UiT
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Unrecognized Myocardial Infarction

Pain tolerance, prognosis and pathogenesis in men and women

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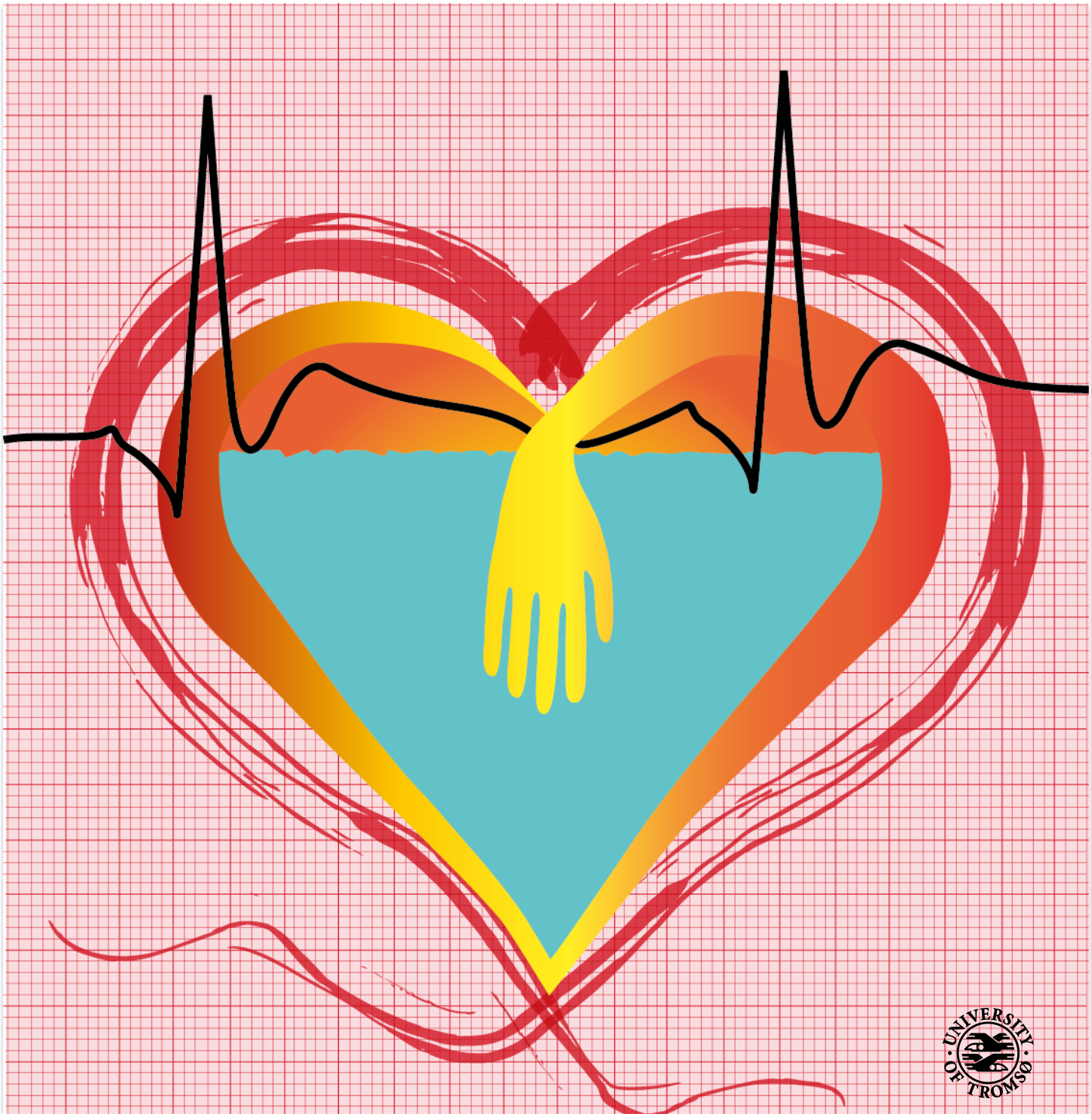
A dissertation for the degree of Philosophiae Doctor – October 2017



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2 LIST OF PAPERS

I. Øhrn A, Nielsen CS, Schirmer H, Stubhaug A, Wilsgaard T, Lindekleiv H. Pain tolerance in persons with recognized and unrecognized myocardial infarction: a population based, cross- sectional study. *Journal of the American Heart Association, 2016. Doi:* 10.1161/JAHA.116.003846.

II. Øhrn A, Schirmer H, Njølstad I, Mathiesen EB, Eggen AE, Løchen MJ, Wilsgaard T, Lindekleiv H. Electrocardiographic Unrecognized Myocardial Infarction Does Not Improve Prediction of Cardiovascular Events Beyond Traditional Risk Factors, The Tromsø Study. Accepted for publication in the *European Journal of Preventive Cardiology* (September 2017).

III. Øhrn A, Schirmer H, von Hanno T, Mathiesen EB, Arntzen KA, Bertelsen G, Njølstad I, Løchen MJ, Wilsgaard T, Merz NB, Lindekleiv H. Small and Large Vessel Disease in Persons with Unrecognized Compared to Recognized Myocardial Infarction: the Tromsø Study 2007-2008. Accepted for publication in the *International Journal of Cardiology* (October 2017).

3 INTRODUCTION

Myocardial infarction (MI) is a leading cause of morbidity and mortality worldwide. Acute MI is mostly associated with severe symptoms leading to emergency medical assistance, but a substantial proportion is accompanied by minimal, atypical or no symptoms. MI without pain was first described back in 1912[1]. Today such events, termed "unrecognized MI", are well documented but still an intriguing entity. Persons that have suffered an unrecognized MI are often unaware of their history with heart disease, and subsequently may not receive medical attention. These patients can be identified retrospectively by electrocardiography (ECG) or with various imaging techniques. ECG was used as detection method in this project. Unrecognized MI constitutes at least 25% of all MI's in men[2-4], higher percentages are usually reported in women[5-8], and it is associated with a similar poor prognosis as recognized MI [3,5,6,9]. Unrecognized MI is a hidden public health issue with unknown underlying causes and no guidelines for screening, treatment or follow-up.

3.1 Risk factors

Risk factors for atherosclerosis such as hypertension [4,6], diabetes [6,10,11], cholesterol[12] and old age [13] are associated with increased risk of unrecognized MI[12,14]. The risk factor level in unrecognized MI seems to be an intermediate between no MI and recognized MI[12,15]. It is, however, not clearly established whether there are risk factors that distinguish unrecognized from recognized MI[14,16]. No significant difference in risk factor profiles between unrecognized and recognized MI groups have been demonstrated.

3.2 Prevalence of unrecognized MI

The true prevalence of unrecognized MI is unknown. Large cohort studies that have used ECG as detection method for unrecognized MI have estimated the prevalence to 1.2 – 6.4%[3,5,13,15,17]. The variability in prevalence between studies may to a large degree be explained by differences in ECG criteria applied to identify unrecognized MI[18,19] and different compositions of study populations. The true prevalence of unrecognized MI in the general population is most likely higher. ECG as detection method of unrecognized MI is less

sensitive compared to imaging techniques such as cardiac magnetic resonance imaging and computed tomography, where studies report a prevalence from 17%-21.5% in older adults and persons with suspected ischemic heart disease[11,15,20].

3.3 Prognosis of unrecognized MI

Increased risk of adverse cardiovascular events and death associated with unrecognized MI have been reported in cohorts of persons with known or suspected ischemic heart disease[21,22], diabetes[23] and in the elderly population[9,13]. These studies show a prognosis associated with unrecognized MI comparable to that of recognized MI. Contemporary data on the prognosis of unrecognized MI in the general population is lacking. At the beginning of the work with this thesis, only two previous studies had assessed the association of unrecognized MI with future adverse events in a general population. The Rotterdam study from 1990-93[5] reported an increased risk of all-cause mortality associated with unrecognized MI in women (HR 1.33, 95% CI 1.11-1.58) and men (HR 1.57, 95% CI 1.30-1.89), adjusted for many cardiovascular risk factors. The Copenhagen City Heart Study from 2001[24] reported an increased risk of death or hospitalization for coronary heart disease (HR 2.8, 95% CI 1.6-5.0), adjusted for a selection of confounders. However, it is not clearly established whether unrecognized MI infers risk for recurrent MI and death in the general population independent of the traditional cardiovascular risk factors. As of today, ECG is not part of the general cardiovascular risk assessment in a primary prevention setting. It is unknown whether ECG diagnosed unrecognized MI might improve the predictive ability of existing risk scores for cardiovascular disease.

3.4 Pain tolerance and recognition of MI

With recognized MI, myocardial ischemia eventually stimulates free nerve endings and gives rise to the conscious perception of chest pain[16]. A person suffering from prolonged chest pain will usually seek medical assistance and receive a proper diagnosis and treatment. The underlying reason for the lack of symptoms, and the following lack of recognition, associated with unrecognized MI is unknown. One possible explanation is decreased sensitivity for pain. Experimental studies have shown that persons suffering from silent myocardial ischemia have

attenuated pain sensitivity compared to persons that experience angina [25,26]. General somatic pain threshold has been reported to correlate with measured pain level during MI[27]. Pain sensitivity was studied in patients hospitalized for acute MI[28] showing decreased pain sensitivity in patients that presented with painless MI compared to painful MI. These studies are limited by small samples and did not include persons with unrecognized MI. However, they imply that decreased pain sensitivity is associated with less symptoms of MI. Knowledge about the association between pain sensitivity and recognition of MI is lacking, but is of interest to better understand the complex underlying causes for unrecognized MI. Pain is one of the main symptoms for seeking medical assistance and is crucial for patients and health workers to recognize and assess the severity of disease. If persons with high pain tolerance is at higher risk for unrecognized MI, this group may be at risk of being underdiagnosed in a large range of diseases.

