td>Cystatin C</td>
<td>2.50</td>
<td>1.12, 5.59</td>
</tr>
</tbody>
</table>

Adiponectin, Lipoprotein(a) and Cystatin C were dichotomized into two equally sized groups, according to the median. $^a$For univariable analyses results are presented for all variables with $p \leq 0.1$. $^b$Stepwise multivariable model incorporating: sex, BMI, SBP, HDL cholesterol, Adiponectin, Lipoprotein(a), Cystatin C, subgroup (MINOCA-control), smoking and estimated glomerular filtration rate. OR, odds ratio; CI, confidence interval of the odds ratio; BMI, body mass index; SBP, systolic blood pressure; HDL, high-density lipoprotein cholesterol.

### 5.4.2 Methodological discussion and additional analyses

Estimated GFR was based on serum creatinine rather than cystatin C, in order to be able to adjust for GFR in a multivariable regression analysis incorporating cystatin C as an independent variable.

There was no difference between the groups without and with CAD with regard to the Framingham risk score (FRS) [74]. However, since the FRS is primarily a tool for primary prevention it might not be appropriate for patients suffering from MINOCA. We therefore performed separate analyses for the control group to investigate whether the FRS was associated with presence or extent of CAD at coronary CTA. Non-parametric testing showed no significant difference in FRS between the groups without and with CAD ($p=0.2$). There was however a statistically significant correlation between FRS and number of diseased segments ($r_s=0.26, p=0.05$) (Figure 8). Significant correlations were also found between FRS and adiponectin ($r_s=-0.41, p<0.01$) and cystatin C ($r_s=0.44, p<0.01$) but not between FRS and lipoprotein(a).
Figure 8. Correlation between the FRS and the number of diseased segments, as determined by coronary CTA ($r_c=0.26$) (A), plasma adiponectin ($r_c=-0.41$) (B) and plasma cystatin C ($r_c=0.44$) (C). Abbreviations: FRS, Framingham risk score; CTA, computed tomography angiography.
6 GENERAL DISCUSSION

6.1 MYOCARDIAL INFARCTION AND NON-OBSTRUCTED CORONARY ARTERIES

Study I of this thesis was the first to use coronary CTA to analyze coronary plaque burden in patients with MINOCA, with a control group matched by age and gender for comparison. We aimed to find out whether MINOCA patients had more CAD than controls, which might indicate that CAD is an underlying mechanism of MINOCA. Instead, we found a similar prevalence of CAD in patients with MINOCA and controls. In addition, a large proportion of MINOCA patients (42%) did not have any signs of CAD at coronary CTA, which strongly suggests there are other underlying causes for a significant number of MINOCA patients. This finding was further supported by the fact that MINOCA patients had a lower rate of large size and mixed type coronary artery plaques; plaque characteristics that have been shown to imply a more vulnerable plaque type, more prone to rupture [112-114].

Coronary CTA has proven to be highly sensitive for detecting atherosclerotic plaques of the coronary arteries [3,4,20]. Still, there are limitations to its spatial resolution, which makes it uncertain to assess very small plaques, in particular if located distally in the coronary arteries. Hence, early CAD, with subtle changes of the vessel wall, as well as distal lesions might remain undetected by coronary CTA [21,115]. In this thesis, a semi-quantitative method that relied on visual assessment was used for coronary CTA plaque analysis. It could be debated whether an automated, non-user dependent method would have been more reliable. There were however no robust and validated automated methods available when this study was conducted. In contrast, semi-quantitative methods for plaque assessment have been described in several studies, demonstrating small inter- and intraobserver variability [116,117]. In clinical practice and in most studies, coronary CTA analysis still relies largely on visual assessment. In a recent study [118] an automated coronary plaque quantification algorithm was compared to expert and non-expert semi-automatic plaque assessment, with intravascular ultrasound as the gold standard. The performance of the fully automated method was good and comparable to non-expert readers but inferior to expert readers. There was a high agreement between expert readings and intravascular ultrasound measurements for all parameters analyzed [118]. Only segments with sufficient CTA and intravascular ultrasound image quality were included in the study and performance of an automated method in the real world scenario with motion, calcifications and other artefacts still needs to be established.

