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# Screening for Atherosclerosis with Magnetic Resonance Imaging and Ultrasound

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### **Abstract**

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Atherosclerosis is a major cause of death and disability worldwide. Although traditional risk factors can identify the healthy or severely affected individuals, sudden lethal outcome is still frequent in those suggested as intermediate in risk for cardiovascular events (CVE). Adding imaging to the traditional scoring systems might improve risk stratification.

This thesis investigates whether the addition of magnetic resonance imaging (MRI) and ultrasound (US) to traditional risk factors might render atherosclerosis suitable for mass screening, selective screening or screening in research settings.

In paper I the carotid arteries were assessed in six different manners (carotid intima media thickness (CIMT) in two different locations, presence of plaque, number of plaques, plaque size and plaque composition) using US. More than 800 Caucasian subjects were assessed at ages 70 and 75, and outcome examined at 80 years of age. Plaques with an area exceeding 10mm<sup>2</sup> in the bulb were found to be most closely related to CVE.

Paper II established that carotid plaque volume measured with MRI did not correlate with carotid plaque area assessed with US. MRI reached the highest levels of reproducibility of the two methods.

Paper III used the previously created total atherosclerotic score (TAS), a scoring system based on whole body magnetic resonance angiography (WBMRA) that assesses global atherosclerosis. TAS was found to predict CVE in 305 PIVUS-subjects at age 70 years during 5 years of follow-up. The risk for CVE was found to be eightfold with TAS>0.

In paper IV CIMT was assessed with US at ages 70 and 75 years. CIMT at baseline, but not the change in CIMT over five years, was significantly related to TAS, thus suggesting carotid changes to correlate with atherosclerosis throughout the body.

In conclusion, in research settings WBMRA and MRI, as well as US, can be used for screening and following up of atherosclerotic changes, as their predictive values and reproducibility are good. US might be feasible in selective screening but none of these methods are as of now suitable for mass screening.

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Declare the past, diagnose the present, and foretell the future.  
- **Hippocrates**

If you can't explain it simply you don't understand it well enough.  
- **Albert Einstein**

*To Anders and Malte.*

The cover depicts a cross stitch embroidery of (from left to right): a cross sectional magnetic resonance image of a carotid artery with a plaque; a magnetic resonance angiography of the arteries in the neck region; and a longitudinal ultrasound image of a carotid artery with a plaque.

# List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I Lundberg C., Ahlström H., Johansson L., Lind L. (2014) A comparison between different indices of carotid artery atherosclerosis at ultrasound regarding risk of incident myocardial infarction and stroke. *Submitted*.
- II Lundberg C., Swärd J., Wanhainen A., Kragsterman B., Li F., Zhao X., Lind L., Ahlström H., Johansson L. (2014) Measuring carotid plaque size in asymptomatic individuals: comparison and reproducibility of MRI and B-mode ultrasound. *In manuscript*.
- III Lundberg C., Johansson L., Ebeling Barbier C., Lind L., Ahlström H., Hansen T. (2013) Total atherosclerotic burden by whole body magnetic resonance angiography predicts major adverse cardiovascular events. *Atherosclerosis*, 228(1):148-152
- IV Lundberg C., Hansen T., Ahlström H., Lind L., Wikström J., Johansson L. (2014) The relationship between carotid intima-media thickness and global atherosclerosis. *Clinical Physiology and Functional Imaging*, 34:457-462

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# Abbreviations

2D	Two Dimensional
3D	Three Dimensional
$\Delta$ CIMT	CIMT over time
AAA	Abdominal Aortic Aneurysm
ABI	Ankle Brachial Index
AMS	Artery Measurement Software
CAC	Coronary Artery Calcification
CCA	Common Carotid Artery
CI	Confidence Interval
CIMT	Carotid Intima Media Thickness
CT	Computed Tomography
CVE	Cardiovascular Events
CVD	Cardiovascular Disease
FRS	Framingham Risk Score
GSM	Gray Scale Median
ICA	Internal Carotid Artery
IMT	Intima Media Thickness
MACE	Major Adverse Cardiovascular Events
MI	Myocardial Infarction
MIP	Maximum Intensity Projection
MRA	Magnetic Resonance Angiography
MRI	Magnetic Resonance Imaging
NWI	Normalized Wall Index
OR	Odds Ratio
PD	Proton Density
PIVUS	Prospective Investigation of the Vasculature in Uppsala Seniors
RF	Radio Frequency
SD	Standard Deviation
SMPA	Swedish Medical Product Agency, Läkemedelsverket
SNBHW	Swedish National Board of Health and Welfare, Socialstyrelsen
SP	Screening Program
T1	Longitudinal Relaxation Time
T2	Transversal Relaxation Time

TAS	Total Atherosclerotic Score
TWV	Total Wall Volume
US	Ultrasound (2D B-mode)
WBMRA	Whole Body Magnetic Resonance Angiography
WHO	World Health Organization

# Introduction

Atherosclerosis is often thought of as a modern condition but has existed through thousands of years.<sup>1</sup> In the western world today it is the number one cause of death.<sup>2</sup>

This slowly progressing inflammatory condition of the arteries starts developing in the youth, but do not give symptoms until decades later in the form of angina pectoris, claudication, strokes or myocardial infarctions (MI). Because of the severity and frequency, as well as the possibility to halt the progression, it is important to identify those in need of preventive treatment. However, atherosclerosis cannot be cured.

One of the first remaining descriptions of atherosclerosis dates back to the early 16<sup>th</sup> century in Italy when Leonardo da Vinci (1452-1519) compared the vessels, revealed at autopsies, between a hundred year old man and a two-year old child.<sup>3</sup> Nearly 300 years later, in 1799, the British physician Caleb Hillier Parry (1755-1822) first believed that plaster from the ceiling had fallen down into his subject while performing an autopsy on a sheep, but realized that the substance came from within the vessel itself.<sup>4</sup> In 1844 the Danish/Icelandic sculptor and artist Bertel Thorvaldsen (1770-1844) suddenly died in the Royal Theatre of Copenhagen. At his autopsy the very plaque that had ruptured with fatal consequence could be identified for the first time in recorded history.<sup>5</sup> In the mid 19<sup>th</sup> century Rudolf Virchow (1821-1902) made the first statement of atherosclerosis being of inflammatory origin,<sup>6</sup> today the inflammatory element in atherosclerosis is well known and substantiated.

With the advent of radiology, contrast agents for intravascular use, and later on the Seldinger technique of catheter introduction into the vessels (introduced in 1952 by Sven Ivar Seldinger)<sup>7</sup>, the lumenographic examinations expanded, revealing inward growing plaques as narrowing irregularities or even occlusions in the vessels. Since Seymour Glagov introduced the model of positive plaque remodeling (outward growing plaques) in 1987<sup>8</sup>, focus shifted toward imaging of the vessel wall, with all radiological modalities available including functional methods, such as positron emission tomography (PET).

In this thesis focus is put on whole body magnetic resonance angiography (WBMRA) and focal assessment of the carotid vessels, either with magnetic resonance imaging (MRI) or ultrasound (US) imaging, and the abilities of these methods to identify atherosclerosis and predict outcome.