3.5 Differences in vessel disease between unrecognized and recognized MI?

With recognized MI, the established working hypothesis is that the MI is primarily an epicardial or large vessel event as a result of plaque rupture and thrombus formation with occlusion and eventual scarring of the myocardium[29]. However, it is not evident that this working hypothesis should be applied in the case of unrecognized MI. It is unknown whether the same mechanism starting with atherosclerotic plaque in a larger coronary artery also applies to unrecognized MI, or if other vascular disease mechanisms play a role. Coronary microvascular dysfunction is a disorder of the smaller vessels of the heart where impaired coronary blood flow can be demonstrated despite no significant atherosclerosis in the larger epicardial coronary arteries[30,31]. Previous studies report findings that are suggestive of underlying differences in vascular pathology, with unrecognized MI possibly more associated with microvascular, or small vessel, disease: Unrecognized MI's are smaller compared to recognized MI [11,32], and more associated with echocardiographic global ventricular dysfunction than regional wall abnormalities[33]. Silent myocardial ischemia in persons with normal coronary arteries is associated with coronary microvascular dysfunction[34], suggesting that myocardial ischemia with less symptoms is associated with disease in the smaller vessels of the heart. A cardiac magnetic resonance study published before the work on this thesis began, reported no association between unrecognized MI and significant atherosclerosis in the

rest of the body, this in contrast to recognized MI[35]. Also, unrecognized MI's have a different distribution of location than recognized MI[24,36] and manifest lower coronary calcium score[15]. Global cardiac dysfunction may influence the increased mortality associated with unrecognized MI more than regional dysfunction[37], which again suggests a more diffuse disease. Also, studies in patients with hypertrophic cardiomyopathy have shown an association between small vessel disease and unrecognized scarring in the setting of no significant epicardial disease[38]. Small vessel disease seems to be a systemic process[39], and the small vessels in the retina has been proposed as a surrogate measure of the coronary microcirculation [40]. Retinal vessel abnormalities are associated with increased risk of cardiovascular disease[41]. A close relationship between carotid artery atherosclerosis and intima media thickness, or large vessel disease, and recognized MI is well established[42-44] and carotid artery atherosclerosis is widely used as a surrogate marker for cardiovascular disease. We used retinal vessel and carotid artery measurements as proxies for the coronary small and large vessels in our study. It is important to elucidate differences in underlying vessel disease between recognized and unrecognized MI to eventually be able to prevent and tailor correct treatment for unrecognized MI's.

3.6 Gender aspects

For many years, knowledge about risk factors, diagnosis and treatment of ischemic heart disease was limited to research on men. The significance of heart disease in women was not appreciated and women were underrepresented in the large epidemiological cohorts designed to understand cardiovascular disease. Although men and women largely share similar risk factors and manifestations of heart disease, significant differences between the male and female heart are becoming apparent, as more women are included in research. We know today that cardiovascular disease is the leading cause of death in women[45], and that there are differences in prevalence, symptoms, prognosis and pathophysiology in ischemic heart disease[46-48]. Women report more angina despite lower rates of significant coronary artery disease[49,50]. Women are significantly less likely to report chest pain or discomfort in the setting of an acute MI[47,51,52], and the lack of chest pain combined with non-chest pain symptoms (shortness of breath, cold sweats, nauseas e.g.) is more often reported in women [51,53,54] and often labeled "atypical".

Women seem to have a larger component of small vessel disease contributing to their ischemic heart disease[55-58], although this phenomenon is also of importance in men[59]. If there are differences in pathophysiology underlying unrecognized and recognized MI, they may reflect general differences in pathophysiological processes in ischemic heart disease between genders.

The proportion of unrecognized MI is larger in women compared to men[5-8], the reason for this is unknown. There may be underlying sex differences in pathophysiology or pain sensitivity and women may be at higher risk for misdiagnosis due to atypical symptoms. Risk scores for future cardiovascular events are based on traditional risk factors, but they often underestimate risk in women[60-63]. Detection of unrecognized MI may provide incremental value to risk scores and in identifying persons who will benefit from targeted preventive measures, maybe particularly in women.

4 AIMS OF THE THESIS

I. To investigate differences in pain tolerance between persons with unrecognized and recognized MI, with a focus on sex differences.

II. To study if the poor prognosis associated with unrecognized MI in men and women is independent of traditional cardiovascular risk factors and if it adds predictive value to existing risk scores for future MI.

III. To study differences in small and large vessel disease in persons with unrecognized and recognized MI in both sexes.

5 MATERIALS AND METHODS

5.1 Data source for papers I-III

5.1.1 Study population

The Tromsø Study is a single-center population-based cohort study conducted in the municipality of Tromsø, Norway. It was initiated in 1974, as a measure to help combat the high cardiovascular mortality in North Norway[64]. Six waves of the study have been carried out 6-7 years apart, referred to as Tromsø 1-6. The population consists predominantly of Caucasians[64]. The sixth survey was carried out in 2007-2008 and consisted of two visits. Total birth cohorts and random samples of birth cohorts were invited to the first visit, and 12,984 (66%) attended[64]. Those eligible for the second visit were first-visit participants in the age groups 50-62 years and 75-84 years, a 20% random sample in the age group 63-74 and participants who also had attended the second visit of the 1994 Tromsø 4 survey and who were younger than 75 years. A total of 7,306 (91.8%) attended, and 6,199 participants were examined with resting 12-lead ECG. Due to capacity limitations and logistic challenges during the first period of the survey, not all participants of the second visit had their ECG recorded. We excluded 71 participants because of ECG issues: 26 ECGs had pathologic non-MI Q waves due to altered conduction (e.g. left bundle branch block and Wolff-Parkinson-White syndrome) or ventricular hypertrophy; 20 ECGs were uncodable (e.g. pacemaker rhythm or missing leads); and 25 ECGs were not available for manual review (ECG files were missing). All three papers included in this thesis are based on the 6,128 persons who had valid ECGs.

5.1.2 Data collection

Baseline information on traditional cardiovascular risk factors was obtained by self-reported questionnaires and physical examinations. Blood pressure was measured using an automated device (Dinamap, GE Healthcare, USA). The cuff was adjusted according to arm circumference, and the blood pressure was measured 3 times in a seated position at 1-min intervals and after a 2-min rest[64]. We defined hypertension as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or use of blood pressure lowering medication. Total cholesterol and high density lipoproteins were measured non-fasting. We defined

diabetes as HbA1c ≥ 6.5% or use of insulin or oral diabetes medication. Smoking was self-reported and defined as "current daily smoker," "former daily smoker," or "never daily smoker" and modeled as a categorical variable for paper I, and binary (current daily smoking yes/no) for paper II and III. For paper I, we also collected information on depression/anxiety and physical activity. Depression/anxiety was measured by Hopkin symptom checklist 10-item version, and modelled as a dichotomous variable (cutoff ≤1.85). Physical activity was self-reported and divided into 3 levels modeled as a categorical variable. For paper II, we defined family history of premature MI as self-reported MI in parents or siblings before 60 years of age. Information on first-ever MI's was registered in the Tromsø Study endpoint registry for all participants of the Tromsø study, identified by linkage to the electronic patient records of the University Hospital of North Norway. The diagnosis was based on symptoms of MI with ECG findings of acute MI and/or elevated cardiac biomarkers. Admissions to other hospitals are infrequent as the nearest hospital is more than 200 km from Tromsø. Each event was reviewed and adjudicated by persons with medical expertise based on local hospital records[64].

5.1.3 The ECGs

A 12-lead resting ECG was recorded in 2007-2008 using a computer-based electrocardiograph (Cardiovit AT-104 PC, Schiller AG, Baar, Switzerland). We used a computer-based algorithm to extract all ECGs with a Q-wave of amplitude ≤ -0.1 mV and duration ≥0.02 s in any lead. My main supervisor, Haakon Lindekleiv, and I independently assessed the 2, 040 extracted ECGs among participants with no prior MI. There were discrepancies in the initial assessment in 140 (6.9%) of the ECGs. Disagreement was resolved after discussion with my co-supervisor Henrik Schirmer, an experienced cardiologist.