Substantial efforts have been made to search for imaging markers of plaque instability that might allow us to identify vulnerable plaques before they rupture and thereby possibly prevent plaque rupture and AMI or sudden death. Pathologic studies have identified the thin-cap fibroatheroma as a vulnerable plaque type with prognostic implications. Studies using intravascular ultrasound and optical coherence tomography have reported that a large necrotic core, a thin fibrous cap and positive vascular remodeling are signs of plaque instability [119]. Several coronary CTA plaque characteristics have been associated with a vulnerable plaque type: positive vascular remodeling, low-attenuation plaque (<30 HU), large plaque area and
non-calcified or mixed plaque types [112-114,120]. There are several limitations when attempting to identify vulnerable plaques by coronary CTA. The limited spatial resolution prevents identification of thin-cap fibroatheromas and makes the measurement of positive remodeling imprecise. Plaque HU measurements do not only depend on plaque composition, but are also affected by technical and imaging parameters. Therefore, we chose to limit plaque characteristic assessment to: degree of stenosis, length of plaque and non-calcified/mixed/calcified plaque.

An important strength of the studies in this doctoral thesis is the high diagnostic quality of the coronary CTA scans, with very few non-evaluable coronary segments. Examinations were performed by dedicated radiographers using a 64-detector scanner, in a centre with several years’ experience of coronary CTA [121]. Evaluation of coronary CTA scans was performed by two experienced readers, separately and in consensus.

Our findings partly contradict findings of a previous study [49], in which only 16% of MINOCA patients did not have signs of CAD when examined with coronary CTA. However, this difference can probably be explained by the difference in inclusion criteria. In the previous study, patients with <50% angiographic diameter stenosis were included, whereas in our study a more rigorous definition was used, including only patients with no or minimal signs of atherosclerosis (<30%). In addition, only patients with evidence of myocardial infarction on CMR were included in the earlier study [49], whereas we also included patients with smaller myocardial infarctions that were proven by biochemical markers but not detectable by CMR. In fact, a recently published meta-analysis [5] demonstrated that subendocardial infarct can only be detected by CMR in one-fourth of patients with MINOCA, which illustrates that the study group of the earlier study [49] might not have been representative of all MINOCA patients. In our study, one-fifth of MINOCA patients had myocardial infarction at CMR, which is similar to findings of the recent meta-analysis [5].

In an intravascular ultrasound study [45], plaque rupture and ulceration was found in 38% of women with MINOCA, suggesting CAD to be an underlying cause for a large proportion of MINOCA patients. Patients with plaque disruption had a higher degree of stenosis (median degree of stenosis 40%) compared to patients without plaque disruption. Interestingly, none of the patients with a normal ICA had plaque disruption [45]. Consequently, it seems likely that the frequency of plaque disruption would be smaller in our study group, with less severe CAD. It has been shown that culprit lesions in AMI are frequently not stenotic. Pathology studies of acute coronary death have demonstrated that the average culprit lesion has a diameter stenosis of approximately 50%, and rarely below 30% [122]. This further supports our conclusion that, for the present study group, CAD is most likely not an important cause of MINOCA.

To the best of my knowledge, to date our study is the largest study of its kind. Still, the sample size was limited. Therefore, the lack of significant differences between the two groups may be caused by a lack of power to detect such differences. However, the data shows a tendency towards more severe CAD in the control group compared to the MINOCA-group,
rather than the other way round. In order to estimate statistical power, one could consider the per segment analysis (n=765+781), a binomial endpoint (segment with or without CAD) and the 5% level of significance. Anticipating a CAD prevalence of 10% in the control group and 15% in the MINOCA group would yield a power of 85%.

We found that coronary CTA detected significantly more MB than ICA. In our study MB was detected by coronary CTA in 47% of the study participants, which is similar to the prevalence (58%) found in a previous coronary CTA study [67], and of the same magnitude as frequencies found in previous autopsy studies [66]. However, most previous studies using coronary CTA showed a lower prevalence of MB [66]. This may in part reflect the superior image quality of newer generations of CT scanners and the fact that evaluations of coronary CTA scans were performed by experienced readers focusing specifically on the presence of MB, and probably also the inclusion of both MB with partial encasement and MB with full encasement. Kim et al. [67] found partial encasement in 19% and full encasement in 39% of subjects, which is similar to our findings (partial encasement 18%; full encasement 29%).