Furthermore, I have chosen to investigate whether screening for atherosclerosis, with the addition of imaging to traditional risk factors, would be possible in different settings; mass screening, opportunistic screening, selective screening or screening in research settings, as the condition has such impact on individuals as well as the health care system. Furthermore methods of identification as well as treatments are well established and continuously refined.

# Background

## Screening

Screening is a process of examining a population in search for individuals with a specific disease or at risk for developing said disease. This can be done in a general population (i.e. mass screening), in a defined population in a clinical setting (i.e. selective screening), or in a research setting. Mass screening can include millions of subjects; defined populations are smaller but can still contain many thousand individuals; whereas research screenings often are restricted to small numbers of subjects (from less than hundred to a few thousands).

In history, and nowadays in less developed areas of the world, screening has been/is often used to identify individuals with a disease in order to treat them and control the spread of the disease, as has been done with pulmonary tuberculosis<sup>9</sup>. With advancing health care systems and epidemics under control, screening has nowadays shifted to becoming a tool used to assess risk for diseases and conditions in an asymptomatic population in order to identify and treat the affected individuals prior to symptoms.

In Sweden we screen in mass settings for a number of diseases, where some screening programs (SP) (such as breast cancer and cervical cancer programs) are in motion throughout the country, and some programs are on trial in only a few communities (e.g. colorectal cancer). Some of the SPs include radiological analyses but as of today only one clinically used screening program focuses on vessels with the use of imaging; the screening for Abdominal Aortic Aneurysms (AAA) in men at the age of 65 years – a program that started in Uppsala 2006 and as of December 2014 is implemented throughout the country.

In research settings atherosclerosis is screened for in numerous epidemiological studies throughout the world with different imaging modalities.<sup>10-14</sup>

Screening should always be linked with a predefined treatment or other preventive measures for it to remain ethically defensible. When a SP discovers pathologies eligible for treatment in an asymptomatic individual the prevention is known as primary as opposed to secondary prevention that aims to prevent further events in an already affected individual.

Primary prevention for atherosclerosis without imaging does exist as “opportunistic screening”, i.e. when patients are screened for signs and symptoms of cardiovascular disease (CVD) when visiting the health care for

whatever reason, and is proven feasible.<sup>15</sup> The Swedish Medical Product Agency (SMPA) estimates that most middle-aged persons see a physician at least once during a two-year period<sup>16</sup>, indicating this set-up as a plausible method.

Selective screening is when individuals found to be at risk due to one or more risk factors are then further screened to try to establish the true risk of outcome.

But is atherosclerotic screening with imaging ready to be launched into real life, i.e. clinical use? Who should be screened, and how often? Does the loss in productive tax-paying citizens really justify the cost of a SP or are we anyway morally obliged to identify all at risk? There are a multitude of questions to answer before setting up a SP.

With SPs it is usually the caregiver who initiates examinations and as the caretaker is without symptoms or suspicion of disease there is need for very high ethical standards. In the evaluation of ethics in medicine four principles, suggested by Beauchamp and Childress in 1979<sup>17</sup>, are often used: respect for autonomy, non-maleficence, beneficence, and justice. Each of these principles should always be fulfilled in any given situation. If two principles contradict each-other one of them must be chosen as the most important.

## The principles of mass screening

In 1968 Wilson and Jungner wrote the “Principles and practice of screening for disease” on behalf of the World Health Organization (WHO).<sup>18</sup> The twelve proposed principles have since been the foundation when evaluating the prerequisites for, or setting up, SPs throughout the world. In a WHO bulletin in 2008 Andermann et al. summed up the additions made to the original principles during the following 40 years.<sup>19</sup> In 2014 the Swedish National Board of Health and Welfare (SNBHW) published a model for assessment, implementation and follow-up of national SP in Sweden.<sup>20</sup> The SNBHW model presents 15 criteria, based on the WHO principles, which are to be met. The three models are displayed in table 1.

In this introduction these criteria will be described divided into six groups: The disease; the test; the treatment; the population; availability (financial and logistical), and; evaluation & continuation. Later on in the discussion the same groups will be used to discuss whether evaluation of atherosclerotic changes examined with WBMRA, MRI and/or US of the carotids can meet the criteria.

<b>Wilson &amp; Jungner 1968</b>	<b>WHO 2008</b>	<b>SoS 2014</b>
Important health problem	SP should respond to recognized need	Important health problem
Accepted treatment	Objectives of screening should be defined at outset	Natural history of the condition understood
Facilities for diagnosis and treatment available	Defined target population	Recognizable asymptomatic stage
Recognizable latent or early symptomatic stage	Scientific evidence of SP effectiveness	Suitable test method
Suitable test or examination	SP should integrate education, testing, clinical service and SP management	Treatment at early stage should be of more benefit than at symptomatic stage
Acceptable test	Quality assurance	SP should reduce mortality, morbidity and/or disability
Natural history of the condition understood	Informed choice, confidentiality, and respect for autonomy	Test and following examinations should be accepted by the population
Agreed policy on whom to treat as patients	Equity and access to screening for the entire target population	Acceptable treatment
Cost-benefit analyzed	SP evaluation should be planned from the outset	Health benefits of SP should outweigh harm
Continuing process	Overall benefits of SP should outweigh harm	SP acceptable ethically
		Reasonable cost-benefit
		Written SP information to all participants
		Clarified SP organization
		Adequate health service provision should be clarified
		SP evaluation should be planned

Table 1. **Criteria to be met when setting up screening programs** as suggested by Wilson & Jungner in 1968, the WHO in 2008 and the SNBHW in 2014. WHO – World Health Organization, SNBHW – the Swedish National Board of Health and Welfare, SP – Screening program.

## The disease

The disease tested for in a SP has to be one that if left untreated causes an important health problem (e.g. premature death, severe injuries or disabilities) either for the society, the individual, or both. Less severe conditions can also be considered for screening if early treatment diminishes suffering and/or saves resources on a larger scale.<sup>20</sup> That is why common diseases with less severe outcome that affect many (e.g. diabetes), and rare diseases with very severe consequences to a few individuals (e.g. phenylketonuria – PKU) can be equally suited for screening.

The natural history of the disease must be known and there needs to be a detectable latent or at least early symptomatic phase. If this phase is very short there is a big possibility that the SP will miss it, while a very long asymptomatic phase can lead to excessive diagnosing and hence excessive, and maybe unnecessary, treatment. This phase, and others, that are part of the terminology when discussing screening are illustrated in figure 1 originally described by Herman et al<sup>21</sup>.