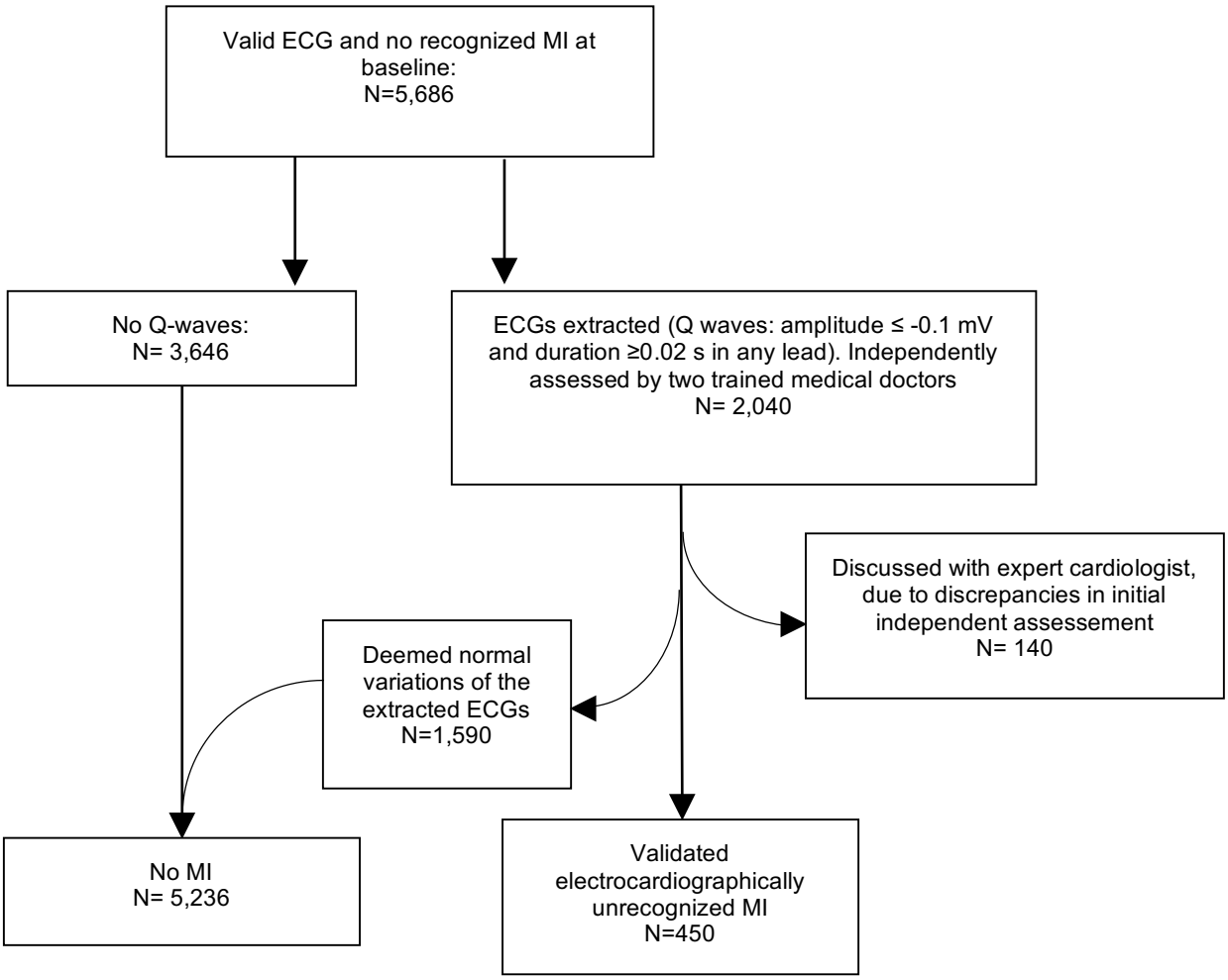

Figure 1. Flow diagram demonstrating extraction and assessment of Q-wave ECGs among participants with no registered MI at baseline. The Tromsø Study 2007-2008

We used the Third universal definition of MI[65] to define prior MI on the ECG as i) any Q wave in leads V2-V3 ≥0.02 sec or QS complex in leads V2 and V3; ii) Q wave ≥0.03 sec or

QS complex in in any two leads of a contiguous lead grouping (I,aVL; V1-V6; II,III,aVF); or iii) R wave ≥0.04 sec in V1-V2 and R/S≥1 with a concordant positive T wave in absence of conduction defect. We defined a Q wave as a negative deflection on the ECG with amplitude ≥0.1 mV without any initial positive QRS deflection. We defined a QS wave as a negative deflection on the ECG with amplitude ≥0.1 mV without any positive deflection in the QRS complex.

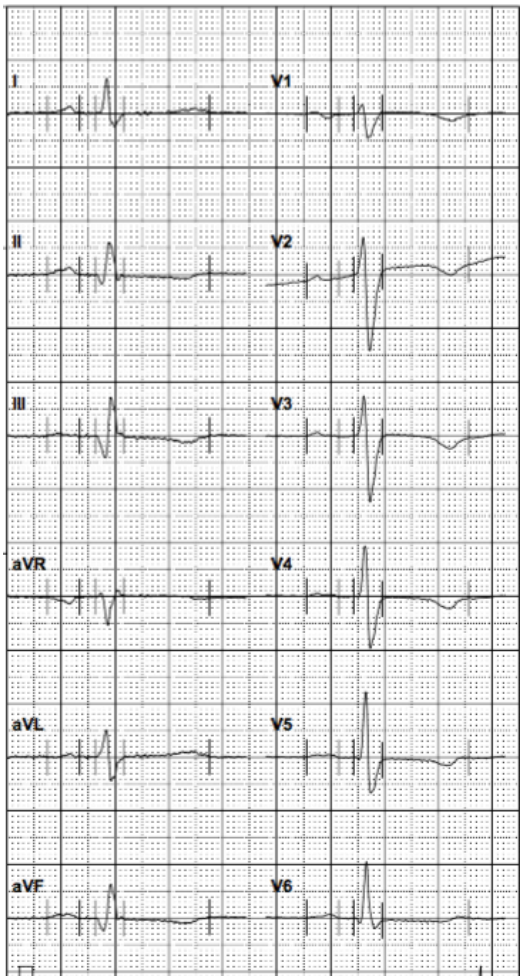

Figure 2a. Inferior infarction (Q-waves in Lead II, III, avF), unrecognized case.

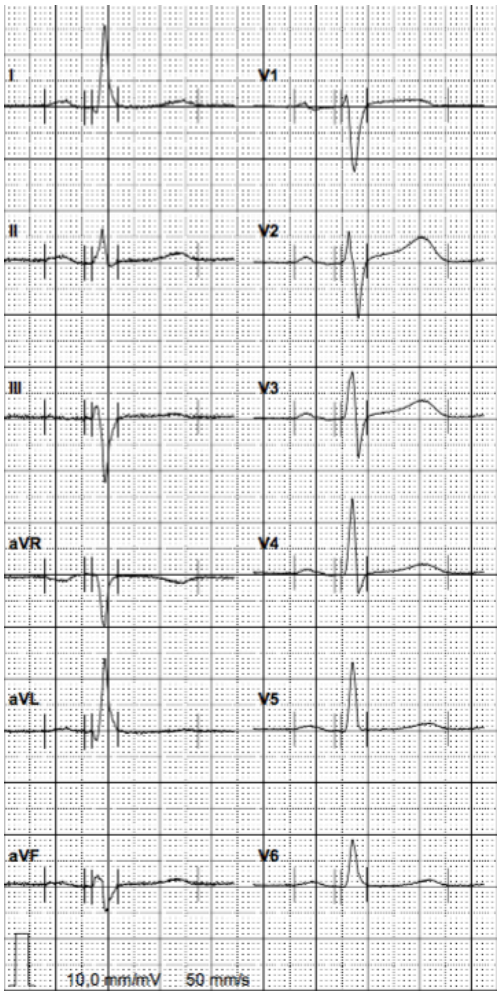

Figure 2b. ECG with no prior infarction.

5.1.4 Unrecognized and recognized MI

We defined participants with unrecognized MI as those with findings of prior MI on the ECG in Tromsø 6 without any clinical event in the MI registry or self-reported MI for paper II. All participants with recognized MI in the MI registry and those with self-reported MI were

excluded. For paper I and III we included those with MI registry diagnosis of silent MI as part of the unrecognized MI group (diagnosed incidentally, or during work-up for symptoms such as dyspnoea, tiredness and swollen ankles, with no history of clinical event combined with findings consistent with previous MI on ECG, echocardiography, or radionuclide angiography). We defined participants with recognized MI as those with a recognized MI in the MI registry for paper I and III. Participants with no MI were excluded.

5.2 Paper I - Pain tolerance in persons with recognized and unrecognized myocardial infarction

5.2.1 Sample

The first visit included testing of pain tolerance with the cold pressor test. The final sample for paper I consisted of 4,849 participants who had undergone the cold pressor test in the first visit and had valid ECGs from the second visit. 4,235 had no MI, 387 had unrecognized MI and 227 had recognized MI. Of the unrecognized MIs, 366 were detected by ECG only and 21 had a diagnosis of silent MI in the MI registry. Participants without MI were excluded from the main analyses.

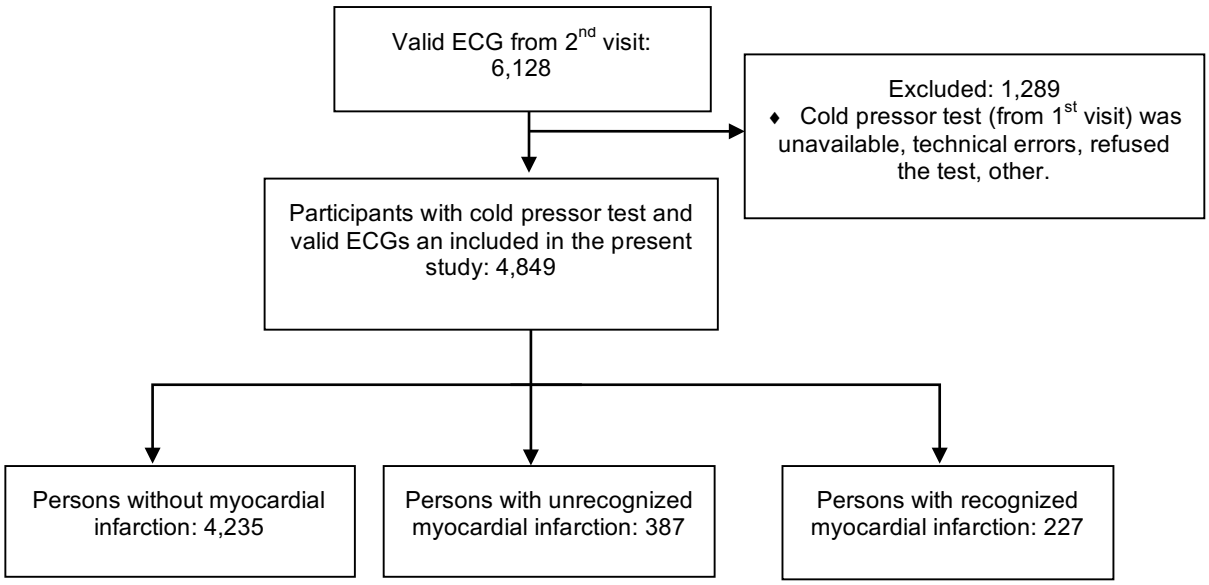

Figure 3: Flow diagram demonstrating inclusion and exclusion of the participants in paper I

5.2.2 Cold pressor pain

The cold pressor test is a common pain assay that has been used in experimental pain research for several decades [66]. The stimulus consists of submerging the hand or foot in circulating cold water and elicits a deep aching pain thought to originate from activation of venous nociceptors [67]. It was historically used as an aid in the diagnosis of angina, and the test is associated with changes in blood pressure and heart rate[68,69]. Participants had the testing procedures verbally explained and were placed in a comfortable chair. They were asked to insert their dominant hand and wrist into a container with circulating cold water at 3°C and a flow rate of 22L/min, and sustain the cold immersion for as long as they could endure, up to a maximum of 106 seconds (s). Cold pressor tolerance was defined as time to withdrawal of the hand from the water.