Study III of this thesis is the first to use coronary CTA in order to compare the prevalence of MB in MINOCA patients with a matched control group. MB was a frequent finding, with a similar prevalence in MINOCA patients and in controls. Therefore, it is unlikely that MB is an important cause of MINOCA. However, in order to find out whether MB might have been an underlying mechanism of the AMI for some individuals, additional testing of the physiological significance of the MB would be necessary, e.g. dobutamine stress coronary angiography or intracoronary acetylcholine testing for coronary spasm and endothelial dysfunction [72,73,123].

The studies of this thesis cannot explain the pathophysiological mechanisms of MINOCA. However, our findings suggest that neither CAD nor MB is a frequent cause. Together with findings of earlier and recent studies [5,10,11] our findings support that patients with MINOCA compose a heterogeneous group, with a variety of underlying causes. For the MINOCA patients with CAD this may, at least for some patients, have been the cause of the myocardial infarction. However, CAD is a less likely cause in the patients with no or minimal angiographic stenosis. Plaque disruption with transient thrombus formation might be one plausible mechanism and vasospasm another [5,45]. There was a higher frequency of smoking in the MINOCA group, which may increase the risk of thrombosis as well as vasospasm. Even though MB is probably not a frequent cause of MINOCA, it should be considered when other potential causes of the MI have been ruled out. TS was a relatively frequent finding (26% of MINOCA patients) and the prevalence might have been underestimated, which will be further discussed. Six out of 57 MINOCA patients showed no signs of CAD, did not have MB and did not get a diagnosis of TS, which means that there is still a proportion of patients where other potential causes of the MI are likely.
6.2 STUDY GROUP

An important strength of studies I and III is the well defined MINOCA group, where all patients underwent CMR imaging in order to exclude those with myocarditis, which commonly mimics AMI [124]. Limitations in the sensitivity of CMR to detect myocarditis may have led to false-negative cases [125]. However, they were most likely few, since most patients presenting with typical clinical features of myocarditis did not undergo ICA and were thus not eligible for the SMINC study in the first place. During the screening period, a majority (64%) of all MINOCA patients in the Stockholm metropolitan area were included in the SMINC study. Of the 100 MINOCA patients included in the SMINC study, only 57 underwent coronary CTA and a possible selection bias must be considered. However, when comparing baseline characteristics of these patients with the 43 MINOCA patients who did not participate in the coronary CTA study, there were no significant differences. Considering my research questions, I do not believe that there was any significant selection bias affecting the results. The upper age limit of 70 years may have reduced the proportion of MINOCA patients with TS, since patients with TS are typically older than other MINOCA patients [7].

The control group was composed of randomly selected volunteers matched by age and gender, free from clinical CVD. The participation rate was approximately 50%. An important question is whether they truly represented the population from which they were recruited. In an ongoing large Swedish multi-centre study of cardiopulmonary disease [126], a pilot study was performed, where randomly selected subjects (50-64 years of age) were invited to take part in a number of examinations, among them coronary CTA. The participation rate was lower in areas of low compared to high socio-economic status (40% vs 68%). Moreover, the pilot study demonstrated significant differences in health related to socio-economic status, with a higher prevalence of risk factors in areas of low socio-economic status [126]. Hence, there seems to be a risk of recruiting controls with a lower prevalence of risk factors than the general population. This effect would probably be even more important since controls of the SMINC study were excluded if they had an abnormal exercise stress test. Since we found in study I that MINOCA patients did not have more CAD than controls, these concerns probably did not compromise our results.

6.3 TAKOTSUBO SYNDROME

For patients with TS we found a prevalence of MB similar to that of the control group. This contradicts findings of a previous study [13] that reported a significantly higher prevalence of MB in patients with TS compared to controls, by coronary CTA as well as by ICA. However, consistent with the findings of our study, a recent ICA study [127] showed a similar prevalence of MB in patients with TS and in a control group. Our findings support that MB is not a cause of TS. In fact, it does not seem plausible that MB would cause the wall-motion abnormality seen in TS, since it does not follow the coronary distribution.