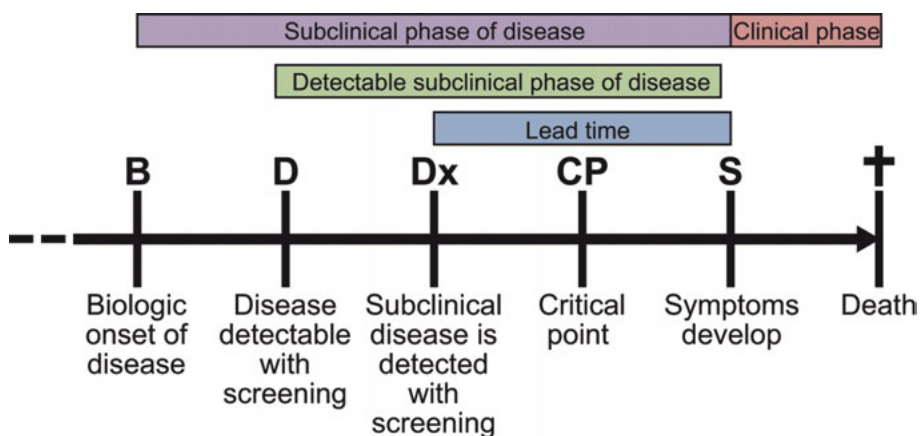


Figure 1. **Diagram of the natural history of disease.** The critical point (CP) represents the point in time where treatment no longer can cure the disease or prolong life (e.g. when a cancer metastasizes). Lead time is the time earned in discovery of the disease by screening.

## The test

The test, or the SP, should if possible be scientifically evaluated, preferably by large randomized population based studies. In order to reach the high ethical standards the test need to be highly sensitive as well as highly specific. Sensitivity and specificity can be illustrated with a simple diagram (figure 2).

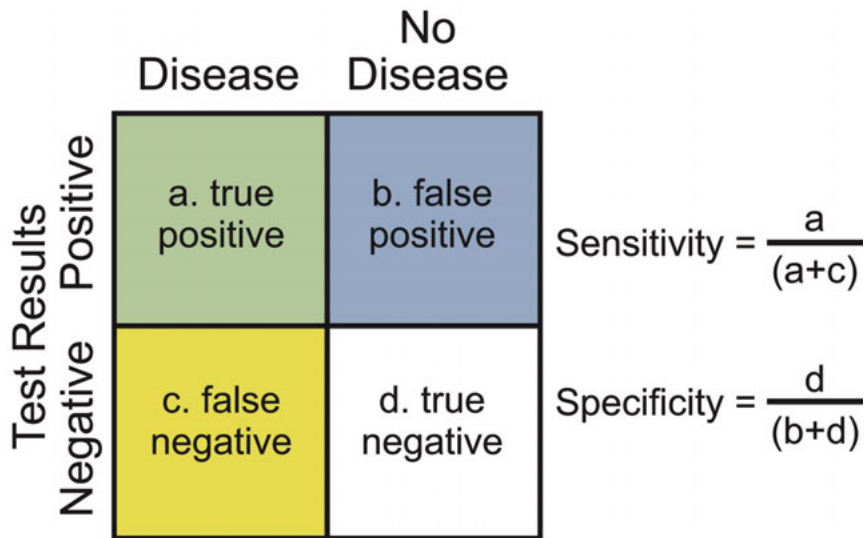


Figure 2. **Specificity and sensitivity.** An illustration of the possible outcomes when applying a test to a disease. The sensitivity equation reveals the rate of true positives, and the specificity equation reveals the rate of true negatives. In an ideal world both these equations should equal 1.

The problem with SPs can also be illustrated as a series of Venn diagrams (figure 3) where a perfect screening test, as in a), will only find true positives (green) and true negatives (white). But those tests rarely exist, normally a test that is sure to identify all subjects with disease or risk for disease, as in b), will result in a lot of false positives (blue), and a test made to not give any false positive results, as in c), will instead have to deal with false negatives (yellow). The most common scenario is depicted in d) where both false positives and false negatives appear.

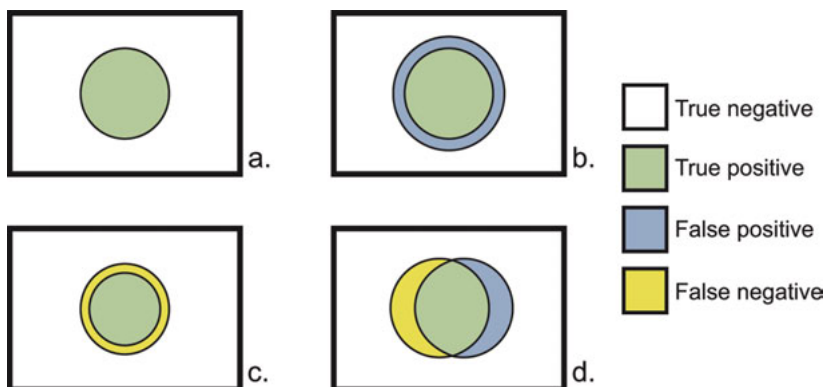


Figure 3. **Venn diagrams of different kinds of screening programs** where a) is ideal with no false results, b) avoids false negatives at the expense of false positives whereas the situation is the opposite in c). The most commonly found result is as in d) with all types of outcome represented.

When setting up a SP one must decide on how many false positives and false negatives one can accept and how to deal with them.

Another prerequisite for an acceptable SP is its reliability, i.e. its reproducibility over time despite different investigators and observers, this is quite a challenge for nationwide SPs. To achieve this, the test needs to be simple and quick to perform, and in order to avoid human errors automated tests are often preferred. If possible the methods of observation should be set up to avoid intra-, and interobserver variability, therefore a clear cut-off value is optimal. The variation in the examined subject (e.g. body constitution) is harder to evade.

The test should not cause discomfort or morbidity. In this aspect screening differs from medical examinations of the supposedly ill patients. In this setting a certain degree of harm (e.g. excessive radiation or painful tests) is warranted if the underlying disease is more harmful than the examination itself. This acceptance is usually lower in a SP.

The ideal screening test should only identify the condition sought after and not find pseudodiseases, which can be categorized into two types: 1) An asymptomatic finding that does not progress or regresses. 2) An asymptomatic finding that progresses with such slow rate that symptoms will not arise before the subject dies of other causes. The main problem with pseudodiseases is that they can be very difficult to differentiate from actual diseases.<sup>22</sup>

Finally the test should also be inexpensive.

### **The treatment**

For a disease to be feasible for screening it has to be treatable, and the treatment has to be curative or improve life expectancy or quality of life. If no such treatment is available the screening will only extend the time of knowledge of the disease, with consequential psychological issues. It is also important to know when in the natural history of the disease treatment should be commenced in order to avoid unnecessary treatment with possible side effects, that is – early treatment should be better than late treatment.

The health care system must have reached a consensus on how to treat the disease before an SP is put into action and the treatment should not have severe side effects or be too expensive.

### **The population**

The population has to be clearly defined. In research settings the cohort often consists of selected groups of individuals and although they may reach large numbers the term mass screening is reserved for screenings of entire populations without exclusions of subgroups. Despite this, mass SPs often have some restrictions such as age and/or gender (e.g. screening of newborns or screening for cervical cancer).

It is important to take into account how a selected population can affect the prevalence of a disease. In clinical studies the population is often selected in some way, e.g. choosing individuals with a certain disease, including visitors to a health care facility or focusing only on the elderly. These populations will most likely be more affected of the disease than the general population with subsequent higher prevalence of the disease, and therefore some study results might not be representative for the general population. Hence, it is important that decisions on whether a disease qualifies for screening or not is based on large prospective and randomized studies running for long times in a population comparable to the one in mind for the SP.