Figure 4. Woman participating in the cold pressor test. Photo: Stina Grønbech

5.2.3 Statistical analysis

We used Cox proportional hazard model to compare cold pressor tolerance between unrecognized and recognized MI. As we could only study the association between cold pressor pain and MI and not causality, and because time to withdrawal of the hand is right-censored data, we used time to withdrawal as the time to event in the Cox model. Data were right-censored if the participant endured the cold pressor test to the maximum 106 s. In the main analysis, we compared persons with unrecognized MI directly to persons with recognized MI and those with no prior MI was excluded. MI was included as a binary variable (prior recognized MI, prior unrecognized MI). We used participants with recognized

MI as reference group. Hazard ratios (HRs) of aborting the cold pressor test were calculated with 95% confidence intervals (CI). Compared to the reference group, HR<1 indicates higher tolerance, whereas HR> 1 indicates lower tolerance for pain. We examined interactions by adding cross product terms of MI group and each of the potential effect modifying variables to the model. Evaluation of Schoenfeld's residuals and inspection of log-log survival plots did not indicate that the proportional hazards assumption was violated.

5.3 Paper II – Electrocardiographic unrecognized MI does not improve prediction of cardiovascular events beyond traditional risk factors

5.3.1 Sample

For paper II, we excluded 442 participants: 326 with validated recognized MI in the MI registry upon attendance, 97 with self-reported MI and 19 with diagnosed "silent" MI in the endpoint registry at baseline (even though the participant had suffered a silent MI, they were recognized and treated accordingly at a time point before baseline). This left 5,236 with no history of MI and 450 persons with unrecognized MI available for analyses. The total population without recognized MI at baseline was 5,686 participants. Participants were followed prospectively for future recognized MI, stroke and all-cause death until December 31^{st,} 2013 (mean follow-up time 5.5 years). All first-ever cases of these events were reviewed and adjudicated by an independent endpoint committee with medical expertise based on the local hospital records and the Cause of Death Registry for deaths outside the hospital [64].

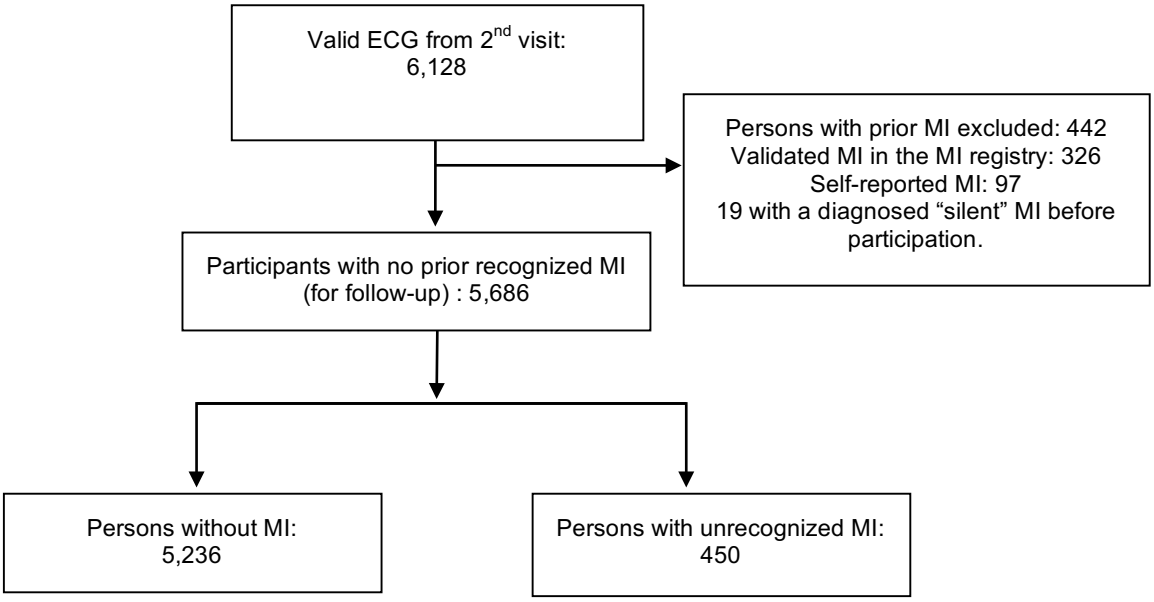

Figure 5. Flow diagram demonstrating inclusion and exclusion of the participants in paper II

5.3.2 Statistical analyses and data management

We used Cox proportional hazard model to examine the association between unrecognized MI on the ECG and future events (MI, stroke, all-cause death, and a composite endpoint of MI, stroke and all-cause death). Hazard ratios (HRs) were calculated with 95% confidence intervals (CI), unadjusted and adjusted for traditional cardiovascular risk factors. We also performed analyses stratified on sex and age >/< 65 years. Evaluation of Schoenfeld residuals and inspection of log-log survival plots did not show violation of the proportional hazards assumption. We calculated Receiver Operating Characteristic curves for future MI using Framingham Risk Score and the Systematic COronary Risk Evaluation (SCORE) [70,71]. We compared area under the curves (AUC) for the standard models to models where we also included unrecognized MI to examine whether addition of unrecognized MI improved prediction of future MI during the follow-up period. We examined interactions by adding cross product terms of unrecognized MI and the potential effect modifying variables to the fully adjusted models.

5.4 Paper III - Small and Large Vessel Disease in Persons with Unrecognized Compared to Recognized MI

5.4.1 Sample

According to the definitions above, the population for the third paper consisted of 5300 participants with no prior MI and 828 participants with prior recognized MI (326) and unrecognized MI (473 detected by ECG and 29 with MI registry diagnosis of silent MI). We excluded 5,300 participants with no prior MI from our main analyses. Of the 828 participants with a prior recognized or unrecognized MI, 17 participants had missing data on carotid ultrasound and 124 participants had missing data on retinal vessel caliber measurements. They were excluded from the analyses of carotid artery pathology and retinal vessel caliber respectively.

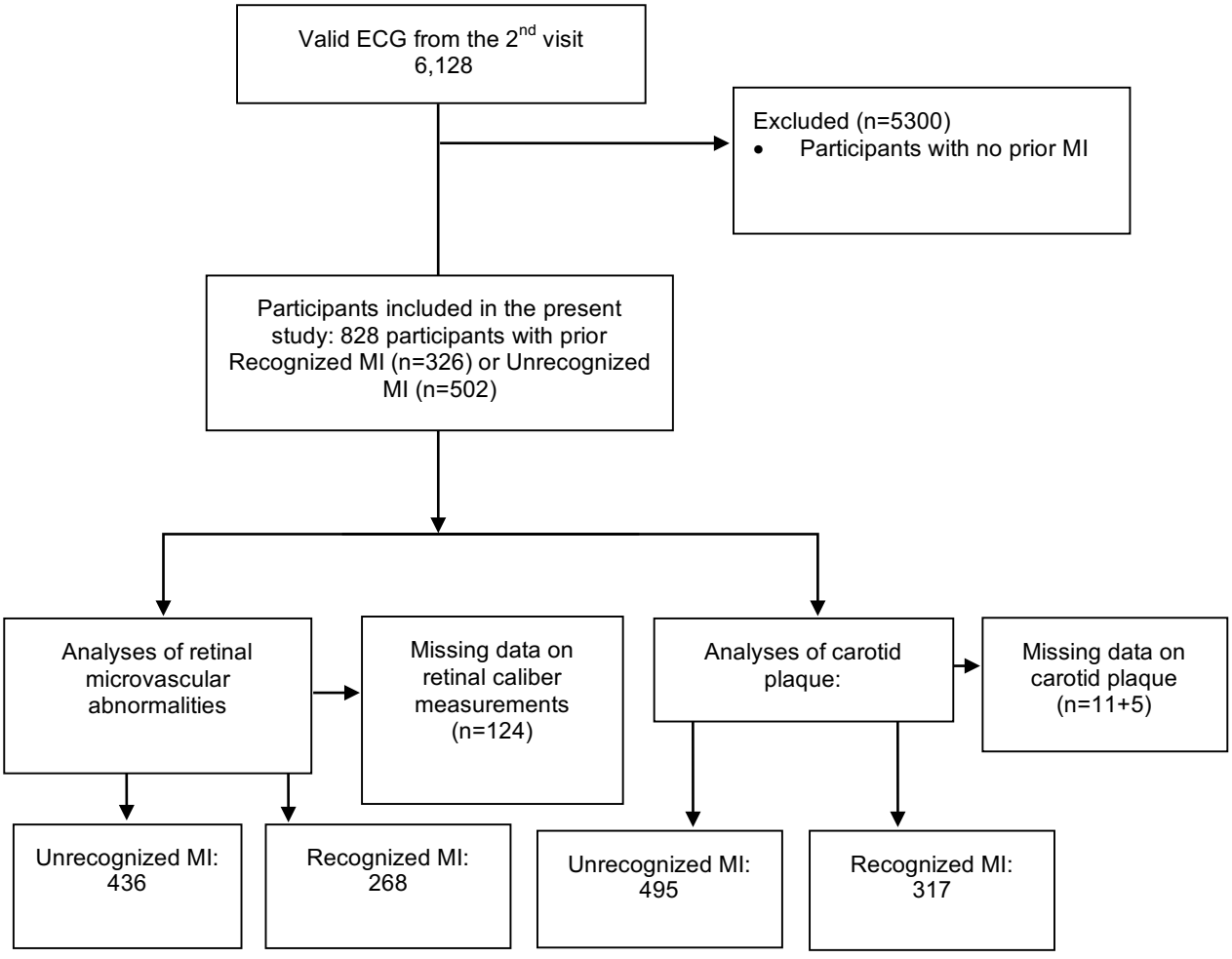

Figure 6. Flow diagram demonstrating inclusion and exclusion of the participants in paper III

5.4.2 Retinal microvascular assessment

We measured retinal arteriolar and venular caliber on retinal photographs. Vessel caliber was measured in one eye, the right eye if eligible, otherwise the left, computer assisted on the disc-centered images with IVAN software. For each image, the vessels coursing through the area of one-half to one disc diameter from the optic disc were measured and the 6 biggest of each vessel type were summarized as the central retinal artery equivalent and the central retinal vein equivalent[72]. Small vessel disease was defined as narrowed retinal arterioles and/or widened retinal venules.