A limitation in studies I and III might be the use of the Mayo clinic criteria [8] for diagnosis of TS. As discussed in the introduction section there are several arguments against these
criteria and updated diagnostic criteria have been proposed [7, 53]. Some of these arguments do not apply to the present study group, who by definition in the study protocol did not have obstructive CAD or myocarditis. However, in addition to the 15 MINOCA patients who fulfilled the diagnostic criteria for TS, there were 10 MINOCA patients for whom there was a clinical suspicion of TS, but the left ventricular wall motion abnormality was atypical and did not fulfil the first Mayo criterion. In some patients, a typical wall motion abnormality might have been missed, if left ventricular angiography was not performed at the time of ICA and if CMR was performed too late, when the wall motion abnormality had partly resolved. Another cause for a “missed” diagnosis might be if the patient had a milder form of disease in the TS spectrum.

6.4 CARDIOVASCULAR DISEASE RISK ASSESSMENT

When interpreting the results of studies II and IV it is important to consider the present study group. The prevalence of CVD risk factors was relatively low, most study participants had low-to-intermediate CVD risk by the Framingham risk algorithm [74] and a majority were women. This contrasts to most other similar studies, where the CVD risk profile was much more pronounced and a larger proportion of study participants were men. In our study group, coronary CTA plaque burden was relatively mild, with almost exclusively non-obstructive CAD. IMT and RH-PAT values were in general in the normal range, as were the levels of circulating biomarkers. It has been shown that subjects with early stage non-obstructive CAD are at increased risk of CVD events compared to subjects without CAD at coronary CTA [14,15,27,28], which is why we found it important to study this predominantly female low-to-intermediate risk group, with no or non-obstructive CAD.

IMT was similar in the groups with and without evidence of CAD by coronary CTA. There was no correlation between IMT and the number of diseased coronary artery segments. Our findings differ from findings of previous studies that focused mainly on patients with more pronounced CVD risk and more severe CAD as determined by coronary CTA or by ICA [99,128,129]. However, our findings are partly supported by a recent study [100] that did not find a significant relationship between carotid artery ultrasound findings and coronary CTA plaque burden. Their study group was composed of asymptomatic subjects referred for risk stratification, with a CVD risk profile and a prevalence of CAD similar to that of our study [100]. As previously mentioned, it has been shown that carotid plaque detection is more accurate than IMT for predicting CVD events [82].

Peripheral endothelial function by the RH-PAT method was similar in subjects with and without evidence of CAD by coronary CTA and there was no correlation between RH-PAT and the number of diseased coronary artery segments. To the best of my knowledge, study II is the first publication comparing endothelial function by the RH-PAT method with coronary CTA plaque burden. A previous study [130] of 140 women with chest pain who underwent RH-PAT testing as well as ICA, demonstrated significantly worse RHI in patients with obstructive and non-obstructive CAD compared to patients with no CAD. In that study, patients with CAD had markedly worse RHI than subjects with CAD of our study. This
difference might reflect a greater risk factor burden of the patients of the earlier study [130]. The fact that we analyzed CAD by coronary CTA instead of ICA might also have affected the results. Since coronary CTA allows for detection of non-obstructive lesions that might not be visible on ICA, more patients with limited disease are likely to be classified as having CAD when coronary CTA is used. Another study [131] reported a lack of association between endothelial function, expressed in terms of flow mediated dilation of the brachial artery and ICA findings, in patients with severe CAD. A possible interpretation of their findings might be that endothelial function testing is of limited value for patients with pre-existing severe CAD. It has indeed been suggested that endothelial function testing, and in particular testing involving the microvasculature as is the case for the RH-PAT method, might be more suitable for subjects with a low prevalence of risk factors [111]. This hypothesis is not supported by our findings. Endothelial dysfunction has been shown to precede the appearance of CAD and to predict the progression of IMT, hence suggesting that it represents an earlier step in the atherosclerotic process [89,132]. However, in our study, a large number of subjects with CAD evident at coronary CTA displayed normal endothelial function.

One explanation for the lack of association between IMT or RH-PAT and CAD might be the fact that the different measures reflect the presence of different underlying CVD risk factors, to which the various vascular beds are more or less susceptible. It has been shown that certain risk factors have different impacts on IMT and CAC, with diabetes mellitus having a relatively higher impact on IMT and blood pressure showing a stronger association with CAC [133]. The finding of a strong correlation between CAC and the presence and extent of CAD as determined by coronary CTA is not surprising, since the two measures correspond to two aspects of the atherosclerotic process of the coronary vascular bed.

Among circulating biomarkers we found that plasma cystatin C was an independent predictor of CAD, with an OR of 2.5 adjusted for confounders, including estimated GFR, based on serum creatinine. Adiponectin levels were significantly lower and lipoprotein(a) levels were higher in the CAD group by non-parametric analysis. However, these associations did not remain in the multivariable logistic regression analysis.