No matter how sensitive or specific a SP might be, the test and the treatment have to be accepted by the affected population and the subjects have to be willing to participate. It has been shown that sociogeographical factors<sup>23</sup> and information<sup>24</sup> matters when it comes to participation in SPs. Therefore information on the condition, the SP and the treatment must be thorough but still understandable for the entire concerned population making the consents well founded. In a national setting this becomes a challenge as the population is inevitably heterogeneous.

Even though the intentions for screening are good the consequences for the individual can be negative with increased levels of anxiety due to information about a condition. A plan for dealing with such consequences must exist.

### **Availability**

When screening a whole population the program has to be equally available to all inhabitants, rural or urban. Therefore easy screening tests spread out through the country are preferred over complicated tests located to the major cities.

Another issue is financial availability; setting up a SP is associated with substantial costs. Not only does the material cost but so does the personnel involved in testing and evaluation, the treatments for found pathology, and the loss in production time of the participants. Even if the medical aim of improving the health of the population may be considered to coincide with the economical aim of lengthening the productive life of the population the cost of every SP must be put in perspective with the total health budget.

### **Evaluation & continuation**

According to Wilson & Jungner, but not Andermann nor SNBHW, a SP should never be a once off occasion but rather a recurring surveillance where the age of inclusion and intervals of examinations must be decided. In order to set such a big machine in operation a nationwide SP needs to be coordinated on a national level to ensure fair spread of resources and equivalent possibilities of participation for all inhabitants.

Any SP must be continuously evaluated and validated.

# Atherosclerosis

## Pathogenesis

All arterial walls consist of three layers. The *intima* is the layer closest to the lumen with a coating of endothelial cells in a monolayer on a basal membrane, followed by an extracellular matrix and a dense elastic membrane (the internal elastic membrane). The *media* consists of smooth muscle cells and extracellular matrix. The *adventitia* mainly consists of loose connective tissue with nerve fibers, small vessels (in the major arteries these vessels are called vasa vasorum and supports half or up to two thirds of the media), and in the larger vessels an external elastic layer as well. The relative thickness of the layers, especially the media, varies depending on vessel type.

The endothelial cells in the inner monolayer have many functions as they maintain a non-adhesive and non-thrombogenic surface, they take part in the modulation of inflammation, they support growth of other kind of cell types – mainly smooth muscle cells, and they affect the reactivity of the underlying smooth muscle cells by producing relaxing or constricting factors. These functions work well under normal circumstances, i.e. normal blood pressure and normal blood flow.

But when exposed to various stress factors, such as turbulent flow, hypertension, elevated blood cholesterol levels, hypoxia, acidosis or bacteria and viruses, a pathological chain of reactions is set in motion. By expressing different factors an inflammatory process begins, causing changes in the intima and damage to the otherwise intact monolayer and basal membrane. Initial mechanical micro-injuries can also be the cause of increased permeability of the endothelial cell layer leading to an accumulation of low density lipoproteins (LDL-cholesterol) within the intima. In response monocytes wander in to the intima and differentiate into macrophages, and by consuming the lipids turn into foam cells. When the lipid filled foam cells get overloaded with cholesterol the lipids are also found in the extracellular space which further accelerates the inflammatory response that can now be measured as elevated hsCRP (high-sensitive C-reactive peptides) in the blood. As the plaque keeps growing the cholesterol filled center gets surrounded by a capsule of smooth muscle cells and fibrins which hopefully stabilizes the plaque. A continuous growth may narrow the vessel lumen and if the inflammatory process increases further cytokines will impede development of the capsule rendering the plaque unstable instead. When unstable, the plaque might not withstand the mechanical strains put on it by turbulent blood flow and the surface might rupture with consequential adhesion of platelets causing thrombosis and focal occlusion, or by emitting thrombo-embolic lesions down the blood stream. This atherosclerotic development takes place over decades and is schematically shown in figure 4.



Figure 4. **Development of atherosclerosis over time.** A longitudinal cross-section of an artery where blue is the adventitia, red is the media and orange is the intima. The yellow color represents a lipid core and the green area represents a thrombotic lesion.

### Risk factors

Some of the risk factors for atherosclerosis are constitutional and cannot be affected, such as age, gender and genetics. Other risk factors can be affected by life style changes or medication, such as smoking, obesity, elevated blood pressure, elevated blood cholesterol levels and type II diabetes.

There are several risk scores available to assess individual risk for cardiovascular events (CVE) and need for medication and/or lifestyle intervention. Most scoring systems effectively rule out the perfectly healthy individuals and find those at high risk, the challenge lies in stratifying risk in the intermediate group, which is of essence since a lot of the sudden CVE with fatal outcome occur in this group.<sup>25</sup>

### Plaque localization

Plaques are often found at specific areas where the vessels bend or divide, since such changes in shape causes turbulence.<sup>26</sup> The laminar flow seen in straight vessels is caused by friction between the blood and the vessel wall. This results in high sheer stress on the endothelial cells that become less permeable and thereby the risk for atherosclerotic changes is reduced. With turbulent flow the sheer stress is lower. This leads to an alteration in the appearance of the endothelial cells due to geometrical changes<sup>27</sup> (figure 5). This increases the permeability of the vessel wall making the area more sensitive to atherosclerotic changes as substances in the blood are more prone to penetrate the intima.<sup>28</sup>

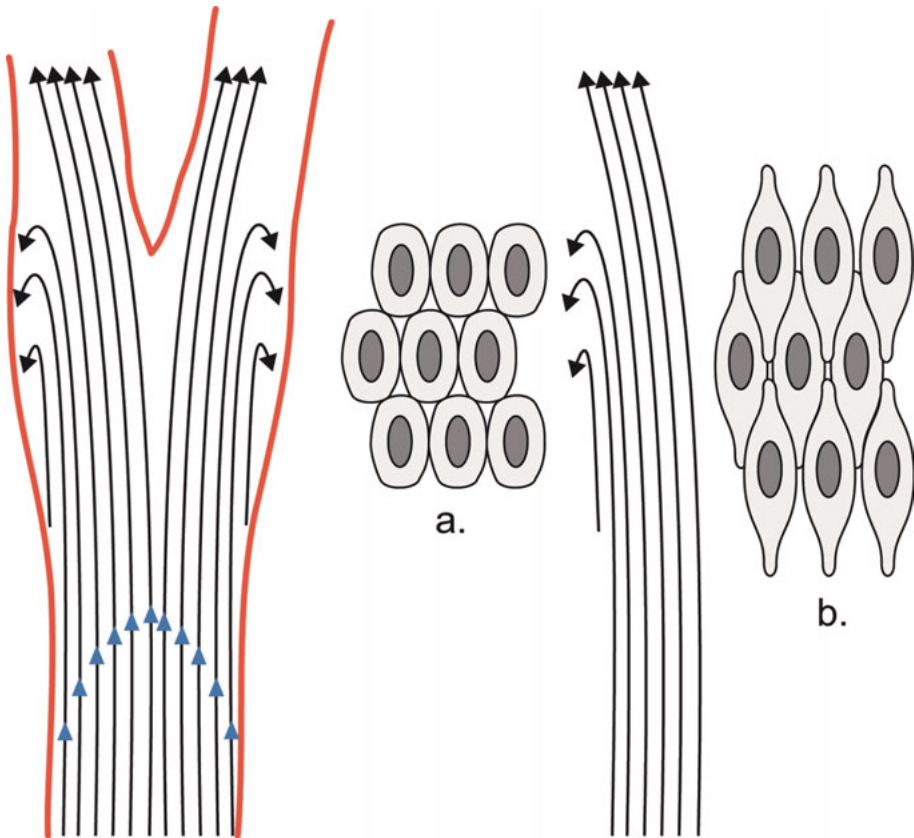


Figure 5. **Illustration of blood flow through the carotid artery.** The black arrows represent the actual blood flow with turbulence at the bulb and bifurcation. The blue arrows illustrate the laminar flow in straight vessels. (a) is a schematic illustration of the more permeable endothelium at an area with low shear stress, whereas (b) is the endothelium in a vessel wall with high shear stress. (a) is more sensitive to atherosclerotic changes.