5.4.3 Carotid artery ultrasound

We measured carotid artery plaque area and intima media thickness of the right carotid artery with carotid ultrasound. The right common carotid artery, the bifurcation (bulb) and the internal carotid artery were scanned for the presence of plaque. A plaque was defined as a thickening of the vessel wall of more than 50% compared to the adjacent carotid intima media thickness. The area of each plaque was registered. Total plaque area was calculated as the sum of all plaque areas. Intima–media thickness was measured in the near and far walls of the distal common carotid artery and in the far wall of the carotid bifurcation. The average of the mean from each of the three sites are presented as the mean Intima–media thickness [73]. Large vessel disease was defined as carotid artery pathology (presence of plaque, increased plaque area and increased intima–media thickness).

Figure 7. Carotid ultrasound demonstration. Photo: Stina Grønbech

5.4.4 Statistical analysis

We used logistic regression to study the relationship between MI recognition and retinal vessel caliber, carotid plaque and intima media thickness. The dependent variable was MI recognition, modeled as a binary variable (recognized=0 and unrecognized=1). We modeled retinal vessel caliber as quartiles and as continuous variables, per 10 μm change. We modeled presence of carotid plaque as a dichotomous variable. We also categorized participants with carotid artery plaque into quartiles based on total plaque area. Intima media thickness was modeled as a continuous variable, per 1 mm increase. Analyses were adjusted for age and sex. Multivariable analyses were adjusted for age, sex, hypertension, diabetes, cholesterol and smoking habits. We performed sex stratified analyses and examined interactions by adding cross-product terms of sex and retinal vessel calibers, plaque present, total plaque area and intima media thickness to the multivariable models.

6 SUMMARY OF RESULTS

6.1 General for paper I-III

The prevalence of unrecognized MI was 8.2% (7.9% in paper II, defined as only ECG detected unrecognized MI, participants with MI registry "silent MI" was excluded) and recognized MI was 5.3% of the 6,128 persons with valid ECG from the Tromsø 6 Study in 2007-2008. Women had less unrecognized MI's compared to men (5.6% vs 11.8% $p<0.001$), but a larger proportion of all MI's were unrecognized in women than in men (66% vs 58%, $p<0.01$). Persons with unrecognized MI were significantly younger, less hypertensive, more often never smokers and had less diabetes compared to those with recognized MI, but all factors were worse than for those without MI.

6.2 Paper I - Pain tolerance in persons with recognized and unrecognized myocardial infarction

Participants with unrecognized MI endured the cold pressor test significantly longer than participants with recognized MI (HR for aborting the cold pressor test; 0.64, CI 0.47-0.88), adjusted for age and sex. After adjustment for additional potential confounding factors (mean systolic blood pressure, use of blood pressure lowering drugs, diabetes, daily smoking, psychological distress and physical activity) the association was attenuated and borderline significant, but the direction of the effect was unaltered. The proportion of women who aborted the cold pressor test before the maximum 106 s was larger than in men (38% vs. 23%, $p<0.0001$). The association between lower pain tolerance and unrecognized MI was stronger in women than in men, and statistically significant in women only, but the sex difference was not statistically significant (p for interaction=0.14). There was no statistically significant interaction between groups of MI and any of the effect modifiers.

6.3 Paper II – Electrocardiographic unrecognized MI does not improve prediction of cardiovascular events beyond traditional risk factors

We observed 148 MI's, 229 all-cause deaths and 106 strokes during 31,152 person-years follow-up. Unrecognized MI was associated with increased risk of future recognized MI (HR 1.84 95% CI 1.15-2.96) all-cause mortality (HR 1.78 95% CI 1.21-2.61) in the unadjusted analyses. The associations did not remain significant after adjustment for traditional risk factors (HR 1.25, 95% CI: 0.76-2.06 and HR 1.38, 95% CI: 0.93-2.05 for MI and all-cause death respectively). Unrecognized MI was not significantly associated with the risk of stroke (unadjusted HR 1.09, 95 %% CI: 0.56-2.17). Addition of unrecognized MI on the ECG did not improve risk prediction for future recognized MI using the Framingham Risk Score (area under the curves 0.68 vs. 0.68, p=0.96) or the European SCORE (area under the curves 0.63 vs. 0.63, p=0.65).

6.4 Paper III - Small and Large Vessel Disease in Persons with Unrecognized Compared to Recognized MI

Compared to recognized MI, unrecognized MI was associated with small vessel disease indicated by narrower retinal arterioles (OR for narrower arterioles in unrecognized MI 1.66, 95% CI 1.05-2.62, highest vs. lowest quartile). Unrecognized MI was less associated with wider retinal venules (OR for wider venules in unrecognized MI 0.55, 95% CI 0.35-0.87, lowest vs. highest quartile). Compared to recognized MI, unrecognized MI was less associated with large vessel disease indicated by presence of plaque in the carotid artery, increasing total plaque area or increasing intima media thickness (ORs for presence of carotid artery pathology in unrecognized MI 0.51, 95% CI 0.37-0.69; 0.46, 95% CI 0.26-0.80; 0.37, 95% CI 0.18-0.75, respectively). All analysis were adjusted for age and sex. We did not find any statistically significant sex differences in the association between MI recognition and small and/or large vessel disease.

7 DISCUSSION

7.1 Methodological considerations

7.1.1 Study design

Paper I & III used a cross-sectional design, where data collected from the population was analyzed at a single point in time. This design precludes causal inference because the temporal order of exposure and outcome is difficult to establish. The results from cross-sectional studies are suitable for establishing associations and lay foundations for hypothesis and a more complex investigation. Paper II used a longitudinal cohort design. Participants were followed prospectively from the date of enrolment in the study, and the design allowed for assessment of multiple outcomes during the study period. The temporal sequence of exposure and onset of disease excluded the possibility of temporal bias (disease being the determinant of the exposure).

7.1.2 ECG as detection method of unrecognized MI

ECG as detection method for unrecognized MI has important limitations. Although cardiac MRI is considered superior to ECG in the detection of unrecognized MI, we believe ECG is of interest in the assessment of unrecognized MI due to its lower cost and widespread availability. Also, accidental discovery of unrecognized MI by ECG is relatively common, and more knowledge in the field is needed for clinicians to make well-founded decisions about their patients.

Q waves on the ECG may be caused by other conditions than myocardial infarction (myocardial fibrosis, cardiomyopathy, left anterior hemiblock e.g.), but the specificity of ECG to detect prior MI have been reported to high, in the range of 76%-97%[18,74-76]. Some studies suggest a lower specificity, especially in inferior leads[77]. We excluded 26 ECGs with Q waves due to altered conduction (e.g. left bundle branch block and Wolff-Parkinson-White syndrome) or ventricular hypertrophy, but false positive ECGs cannot be excluded.

The sensitivity of ECG as detection method of MI is low, estimated to lie in the range of 21-58%[11,18,74,76], and accordingly many unrecognized MI's in our study was probably not detected. These include MI's where Q waves disappeared over time[78] and unrecognized non-

Q wave MIs[21]. We used the Third universal definition of MI, whereas other studies have used the Minnesota code[79] or Novacode[80] as basis for the classification. As the classification systems differ, a direct comparison of results is difficult. The Third universal definition includes smaller Q waves than the Minnesota or Novacode, which most likely have led to a more sensitive and less specific detection of unrecognized MI compared to other population studies. This may partly explain a prevalence of unrecognized MI in our study of 8%, while other studies report a prevalence of 0.2-%-7.6% [3,13,17,18,81,82]. However, a recently published study reports that small- and large Q-wave unrecognized MI are confirmed by imaging techniques with comparable frequencies [82]. This finding indicate that the specificity might largely be unaffected by inclusion of smaller Q-waves. Also, the specificity of ECG as detection method was shown to decrease when only large MI's were considered (and small MI's excluded)[74].

During the work with my thesis, a study from the Lifeline cohort in the Netherlands was published[83]. The Third Universal definition of MI was used to define unrecognized MI's by ECG also in this study, but they reported an overall prevalence of 0.3% versus our result of approximately 8%. We believe the large discrepancy between this study and our study may be due to a much younger population in the Lifeline cohort compared to our study (the Lifeline cohort included persons from 18 years of age, whereas our sample consisted of middle-aged and elderly persons). Also, the true prevalence of unrecognized MI may be higher in our study population, as the incidence of cardiovascular disease in North Norway has been among the highest in Europe[84] and the Norwegian population see their doctor less frequent compared to the rest of the European population[85].

If the sensitivity is low and the specificity is high, one would expect the prevalence to be underestimated but most identified MI's to be true cases. However, the estimations of sensitivity and specificity of ECG in detection of MI are based on prior recognized MI's and it is not clear whether ECG has similar sensitivity and specificity for unrecognized MI. A recent imaging study of 936 persons from the ICELAND study reported that myocardial scarring was found in only 42% of ECG diagnosed unrecognized MI's[15]. The high rate of apparently false positive ECGs indicates a lower specificity for unrecognized MI than reported for recognized MI (76-97%). However, this study was done in older adults and a large proportion was selected persons with diabetes (28%), and ECG may not perform with same test characteristics in this group as in the general population.

It may be that some MI's registered on ECG, but not on magnetic resonance imaging, are still true MI's. The extent of this has to my knowledge not been studied. Over the last decade, several studies have questioned the traditional notion that Q-waves are indicative of transmural MI.[21,86,87]. Electrocardiographically determined unrecognized MI manifest less structural abnormalities and regional wall motion abnormalities on echocardiography compared to recognized MI[33,88]. It may be that smaller, more diffuse scarring of the myocardium may alter the electrical conduction and give rise to Q-wave pattern on ECG. Imaging techniques such as cardiac magnetic resonance have developed methods of MI detection based on patterns of recognized MI. Myocardial scarring is usually considered sign of MI if there is endocardial involvement and if it follows the coronary artery distribution[12]. Other scar patterns are usually considered atypical for MI and not deemed as MI. The necrotic myocytes in unrecognized MI may be distributed more diffusely over a larger area and accordingly not detected[89]. With regards to diagnosing myocardial fibrosis, which is also myocardial scarring, cardiac magnetic resonance is considered the non-invasive gold standard. However, the correlation between MRI and histological in quantifying fibrosis, especially for diffuse myocardial fibrosis, is only considered moderate[90]. Further, the ability to detect structural abnormalities may depend on the strength of the magnetic field. Cardiac magnetic resonance at ultrahigh field strength has better spatial resolution and is able to detect structural abnormalities that are not detectable at conventional clinical field strengths [91].