An association between elevated plasma levels of cystatin C and CVD has been demonstrated in patient groups with elevated CVD risk as well as in the general population [134,135], including in subjects without chronic kidney disease (estimated GFR ≥60 ml/min/1.73m2) [136]. Cystatin C is an accurate marker of renal function and its association with CVD might be related to compromised renal function, possibly also within the normal GFR range. The pathophysiological mechanisms are incompletely understood and other effects of cystatin C on for instance atherogenesis have been suggested [137]. Two previous ICA studies [138,139] showed associations between cystatin C levels and extent of CAD and number of stenotic vessels (≥51%). In both studies, a majority of the study participants were men, with a higher prevalence of CVD risk factors than in our study group. The extent and severity of CAD was considerably more pronounced and concentrations of cystatin C were in general
higher than in our study. To the best of my knowledge, there is only one previous publication [103] that has examined the association between plasma levels of cystatin C and CAD determined by coronary CTA. That study differs fundamentally from our study since it included only patients with evidence of CAD at coronary CTA (stenosis <50%). In addition, a large proportion were men (59%) and the prevalence of diabetes mellitus and hypertension was higher. Imai et al. [103] showed that cystatin C was associated with the number of diseased segments and that increasing plasma concentrations of cystatin C had a stronger impact on non-calcified compared to calcified plaques, suggesting a possible role for cystatin C in the early stage of CAD. Our study provides additional information by demonstrating an association between plasma levels of cystatin C and absence or presence of CAD at coronary CTA, in patients with normal or mildly reduced kidney function. Our findings suggest that there is an association between cystatin C and CAD even in subjects with lower CVD risk than previously reported. Our study, in contrast to most previous studies, reported plasma levels of cystatin C that were relatively low (mainly within clinical reference ranges), which indicates that the predictive value of cystatin C applies to both low and high plasma concentrations.

Adiponectin was lower in the group with CAD by non-parametric testing, but the difference did not remain in the logistic regression analysis. Only a few studies [101,102,104] have investigated the association between plasma adiponectin and coronary CTA findings, and they have demonstrated conflicting results. One study [101] demonstrated an inverse association between plasma levels of adiponectin and non-calcified plaque and another [104] showed that adiponectin was an independent predictor of multivessel CAD, whereas a third study [102] did not find a significant association between levels of adiponectin and coronary CTA findings. In all three studies, the study participants had an elevated CVD risk and more pronounced coronary CTA plaque burden compared to the participants of our study. Due to the small number of subjects with exclusively non-calcified plaque of our study, analyses stratified by plaque type were not performed.

In multivariable analysis, lipoprotein(a) was not associated with CAD, which is consistent with two previous coronary CTA studies [101,102]. In contrast, numerous prospective studies [140] have shown a moderately strong association of lipoprotein(a) with CVD events, largely independent of classical CVD risk factors. This suggests that lipoprotein(a) might have effects on CVD risk that are not mediated through an increased coronary plaque burden.

Within the study group, ranges of plasma biomarker concentrations were quite small, which may partly account for the lack of associations between CAD and circulating biomarkers. Due to the limited sample size, I focused on correlation and binary logistic regression analyses and did not perform additional statistical analyses such as the C statistic or area under the receiver operating characteristic curve.

In order for a biomarker to be clinically useful it should meet several criteria. To begin with, measurement should be easy. In addition, the biomarker should provide new information on top of traditional risk factors and it should have an effect on patient management [79,141].
addition to a statistically significant association with the outcome, the test needs to have an ability to discriminate (between those who will and those who will not get the disease). It needs to be calibrated (tested for the agreement between predicted and observed risk in groups with different baseline risks) and tested for its ability to reclassify subjects from one risk category to another, thus affecting treatment decisions [75,79]. Ideally, the accuracy of the reclassification should also be explored. Hence, in order to establish the clinical usefulness of a novel biomarker large-scale studies are necessary.
7 CONCLUSIONS AND FINAL REMARKS

The general aims of this thesis were to investigate the underlying mechanisms of MINOCA and to examine the association between coronary CTA plaque burden and other risk markers of CVD.