## Plaque composition

Plaque composition and vulnerability is not focused on in the papers building this thesis but as it is mentioned in the discussions in both the thesis and some of the papers it deserves a few sentences.

CVE can be caused by narrowing of the vascular lumen, either by plaque growth or by focal thrombosis due to severe platelet adhesion on a ruptured plaque. CVE can also be caused by a plaque emitting emboli that occludes the vessel further down the blood stream. The growth of the plaque can be slow or sudden by reason of intra-plaque hemorrhage.

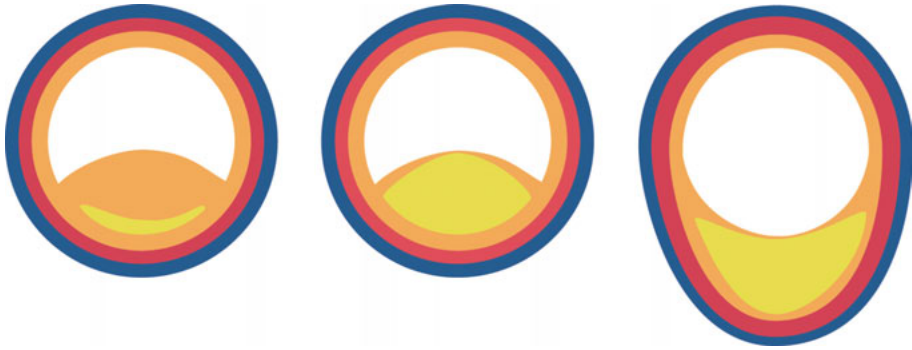


Figure 6. **Different types of plaque.** a) and b) are identical in size but b) is considered vulnerable as the lipid core is big and the fibrous cap very thin in comparison with the stable plaque in a). c) also demonstrates a vulnerable plaque but the vessel lumen is intact since the plaque is positively remodeled.

Since Glagov established, in 1987, that plaque growth starts outwards before affecting vessel lumen<sup>5</sup> focus shifted towards plaque composition in contrast to mere size.

It has been established that some plaques are more unstable than others and therefore prone to eruption or rapid growth with severe clinical outcome as a result, these plaques have been termed vulnerable plaques. The most common description of a vulnerable plaque is a plaque with a large lipid core, increased inflammation and only a thin fibrous cap (figure 6b) also called thin capped fibroatheroma (TCFA), whereas the stable plaque has a thick fibrous cap dense with collagen, minimal inflammation and a small or even negligible lipid core (figure 6a). Tendencies to intra-plaque hemorrhages, that can cause sudden increase in plaque size with vessel blockage or plaque rupture as a consequence, can also be included in the term of vulnerability.

## Cardiovascular events

Atherosclerotic changes finally lead to symptomatic ischemic events that can be of a chronic nature as in (stable) angina pectoris where the luminal narrowing severely increases the risk for ischemia. The events can also be acute with sudden clogging of vessels at the site of a ruptured plaque (thrombosis) or in the periphery (embolism). This can cause stroke, MI or peripheral ischemia depending on vessel location.

The term cardiovascular events (CVE) include events within the heart as well as all other vessels, whereas cerebrovascular events are those affecting the vessels that support the brain. Cardiac events concern the heart and peripheral arterial events are affecting the limbs. It is important to remember that although atherosclerotic changes are present there might not be any

events and this asymptomatic period can vary enormously in length in between individuals.

Major Adverse Cardiovascular Events (MACE) and CVE are definitions with different meanings that must be defined when used. In paper III MACE is defined as cardiac death, MI, stroke and/or coronary revascularization with coronary artery bypass graft (CABG) or percutaneous cardiac intervention (PCI). In paper I only MI or ischemic stroke is used in the definition and the term CVE was used. In the discussion part of this thesis CVE will be used in a general setting.

Since the carotid artery in the neck has a bifurcation (giving rise to turbulence and hence atherosclerosis), provides blood to a sensitive area (the brain) and is easily accessible, it is a highly interesting area often focused on in clinical and research settings. Furthermore, a correlation is seen between atherosclerotic changes in the carotids and similar changes in the coronary arteries<sup>29</sup>, as well as with overall atherosclerosis<sup>30</sup>. Therefore outcome in the heart can be addressed even though the vessels of the heart have not been viewed in any of the papers.

## Imaging of atherosclerosis

To date there is no imaging technique that illustrates all components of vulnerability of the plaque as demonstrated in figure 7. In this thesis focus is put on MRI, WBMRA and US.

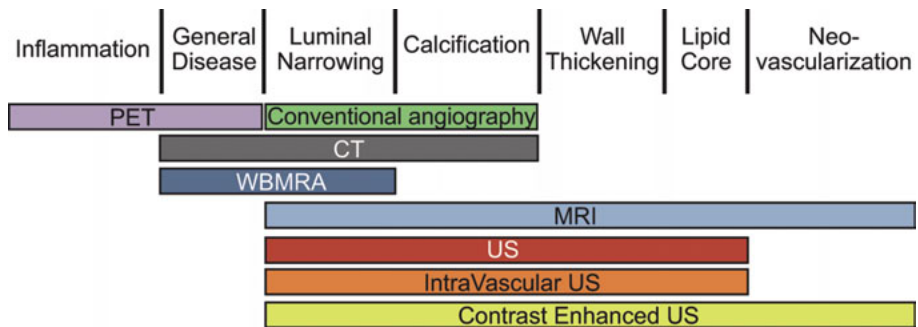


Figure 7. **Different imaging methods used in depicting atherosclerosis, and their strengths.** “General Disease” is the ability to visualize all affected areas simultaneously. Abbreviations: PET – Positron Emission Tomography; WBMRA – Whole Body Magnetic Resonance Angiography; CT – Computed Tomography; MRI – Magnetic Resonance Imaging; US – Ultrasound.

## Magnetic Resonance Imaging

The Magnetic Resonance Image (MRI) is based on the nature of the hydrogen atoms throughout our body. As these positively charged atoms spin around their own axis a tiny magnetic field is induced around each one. The protons spin in random directions causing the tiny magnetic fields to cancel each other out (figure 8), but by placing them in a very strong external magnetic field ( $B_0$ ), such as an MR scanner, the protons align with the stronger field (figure 9).