7.1.3 Internal and external validity of the studies

The concept of validity can be divided into two main categories: internal and external validity. Internal validity implies validity of inference of the findings in the study to the source population [92]. This requires a correct sampling and accurate measurement of effects apart from random variation. There are three major violations that can threaten internal validity: selection bias, information bias and confounding[93]. Selection bias occurs when there is a systematic error in recruitment of study population. Information bias concerns errors in measurement of exposure and/or disease in study subjects. Confounding occurs when an association between exposure and disease is observed because of extraneous variables that are associated with both the exposure and outcome variable. External validity pertains to the generalizability of the findings to the background population or other populations, and is also dependent on the study's internal validity.[92]

7.1.3.1 Selection bias

Selection bias is a potential threat to the validity of population based observational studies. If the relation between exposure and outcome in persons who attend the study differs from the rest of the eligible population this will result in selection bias. As estimates of effect are conditioned on participations, it will distort the measured association between exposure and outcome[92].

Tromsø study population

The selection criteria and the relatively high attendance rate are factors that contribute to control selection bias in the Tromsø Study. However, a participation rate of 66% in Tromsø 6 indicates that selection bias most likely is present to some extent. The participation rate was lowest in the youngest and oldest age groups, and lower in men in all age groups. The educational level of the participants was higher compared to the general Norwegian population. Also, the participation rate was lower in first time invited compared to participants who had participated in previous surveys[73]. Participants of the Tromsø Study may represent a more compulsory, health-concerned, and healthier group compared to non - participants. As participation required physical attendance at study site, it is possible that selection bias have occurred due to lower attendance rates in ill or disabled persons. On the other side, the study may also attract persons who are already aware of their increased cardiovascular risk e.g. (hypertension, hypercholesterolemia, family history of MI e.g.) and who consider the survey an opportunity for an extensive medical examination. The Norwegian data protection authority does not allow detailed analyses of mortality or morbidity of persons that did not give consent, thus a comparison of attendees and non-attendees is not possible. However, analyses from previous surveys of the Tromsø Study demonstrates a lower mortality among consistent participants of the study compared to one time participants[64]. This may indicate that those who participate are healthier or represent a cohort bias; attendance may shift behavior towards a heathier direction.

Survival bias

There is an inherent survival bias in this study that must be acknowledged. Participants have survived the index infarction and lived to the time of study entry. The risk of dying of a myocardial infarction is highest the first 6 months [16], but most of the subjects with a myocardial infarction (both unrecognized and recognized) in the study had emerged the high-

risk period at study entry. Also, it is possible that those with a recognized infarction have a higher short-term mortality than those with an unrecognized infarction, and that a larger proportion of those with unrecognized MI lived to study entry compared to recognized MI. This may have affected the findings of paper I and III where we compared participants with unrecognized and recognized MI. However, the presence of survival bias does not diminish the relevance of the data because it is the survivors of myocardial infarction who are encountered clinically.

Missing data and loss of follow-up

A total of 7,306 (91.8%) attended the second visit of Tromsø 6, of whom 1,107 did not have their ECG recorded. This was largely due to capacity problems during peak hours, technical problems or other logistic reasons. There was no statistically significantly differences between those who did and did not have their ECG recorded with regards to age (p= 0.9), sex (p= 0.8), systolic blood pressure (p=0.8), smoking habits (p=0.3) or diabetes (p=0.8), but those without ECG had lower total serum cholesterol ($p<0.05$).

For paper I, an additional 1350 were excluded because they were not examined with the cold pressor test, also mostly due to logistic challenges. Excluded participants were older (66 versus 63 years), more often women (60% versus 55%), hypertensive (64% versus 58%), and above the cut off of 1.85 for the HSCL score for psychological distress (14% versus 11%). This may partly be because technicians were asked to prioritize participants <60 years due to the lower sampling rate in the younger age groups. Proportionally more women with increased risk factor level were excluded from analyses- this may have attenuated the study's ability to detect sex differences. The main results of paper I indicate that the ability to detect differences in pain tolerance between persons with unrecognized and recognized MI, and possibly sex differences, were attenuated because of these differences. We do not have reason to believe that the differences have led to erroneous conclusions.

Of the 828 with prior MI included in paper III, 124 persons had missing data on retinal vessel caliber measurements. There were no statistically significant differences between those who had missing vs. no missing data on retinal measurements with regards to sex (p=0.4), smoking habits (p=0.08), diabetes (p=09) and total serum cholesterol (p=0.12), but they were older (69.6 vs 66.4, $p<0.1$) and had higher systolic blood pressure (146.8 vs 142.5, p= 0.03). Only

16 persons had missing data on carotid ultrasound measurements. We do not have specific reason to assume that this has caused bias.

For paper II, only 19 of the 5,705 participants at baseline were lost to follow-up due to emigration. This was handled by censoring as it was assumed that emigrated persons have the same risk of end-point event (during their limited participation time) as persons remaining in the study.

7.1.3.2 Information bias and misclassification

Information bias may occur due to error in the collection of information on exposure and outcome. It can result from imperfect definition of study variables or flawed data collection procedures. These errors may lead to misclassification of exposure and/or outcome for a significant proportion of the study population [93]. Misclassification due to information bias may distort the magnitude of a risk estimate. If the misclassification rate differs between the study groups, it is called differential, and the effect on the risk estimate is difficult to predict[92]. If the rate of misclassification is equal across study groups, it is called nondifferential, and will usually lead to an attenuation of the risk estimate. If the true RR or OR is close to 1.0, a nondifferential misclassification may mask the association. Both failing to demonstrate an actual association or infer an association where there is none may have serious public health implications. Exposure and outcome variables in all papers included in this thesis have potential sources of misclassification. Assessment of MI status, cold pressor pain and small and large vessel disease are important sources.

Classification of unrecognized and recognized MI

The limited specificity and sensitivity of ECG as detection method for unrecognized MI is a source of misclassification, as discussed in the section *ECG as detection method for unrecognized MI*. The limited sensitivity will have led to false negative unrecognized MI's, and the limited specificity is a potential source of false positives. This is likely to have diluted our findings. For paper I & III, unrecognized MI is compared directly to recognized MI. The classification of recognized MI is based on criteria (symptoms, ECG, cardiac biomarkers) that combined give a relatively higher specificity and sensitivity[94] for detection of recognized MI compared to ECG as detection method of unrecognized MI. There is probably a larger proportion of non-Q-wave MI's among participants with recognized compared to unrecognized MI. There is more Q-wave development in ST elevation compared to non-ST-

elevation MI's (49.7% vs. 9.6%)[95], but there are no strong reasons to believe that the outcomes for paper I and III differ between Q- and non-Q-wave recognized MI's and have affected our results. Markers of large vessel disease were found to be equally distributed between the groups[96]. With regards to differences in microvascular dysfunction and non-ST and ST-elevation MI's, evidence is sparse and contradictory[97,98]. For paper I, there is no reason to believe that pain tolerance measured subsequently to the MI is affected by the Q-wave status. For paper I and III we did not include persons with self-reported MI in the recognized MI group. Most of these were excluded as part of the No MI group, as we preferred the more rigorous validation through the endpoint registry. However, a proportion of recognized MI's might have been misclassified as unrecognized MI; if a participant suffered a recognized Q-wave MI outside of the hospital area of Tromsø University Hospital and did not report having had a MI on the questionnaire. This too may have diluted our findings. For paper II, participants with self- reported recognized MI were excluded from analysis together with validated recognized MI's.

Cold pressor pain

The cold pressor test is an experimental pain assessment method that is potentially vulnerable for technical and procedural errors. The instructions to participants, technical procedures and documentation of results were standardized to reduce the probability of error. Technicians were blinded to MI status information on the research question was not available. The reliability of pain assays within the same session is found to be high[99]. One PhD thesis based on data from Tromsø 6 describes a high test-retest reproducibility for the cold pressor test (Chronbach'a alpha >0.8) with approximately 3 months between test and retest[100], which indicates high instrument reliability. Data with longer follow up on the reproducibility of the cold pressor test is lacking. It is also unknown whether pain tolerance is a stable characteristic in the individual over time or whether it is fluctuating through life or is affected by unknown factors.

Retinal caliber measurement

The IVAN method and software used for measurement of retinal vascular caliber is considered a method with high reproducibility[101,102]. Graders were blinded to any information on the participants. Intra- and intergrader reliability in Tromsø 6 was evaluated in 5% of the images, randomly selected. Reliability testing yielded intraclass correlation coefficient (ICC)

of >0.95 for intragrader reliability and >0.93 for intergrader reliability[103], which indicates satisfactory classification of central retinal artery and vein equivalent in Tromsø 6.

Carotid artery ultrasound

Ultrasound measurements of carotid artery pathology are potential sources of misclassification in paper III. Only the right carotid artery was examined. Inclusion of left carotid artery atherosclerosis would have yielded a better estimate of the true atherosclerotic burden in the large vessels. One recent population-based study found that a large majority have bilateral carotid plaques, although left sided unilateral plaque was more prevalent than unilateral right sided (67% vs 34%, p<0.001) and left sided plaque was thicker[104]. Although 85% had bilateral plaque, this may have caused an attenuation of our results (unrecognized MI may be even less associated with large vessel disease compared to recognized MI). The examinations were performed by trained sonographers, and the procedures and documentation of results followed were standardized to minimize errors. A reproducibility study was conducted in 76 subjects, of whom 71 was rescanned 1-2 weeks later[105] . This yielded kappa values for plaque detection of 0.65 in the intergrader study and 0.65 in the intragrader study. Both values are considered to represent "substantial agreement"[106].