Our results suggest that:

- Non-obstructive CAD is not a frequent cause of MINOCA in patients with angiographically normal or near-normal coronary arteries.
- MB is frequent and is most likely not a common cause of MINOCA or of TS. Coronary CTA detects significantly more MBs than ICA.
- Neither IMT nor RH-PAT can reliably be used to predict subclinical CAD at coronary CTA.
- Circulating cystatin C is an independent predictor of presence and extent of CAD at coronary CTA.

Since the SMINC study was initiated in 2007, numerous studies have been published, examining the prevalence, risk factors and possible pathophysiologic mechanisms of MINOCA. There is strong evidence that the condition is heterogeneous, with a variety of underlying causes [5,10,11]. Thus, MINOCA should not be considered a definitive diagnosis, but rather a working diagnosis, warranting additional diagnostic evaluation. Myocarditis is one of the conditions that may manifest itself as MINOCA. Findings of the SMINC study, where myocarditis was excluded by CMR, suggest that TS is an important cause of MINOCA [10,11]. Other potential causes include CAD with rupture of a non-stenotic lesion, coronary artery spasm, thrombotic disorders and microvascular dysfunction [5,44,142].

Identifying the cause of MINOCA is crucial, since these conditions have different prognostic implications and different treatment strategies are required in order to improve prognosis. Additional research is needed to define optimal diagnostic strategies for patients with MINOCA. At ICA, provocative testing may confirm coronary artery spasm and left ventricular angiography may demonstrate the wall motion abnormality of TS [5,44]. In addition, intravascular ultrasound may demonstrate the presence of vulnerable or ruptured plaques [45] and if MB is suspected, testing of its physiological significance may be performed [72,73,123]. CMR imaging will probably have a central role, since it allows for assessment of TS as well as differentiation between myocardial infarction and myocarditis [11,62,63]. In some cases, coronary CTA may be useful to confirm or rule out the presence of non-obstructive CAD [143].

Regarding TS, this thesis contributed by demonstrating that MB is most likely not an underlying cause, which highlights that the underlying pathogenic mechanisms still need to be clarified. Establishing consensus on the diagnostic criteria will facilitate future research on pathophysiological mechanisms, diagnosis, prognosis, and patient management [7].
CVD is the leading cause of death worldwide and causes considerable morbidity each year. Improving CVD risk prediction might enable more individualized preventive measures, which may in turn have a large impact on health and healthcare costs. However, the relationship between different risk markers and adverse CVD events is complex and the contribution of individual risk factors or markers is difficult to isolate. This study provides novel insights into the association of cystatin C with non-obstructive CAD determined by coronary CTA, in a study group with a predominantly low-to-intermediate CVD risk profile and with normal or only mildly reduced kidney function. Cystatin C might thus have a potential to serve as a screening test aiming to identify patients with subclinical CAD, who are at increased risk of future CVD events. However, large-scale studies will be necessary to establish its clinical usefulness.

A prognostic value of the total coronary CTA plaque burden (obstructive and non-obstructive plaque) has been established [14,15,27,28] and several studies have suggested that identification of vulnerable plaque characteristics may provide additional predictive information [112-114,120]. However, there are several factors that may limit the usefulness of coronary CTA to determine vulnerable plaque characteristics, a few of them previously discussed. Keeping radiation exposure low in coronary CTA is essential for patient safety. Even though low-dose CT protocols yield a high diagnostic accuracy for diagnosing stenoses, evaluation of non-calcified plaque and plaque components becomes more uncertain with very low radiation doses. One future application of coronary CTA in identification of high-risk plaque may instead be the combined anatomic imaging of coronary CTA with imaging of metabolism and inflammation using $^{18}$F-2-deoxyglucose (FDG) positron emission tomography (PET) [144]. Recently, there has been increasing emphasis on identifying “the vulnerable patient”, rather than the vulnerable plaque, since CAD has become considered more as a generalized disease than a focal disease. Instead of focusing on identifying individual high-risk plaques, it may prove to be more useful to consider the extent and inflammatory activity of CAD together with other risk markers, including markers of a prothrombotic state [122,144]. Hence, large-scale studies with a comprehensive integrative approach will be necessary to investigate which combination of imaging parameters and other risk markers yields the most accurate individual risk prediction. An accurate tool for CVD risk prediction will allow for the assignment of individuals to risk categories that can guide management and treatment and, in the long run, lower CVD morbidity and mortality.
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