A few more align themselves in a parallel direction than anti-parallel, creating a new magnetic field called the net magnetization ( $M_0$ ). The protons start precessing with a frequency proportional to the strength of  $B_0$  (figure 10). By affecting the protons with a radio frequency (RF) pulse, with the same frequency as the precession,  $M_0$  is tilted and can now be detected (figure 11).

There are three ways of separating different tissues from each other in the making of an MR image. Primarily, the varying amount of protons in different tissues emits different amounts of detected signal known as proton density (PD). The two other ways are results of what happens after the RF pulse has tilted the protons and they return to their original position. During this relaxation time a signal from each proton is emitted and detected. The relaxation occurs in two directions known as longitudinal relaxation time (T1) and transversal relaxation time (T2). The differences in these three variables (PD, T1 and T2) are the foundation in producing MR images with extremely good abilities to discriminate between soft tissues.

As the precessing frequencies of the protons are proportional to the surrounding magnetic field an extra magnetic field, graded from low to high in strength, is added in the MR machine. Thereby the frequency of the emitted signal is dependent on the position it had in the body. This brings order to the signals detected and an anatomically correct image can be made.

In conclusion, the MR images are made by sending in RF pulses and listening to their echoes. By altering when the pulses are sent in and when the echoes are listened to, in combination with a gradient field, different kind of images can be made.

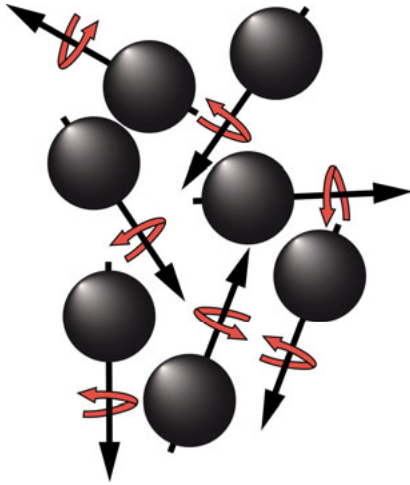


Figure 8.

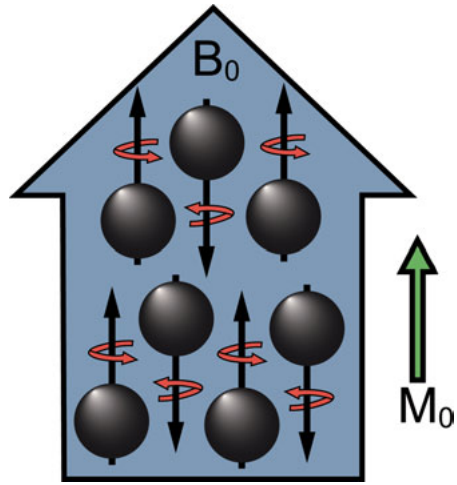


Figure 9.

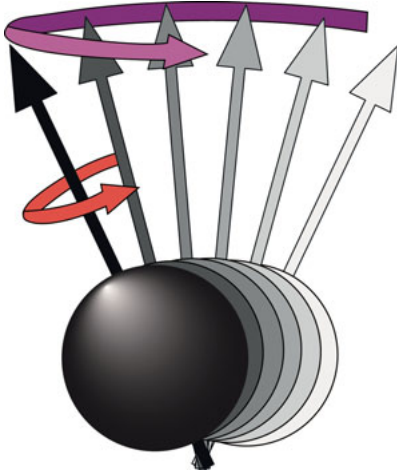


Figure 10.

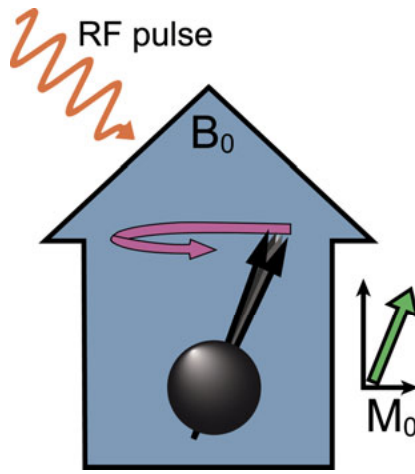


Figure 11.

Figures 8-11. Figure 8 demonstrates randomly spinning protons. In figure 9 the protons are aligned by a strong external magnetic field ( $B_0$ ) thus creating a new magnetic field ( $M_0$ ). As seen in figure 10 each proton starts precessing with a frequency proportional to that of the strength of the external magnetic field. In figure 11 the protons are tilted by a radio frequency (RF) pulse of the same frequency as the precession. This causes the net magnetization ( $M_0$ ) to tilt and thus making it detectable.

## Magnetic Resonance Angiography

To acquire an angiographic image easy to evaluate the subtraction technique is used. The main principle is to create an initial scan (figure 12a), without added contrast enhancing agents, and use this as a mask to subtract all tissues from the scans where contrast agents have been added (figure 12b). The

result is an image where nothing but the blood, due to how the contrast agent changes the blood's signal, and thereby only the lumen of the vascular tree is seen (figure 12c). The final image (figure 12d) is a MIP-image (Maximum Intensity Projection), a volume-rendering image that consists of all coronal scans within one sequence, stacked on each other with only the pixels with maximum intensity seen. Should subtraction not have been made (figure 12d) the enhanced blood would be difficult to separate from the subcutaneous fat, especially below the knees, in such a MIP-image.