Nondifferetial misclassification

We have no reason to assume that misclassification of pain tolerance or small and large vessel disease is dependent on MI status or the other way around, and should hence be considered nondifferential. For paper II, misclassification of unrecognized MI (as exposure variable) was not dependent on the outcome variables under study (stroke, death and recognized MI in the endpoint registry). The diagnosis of unrecognized MI was unknown to the assessors of outcomes in endpoint registry and could not have affected the endpoint classification. The misclassifications in paper II should also be considered nondifferential.

7.1.3.3 Confounding

Confounding is an important issue in observational studies. Confounding happens when an association (noncausal) between an exposure and the outcome is observed as a result of a third variable [93]. A confounding variable is an exposure variable, other than the one under study, that may affect the outcome. This may lead to erroneous conclusions about the relationship between the exposure and the outcome: it may induce, strengthen, weaken or

eliminate the association by a third variable or groups of variables. There are 3 classical criteria that should be met[93]:

1. The confounding variable is causally associated with the outcome, and
2. Noncausally or causally associated with the exposure, but
3. Is not an intermediate variable in the casual pathway between exposure and outcome.

Due to a wealth of genetic and environmental factors, no individual is exactly like the other. This means that when we compare individuals that differ in a given exposure, the individuals (or groups of individuals) will also differ in other exposure variables (potential confounding variables) that we may or may not be aware of. Statistical techniques are available to control for confounding, where the basic idea is to estimate the association between exposure and outcome given a constant level of the confounding variable[93]. Potential confounders are then included as covariates in multivariable regression models, and the output will be the adjusted or corrected estimates of the "pure" effect of the exposure on the outcome.

Potential confounders

In paper I and III we examined associations that has never been studied before. Potential confounders were selected on the basis on extensive literature search. For the association pain tolerance and MI recognition in paper I, we chose age, diabetes, sex, hypertension, depression, anxiety, physical activity, and smoking as potential confounding variables. Age, diabetes, hypertension and female sex have been reported to be associated with risk of unrecognized MI[9,12,14], and these variables are also associated with pain perception[107-109]. Depression and anxiety was reported to be differently associated with unrecognized and recognized MI[110], and is associated with increased risk of pain disorders[111]. Physical activity is protective of coronary heart disease[112], whereas smoking is an established cardiovascular risk factor, and they are both linked to pain sensitivity [113,114]. For the association between MI recognition and small and large vessel disease we selected age, sex, hypertension, diabetes, cholesterol and smoking as potential confounders. Age[115,116], diabetes[117] and hypertension[118,119] are associated with small and large vessel disease. Cholesterol is associated with increased risk of unrecognized and recognized MI[12] as well as small[120] and large[44] vessel disease. Smoking is associated with pain sensitivity and paper I demonstrated an association with MI recognition, and smoking is also associated with small[121] and large[119]

vessel disease. In paper II we assessed the risk for adverse cardiovascular events in persons with unrecognized MI compared to persons with no MI, and we adjusted for the established cardiovascular risk factors. Nevertheless, there may still be significant residual confounding present due to unidentified confounders or imprecise definition of confounders in all papers.

Temporal issue

During the work with the cross-sectional studies (paper I and III), we identified a temporal issue concerning the collection of outcomes and potential confounders. Upon attendance in the study participants were identified as unrecognized or recognized MI based on criteria previously described, hence the MI had happened at a time point before Tromsø 6. This means that potential confounders were measured subsequently, and it is highly likely that values of diabetes, hypertension, smoking and cholesterol were affected differently in persons with unrecognized and recognized MI. Because of this temporal issue, we decided that the unadjusted and age and sex adjusted effect estimates were most appropriate to present as our main results. We encourage that the multivariable adjusted results should be interpreted with caution. However, none of the potential confounders stood out as important confounders for the associations under study in paper I and III. The effect estimates did not differ much between the age and sex adjusted/unadjusted models and the multivariable adjusted models.

7.1.3.4 External validity

External validity is the extent to which the results of the study are generalizable to the source population or other populations. The population of Tromsø was the source of invitations, and the age and sex distribution in the Tromsø study reflects the general adult population of Tromsø[73]. The Tromsø Study is based in the seventh largest Norwegian city and population consist of predominantly Caucasians of Norwegian origin. Tromsø may be considered representative of a Northern European white, urban pupulation[73]. With regards to considerations of the internal validity of the study, we believe that the findings of our study will be applicable to other northern European, urban populations.

7.2 Discussion of main results

7.2.1 Pain tolerance

The findings of paper I indicate that increased pain tolerance may partly explain the lack of symptoms associated with unrecognized MI. Pain tolerance in persons with unrecognized and recognized MI has previously not been studied. In agreement with our findings, however, are two studies indicating that increased tolerance for pain is associated with less symptoms during an acute MI[28,122]. Also, experimental studies have shown that persons with silent myocardial ischemia have an attenuated pain response[123-125]. We speculate that the reason for our finding is that myocardial ischemic pain is modulated thorough the same processes as other pain modalities, such as cold pressor pain. There is some evidence that this is mostly due to differences in central processing of pain signal. Activation of the thalamus was registered by positron emission tomography during myocardial ischemia in both persons that present with angina and silent ischemia[126,127]. For the conscious sensation of pain (in angina), activation of the frontal cortex in addition was necessary, which was not seen in silent ischemia. The thalamus act as a gate to incoming pain stimulus, and in some persons the stimulus may have to be more intense or longstanding to pass the thalamus and reach the frontal cortex[127]. Comparison of peripheral nerve conduction in patients with angina and silent myocardial ischemia showed no significant difference[124], which supports the assumption that central modulation of pain is most important. Interestingly, this do not support the common notion that unrecognized MI is more common in patients with diabetes due to peripheral diabetic neuropathy. Symptom severity may also be influenced by the size of the MI. The myocardial scarring was reported to be smaller compared to recognized MI's [11]. However, other studies indicate no association between symptom severity and MI size[27,122]. We did not have the opportunity to study the association between MI size and MI recognition in our data. The doctors' and patients' perception of cardiovascular risk may also influence the perception of pain and symptoms, and it probably affects the likelihood of a correct diagnosis. Women do not relate chest pain to heart disease nearly as often as men[128,129]. Also, public information and medical training of health personnel focuses on recognition of male pattern symptoms, leaving women at greater risk of not having their heart disease recognized.

We found that the association between unrecognized MI (compared to recognized MI) and lower pain tolerance was stronger in women. In stratified analyses, the effect estimate was

significant in women only. Experimental pain studies most often show that women tolerate less pain[108,130], and it may then seem contradictory that women have a larger proportion of unrecognized MI's compared to men. As we have compared pain tolerance in persons with unrecognized MI relative to recognized MI, the stronger association in women may be because it is women with low pain tolerance that presents with the most severe symptoms that are most likely to have their MI recognized. It may also be that MI's of smaller size and more diffuse character are more prevalent in women and that they, because of that, present with less symptoms and are more often unrecognized.

One limitation in this paper is that we did not have data on symptoms. This limitation pertains to all population-based epidemiological studies of unrecognized MI, as it is difficult to retrospectively assess whether a person had symptoms related to the unrecognized MI. In our study, as in previous studies, we defined unrecognized MI as electrocardiographic evidence of MI without a history of hospital admission for MI. We have, as previous reports on the topic, thought of this as evidence of less symptomatic and less painful MI. That unrecognized MI is often painless or less painful is supported by hospital based case-series[131].

The most likely explanation for the lack of recognition of the MI is a combination of multiple factors; personal characteristics (gender, denial, cultural surroundings, genetics, sex), the severity of the infarction and the persons' surroundings at the time of event e.g. In addition, our results indicate that increased tolerance for pain may partly explain the lack of symptoms. Unrecognized MI is an entity that needs further mapping, and this study opens to the possibility that differences other than strictly cardiovascular factors is of interest, and factors associated with pain tolerance should be included in future studies of unrecognized MI. Our findings need validation in study with a prospective cohort design, as cross sectional studies preclude causal inference and is vulnerable to temporal bias.

7.2.2 Prognosis

Paper II foud that unrecognized MI was associated with increased risk of future MI and all-cause death in the general population. The associations were not significant after adjustment for age, sex, hypertension, cholesterol, diabetes, smoking and family history of premature cardiovascular disease. Although the adjusted risk estimate for all-cause mortality did not reach statistical significance in our study, the confidence interval (HR 1.38, 95% CI: 0.93-2.05) suggests that an independent risk may be present. However, our data indicate that the

increased risk of future MI and all-cause mortality associated with unrecognized MI is largely explained by traditional cardiovascular risk factors. Addition of unrecognized MI to established risk scores did not improve prediction of future MI during the follow-up period.

Most previous studies of prognosis associated with unrecognized MI were not done in the general population, but in elderly, men only, women only, persons with known or suspected ischemic heart disease or diabetes. However, four population based cohorts have studied the assoociaton of electrocardiographic unrecognized MI with future adverse events in samples of the general population. The ARIC Study[81] from 1987-89 found an almost identical risk of all-cause mortality associated with unrecognized MI as in our study adjusted for the cardiovascular risk factors (HR 1.34 95% CI 1.09-1.65), although they included persons aged 45-64 only. The Rotterdam Study from 1990-93[5] reported that unrecognized MI was associated with increased risk of all-cause mortality in (HR 1.33, 95% CI 1.11-1.58) and men (HR 1.57, 95% CI 1.30-1.89), adjusted for the cardiovascular risk factors. Both studies had longer follow-up and more events compared to our study, this may explain the statistically significant multivariable adjusted risk estimates compared to our results. Also, the ARIC population had a different ethnic composition. In contrast to the two studies, we conducted our study more recently and in the era of widely available angiography and widespread statin therapy, which may have contributed to a lower risk of death and MI in our study. The Copenhagen City Heart Study from 2001[24] reported an increased risk of death or hospitalization for coronary heart disease (HR 2.8, 95% CI 1.6-5.0), adjusted for age, hypertension, glomerular filtration rate and diabetes only. They had a different endpoint and did not adjust for all the risk factors. Mid 2017, a study from the Lifeline Cohort Study [83] reported an independent risk for mortality associated with unrecognized MI (OR 2.21 95% CI 1.12-4.37), adjusted for age, sex, diabetes and heart rate. Unrecognized MI was reported to be a stronger predictor for mortality in persons < 65 years, whereas we found that unrecognized MI was a stronger predictor for future recognized MI in women < 65 years. This may partly explain the independent mortality risk compared to our findings as they studied a younger population.