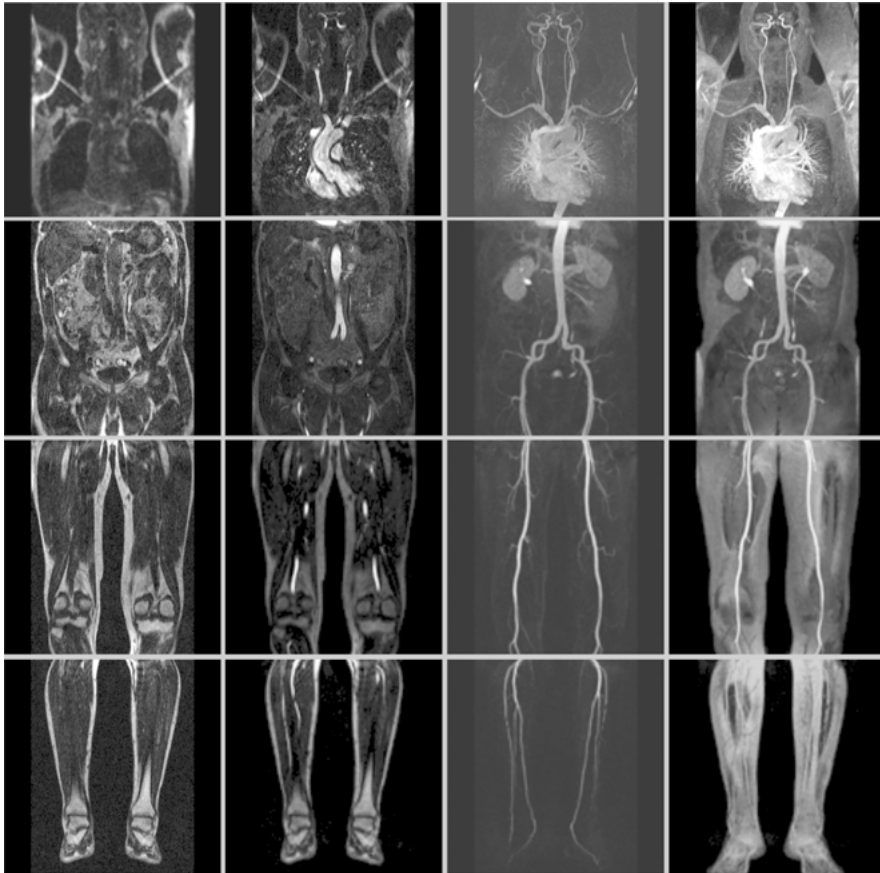


Figure 12. **The principle of subtraction angiography in Whole Body Magnetic Resonance Angiography.** An initial image without the addition of a contrast agent (a) is subtracted from the following images where an intravenous contrast agent has been given (b), resulting in an image only showing the arterial blood flow – i.e. the lumen of the arteries (c). Without subtraction the vessels would be harder to separate from fatty tissue as seen in (d). (c) and (d) are MIP-images showing a volume whereas (a) and (b) only show a single coronal slice of the body. Abbreviations: MIP – Maximum Intensity Projection.

## Ultrasound Imaging

An ultrasound (US) image is created by transmitting high frequency sound waves (usually 2-20 MHz) into the body with a transducer that acts as a combined transmitter and receiver. The waves are partially reflected on any change in tissue density. The time for the echoes to return to the transducer gives information on where in the examined area the reflection took place. The reflected sound waves are illustrated as bright spots on a dark background, thus creating an US image. Some matters (e.g. air, bone, calcifications or metal) reflect the entire sound wave making it impossible to depict tissues behind said matters. Free fluids, such as water and flowing blood, do not reflect any sound waves. The higher the frequency transmitted the higher the resolution in the images but the lower the penetration. When analyzing the layers in a vessel wall there is need for very high resolution, and thereby only superficial vessels, such as the carotids or the femoral arteries, can be assessed due to poorer penetration. Despite the high resolution it is hard to differentiate the intima from the adventitia by US as these layers are close in echogenicity. Fortunately it is easy to identify the border between blood and intima as well as between media and adventitia making the complex of the two layers suitable for measuring (figure13).

An often stated limitation to US examinations is the dependency of a trained examiner, but it has been proven achievable to train novices in performing measurements of Carotid Intima Media Thickness (CIMT) to a solid degree in a fairly short amount of time.<sup>31</sup>

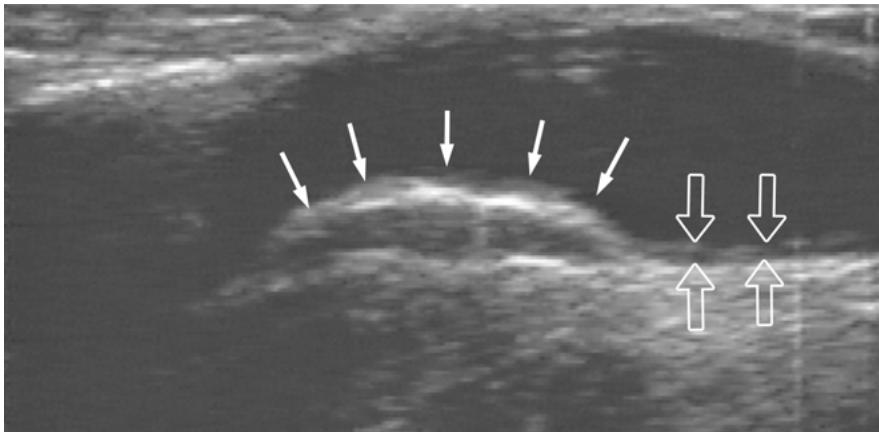


Figure 13. **Ultrasound image of a carotid plaque.** The image shows a longitudinal section of the carotid artery where the small white arrows point to a plaque. The larger arrows points to the combined intima-media layer where the border between black blood and dark grey intima is seen as easy as the border between the bright adventitia and the dark grey media.

# Aims

## General aim

The general aim is to investigate whether MRI and/or US are suitable screening tools for detecting atherosclerosis in a general population (i.e. mass screening), a selective population, and/or research settings. This is done by evaluating the reproducibility of the methods, their ability to predict CVE, and by assessing whether the methods can fulfill the prerequisites for screening.

## Specific aims

### **Paper I**

The aim was to compare three methods of vessel analysis with US (CIMT, presence of plaque and GSM), and assess their ability to predict MACE in 887 subjects at the age of 70 years during a follow-up period of 10 years.

### **Paper II**

The aim was to evaluate the correlation between MRI and US assessment of carotid plaque size, and to compare the reproducibility of MRI and US, in a population of 37 men with known carotid plaques.

### **Paper III**

The aim was to investigate the relationship between general atherosclerosis, defined as TAS, and the risk of receiving a MACE, in a population of 305 70 year old Caucasians with a follow-up period of nearly 5 years.

### **Paper IV**

The aim was to investigate the relationship between CIMT (at baseline as well as its change over 5 years) and TAS assessed by WBMRA in a population of 272 Caucasians at the baseline age of 70 years.



# Material and Methods

## Study populations

### Paper I, III and IV

In 2001 the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study took off by inviting 2025 randomly chosen subjects, resident in the municipality of Uppsala, shortly after their 70<sup>th</sup> birthday, to participate. By June 2004, 1016 subjects had agreed, given written consent, and been enrolled in the study. 887 of these individuals had no MI or ischemic stroke prior to baseline and were analyzed in paper I.

Starting in October 2002, 306 of the original 1016 subjects were consecutively asked, and agreed, to undergo WBMRA. By November 2005, 305 of these examinations were successfully performed, and make up the study cohort in paper III and IV.

Within a month of their 75<sup>th</sup> birthday 827 subjects from the original PIVUS cohort accepted an invitation for re-examination; 52 had died, and 137 subjects declined the invitation. The time between the examinations was 5.13 (SD 0.10) years. These 872 subjects are included in paper I. A total of 272 subjects among those submitted to WBMRA had also undergone successful measurements of CIMT at both age 70 and 75 and are included in the study cohort in paper IV.

At 80 years of age 606 subjects returned for yet another re-examination; they are included in paper I.

### Paper II

In paper II 37 male subjects, at the age of 65 years, with at least one known carotid plaque were examined. This cohort was a subgroup obtained from a larger intervention study where subjects were recruited from a population screening project of AAA at the Vascular Laboratory at the Uppsala University Hospital. In this screening study a total of 5936 male subjects at the age of 65 years, living in the municipality of Uppsala, were invited. 4657 subjects accepted and were assessed by ultrasound for AAA and the presence of plaque in the carotid region. Of these, 1161 subjects met the inclusion criteria of having at least one plaque measuring at least 6 x 2 mm on ultrasound exam in either carotid artery. As 513 subjects were excluded due to statin

use and/or diabetes mellitus 828 subjects remained. From this group the aim was to consecutively recruit a total of 74 subjects (32+5 in two groups receiving Rosuvastatin or placebo treatment). The subjects were contacted by phone and consecutively recruited. A total of 138 subjects underwent MRI-scanning, 65 subjects were excluded from further investigation due to not having a plaque big enough on MRI. The 73 remaining subjects were randomized into the two treatment groups; in paper II the 37 subjects from the placebo group were analyzed.