It has been suggested that the similar prognosis associated with unrecognized and recognized MI is due to the extent of myocardial damage and diminished cardiac function[132]. On the other hand, studies have shown that unrecognized MI is an intermediate between no prior MI and recognized MI when it comes to the association with traditional risk factors, coronary

calcium [15] and structural abnormalities,[33,37]. The degree of myocardial damage cannot fully account for the similar poor prognosis.

The lack of secondary preventive measures and less aggressive treatment of hypertension and cholesterol e.g. in persons with unrecognized MI is probably part of the reason for the prognosis. However, knowledge in the field of unrecognized MI is lacking. As described above, and elaborated on in the section on differences in underlying pathophysiology, there are important differences between unrecognized and recognized MI. We therefore consider it premature to recommend similar post infarction treatment as with recognized MI, such as beta-blockers and anti-platelet therapy e.g., the risk-benefit ratio is still uncertain. Our findings, support the notion that discovery of unrecognized MI should lead to careful work-up of cardiovascular risk factors. Treatment of these in line with guidelines for established cardiovascular disease[133] should be considered.

7.2.2.1 Sex differences

Some previous studies have found that unrecognized MI confers a lower risk of death and cardiovascular events for women compared to men [5,8,134]. In contrast to this, we found that the highest risk of future MI was in women < 65 years, and the associations remained significant in this group only, which implies an independent unexplained increased risk in this group. Prognosis did not differ significantly with sex and age group (>/< 65 years, p for interaction <0.1), and the number of events was low, so it is possible that the results of the age-and sex stratified analyses were due to chance because of multiple comparisons. The finding is, however, in line with the ARIC study[135] reporting a higher risk for both cardiovascular associated – and all-cause death associated with unrecognized MI in women. For women, a large proportion of MI's occur in the absence of these risk factors[136], and many women with risk factors do not experience adverse cardiovascular events[137]. There is continous work to develop risk scores specific for women[138,139], as existing risk scores are developed based on data on male cohorts and often misclassify women[62,63]. Although addition of unrecognized MI did not improve prediction of MI in women in our study, the potential should be further investigated in larger studies with longer follow-up.

7.2.3 Vessel disease and MI recognition

Paper III found that unrecognized MI was more associated with small vessel disease, and less associated with large vessel disease compared to recognized MI. To our knowledge this was the first study to examine the relationship between small and large vessel disease and MI recognition.

Narrower retinal arterioles and wider retinal venules are considered unfavourable in terms of cardiovascular disease[55]. A little surprising, we found that the two measures of small vessel disease were differently associated with MI recognition. Narrower retinal arterioles were more associated with unrecognized MI whereas wider retinal venules were not. An explanation for this may be that it is narrower retinal arterioles, and not wider retinal venules, that is associated with myocardial small vessel disease. The Multi-Ethnic Study of Atherosclerosis found that retinal arteriolar narrowing, but not venular widening, was associated with reduced myocardial perfusion in persons without recognized cardiovascular disease[40]. On the other hand, a previous study found that venular widening is associated with many markers of large vessel disease, including higher carotid plaque score, more aortic calcifications, lower ankle-arm index and higher total serum cholesterol[140], whereas narrower arterioles was associated only with increased carotid intima thickness. We speculate that wider venules are associated with increased cardiovascular risk through other pathophysiological mechanisms, and may be more associated with classical epicardial coronary disease.

We have compared the presence of small and large vessel disease in persons with unrecognized MI relative to recognized MI. This suggests that the relative burden of small vessel disease compared to large vessel disease is larger in unrecognized MI. It does not, however, exclude the simultaneous presence of large vessel disease. A couple of cardiac magnetic resonance studies of atherosclerotic burden in persons with unrecognized and recognized MI have been conducted, but they report conflicting results. A study by Barbier et.al. found that unrecognized MI was not associated with significant atherosclerosis in the rest of the body, whereas recognized MI was[35]. The authors speculated that unrecognized MI might not be associated with atherosclerosis. One study published by Hammar et.al. after the work with this thesis started, showed an association between unrecognized MI and presence of stenotic coronary artery disease in the relevant supplying coronary artery[141]. This demonstrates the presence of large vessel disease in unrecognized MI. Interestingly, prognosis

associated with unrecognized MI was similar in those with and without significant stenosis[142], which may imply the presence of underlying small vessel pathology in addition to epicardial coronary artery (large vessel) disease. On the other side, there is also some evidence that recognized MI is associated with small vessel disease. Narrower retinal arterioles and wider retinal venules were reported to be predictive of recognized coronary heart disease[143-145], although consistent in women only[55,143]. A few studies also indicate that small and large vessel disease may affect each other in a negative way. Animal studies have shown that atherosclerosis in the larger epicardial vessels of the heart have negative effects on the coronary microcirculation[146,147]. Also, microvascular dysfunction may affect the atherosclerotic process in the epicardial vessels[148,149]. The apparently contradicting results in the literature regarding vessel disease and MI recognition may reflect an overlapping continuum of underlying vessel disease and symptoms of ischemic heart disease. The importance of small and large vessel disease probably varies across the spectrum of the different presentations. Our findings suggest that small vessel disease could play a larger role compared to large vessel disease in the pathophysiological background of unrecognized MI.

7.2.3.1 Sex differences

We did not find significant sex differences in the relationship recognition and small and/or large vessel disease. A growing body of evidence suggest that small vessel disease may play a larger role in the pathogenesis of ischemic heart disease in women as compared to men[31,55,57]. As the association between arteriolar narrowing and unrecognized MI is stronger compared to recognized MI, and the proportion of unrecognized MI's is larger in women, this study adds to previous reports of microvascular disease most often suggesting a larger burden of microvascular disease in women.

7.2.3.2 A new hypothesis: unrecognized MI as a precursor for recognized MI?

The contradicting results regarding vessel disease and MI recognition discussed above suggest that small vessel disease may precede and/or coexist with large vessel disease. We show that unrecognized MI is related to younger age and intermediate risk factor levels. Also, a recent imaging study found old unrecognized MI in 13% of acute recognized MI patients[150]. This is the foundation for the hypothesis that unrecognized MI may be a detectable precursor of recognized MI. Small vessel disease, which is associated with unrecognized MI, may contribute to the development of large vessel atherosclerosis underlying recognized MI later

in life as discussed above. In addition, subclinical episodes of plaque disruption[151,152] may occasionally lead to unrecognized MI and negatively affect the coronary microcirculation, and over time, contribute to the progression of atherosclerosis into more severe epicardial coronary artery disease. Small vessel disease was initially thought to develop in the aftermath of an acute coronary event, more as a passive bystander[148]. There is an increasing body of evidence supporting coronary microvascular disease as a well-defined condition that has been shown to be both independent of[153] and associated with[147,148] obstructive coronary heart disease. Paper II in this thesis found that unrecognized MI was associated with future MI. Nordenskjold et.al reported that unrecognized MI was associated with adverse outcomes, but not after adjustment for severity of CAD[142]. This coincides with our finding that the poor prognosis associated with unrecognized MI did not remain significant after adjustment for traditional risk factors and may imply that the poor prognosis is mediated through the further progression of atherosclerosis.

The higher proportion of unrecognized MI in women could be due to different trajectories for cardiovascular disease in men and women. Women develop recognized MI at a later age despite similar risk factor levels[154] and women have less carotid plaques at any age than men[155]. In women, an unfavourable retinal microvascular profile confers increased risk of incident MI and is associated with more diffuse and severe coronary artery disease[58]. This, again, suggests a timeline in development of coronary heart disease, with small vessel disease contributing to development of atherosclerosis, especially in women. Our findings of more small vessel disease in unrecognized MI suggest that unrecognized MI may precede recognized MI, which is more associated with large vessel atherosclerosis. This idea is also supported by our finding that the risk of a future MI following an unrecognized MI was strongest in women < 65 years.

8 CONCLUSIONS

Persons with unrecognized MI have increased pain tolerance compared to persons with unrecognized MI. The association between unrecognized MI and increased pain tolerance was stronger in women, but the sex difference was not statistically significant.

In men and women, unrecognized MI was associated with increased risk for cardiovascular events and death, but not independent of cardiovascular risk factors. Unrecognized MI did not add predictive value to risk scores for future MI.

In men and women, unrecognized MI was more associated with small vessel disease and less associated with large vessel disease compared to recognized MI.

9 FUTURE RESEARCH

Unrecognized MI is a hidden public health issue with unknown underlying causes and no guidelines for screening, treatment or follow-up. Although imaging techniques are superior to ECG, most unrecognized MI are detected by ECG and constitute a diagnostic and therapeutic challenge for clinicians. Future research should aim to reach consensus on appropriate follow-up and treatment of these patients. Will patients with an electrocardiographically detected unrecognized MI benefit from further imaging, stress tests or biochemical tests? If not, are there subgroups that may benefit? Should cardiovascular risk factors be treated more intensively in this group compared to the general population, and will they benefit from anti-platelet treatment or beta-blockers? One should also aim to further investigate differences in vessel disease between unrecognized and recognized MI, possibly with more direct measures of small and large vessel disease in the heart. It is now possible to measure coronary microvascular function independent of presence of obstructive coronary heart disease[153], which makes for an ideal situation to assess development and contribution of small and large vessel disease in unrecognized. Also, a study of coronary angiography (frequency and findings) in men and women with unrecognized MI is of interest.

The 7th wave of the Tromsø Study was just completed, and ECGs was registered during this second visit as well. This opens for the possibility to conduct prospective cohort studies on the research questions for paper I and III (pain tolerance and small and large vessel disease). Finally, the potential for enhanced risk prediction for future MI in women with unrecognized MI should be investigated in a study with longer follow-up.

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PAPER I

PAPER II

PAPER III