## Analyses with MR imaging

### WBMRA

With the continuous development of the MRI-technique it has become possible to scan the whole body in a single setting, visualizing the arterial tree by the use of an intravenously administered contrast agent that visualizes the blood as it spreads throughout the body, and thus creating the WBMRA (figure 12).

The WBMRA examination used in our studies was performed with a built-in body coil in a 1.5 Tesla scanner (Philips Medical System, Best, the Netherlands). The subjects were scanned in the supine position with arms over head and feet on an extension attached to the table top. The examination was divided into four stations (figure 14):

1. Circle of Willis to the diaphragm
2. The abdomen
3. Lower part of the pelvis to the knees
4. Lower legs

An overlap of 3 cm between each station gave a maximum total scan length of 171 cm. Each scan took 87 seconds to perform and breath-holding was only necessary in the second station. A 3D RF-spoiled T1-weighted gradient echo acquisition was performed in all stations from feet to head to obtain subtraction images. An injection of 40 ml of the contrast agent gadodiamide (Omniscan, GE Healthcare, Oslo, Norway) was given intravenously and the stations were scanned in the same manner from head to feet. After reconstructions the voxels in the obtained images measured  $0.88 \times 0.88 \times 2.0$  mm, giving a voxel volume of  $1.54 \text{ mm}^3$ .<sup>32</sup>

Despite the term whole-body neither the cardiac or cerebral arteries, nor the arteries in the arms were analyzed.

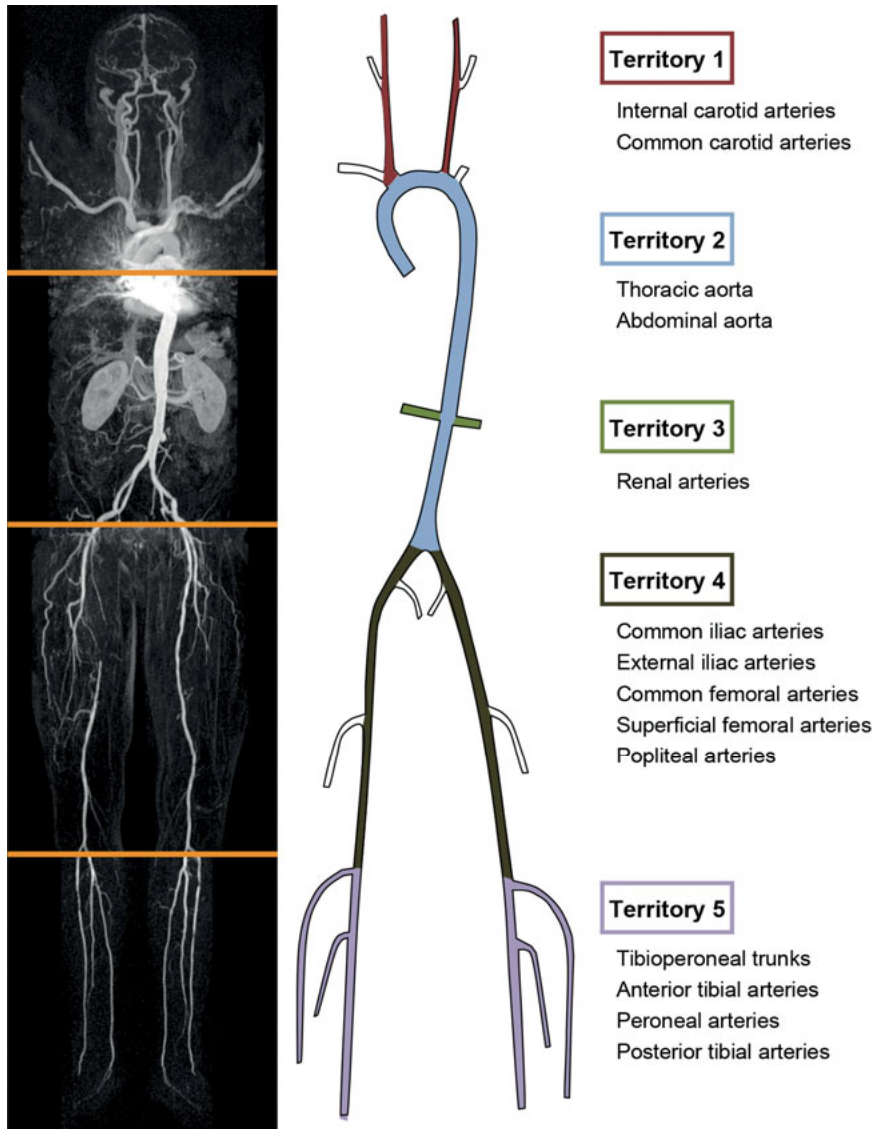


Figure 14. **WBMRA and TAS.** The four stations used in WBMRA acquisition are separated by yellow lines in the left image. The right picture is a schematic overview of the territories and their segments used to make up the TAS. Abbreviations: WBMRA – Whole Body Magnetic Resonance Angiography; TAS – Total Atherosclerotic Score.

## TAS

In order to evaluate the total atherosclerotic burden throughout the body the Total Atherosclerotic Score (TAS) was developed by Hansen et al.<sup>33</sup> The arterial tree was divided into five territories containing 2-10 arteries (segments) each (figure 14).

Each segment was evaluated and the most severe stenosis within it was graded with 1 point if the stenosis occluded less than 50% of lumen and 2 points if 50% or more of lumen was occluded. Even the slightest irregularity received a point. Normal vessels scored 0 points. The received sum in each territory was divided by the highest possible sum and multiplied with 100. This gives a maximum sum of 100 in each territory and by adding the 5 territories the maximum TAS is 500, and the lowest pathological score is 5.

By dividing the arteries into territories with different amounts of segments each artery is weighed in importance. For example; a severe stenosis in the aorta or one of the renal arteries has five times the influence on the total score as does an equally severe stenosis in one of the arteries in the lower legs.

## Plaque size

In order to do accurate measurements of plaque size, the images have to be of high quality. In paper II this was achieved by scanning the subjects in a 3Tesla MRI scanner (Philips Medical System, Best, the Netherlands) with a carotid surface coil. The images were centered at the bifurcation with a longitudinal coverage of 20 mm in each direction. To simplify delineation between plaque and vessel lumen black blood images was achieved by cancelling the signal from the blood. Field of view was 14x14 cm, which in a 256x256 matrix results in voxel sizes of 0.55x0.55 mm, and the slice thickness was 2 mm.

Two kinds of volumes were calculated from sequential axial images; the total wall volume (TWV) and the Normalized Wall Index (NWI) where the latter takes the vessel size into account (figure 15). TWV was calculated by adding the wall area (lumen area subtracted from total vessel area) in each slice containing plaque, and multiplying those by the slice thickness of 2mm. NWI was calculated by dividing the total wall area with the total vessel area from the same slices.

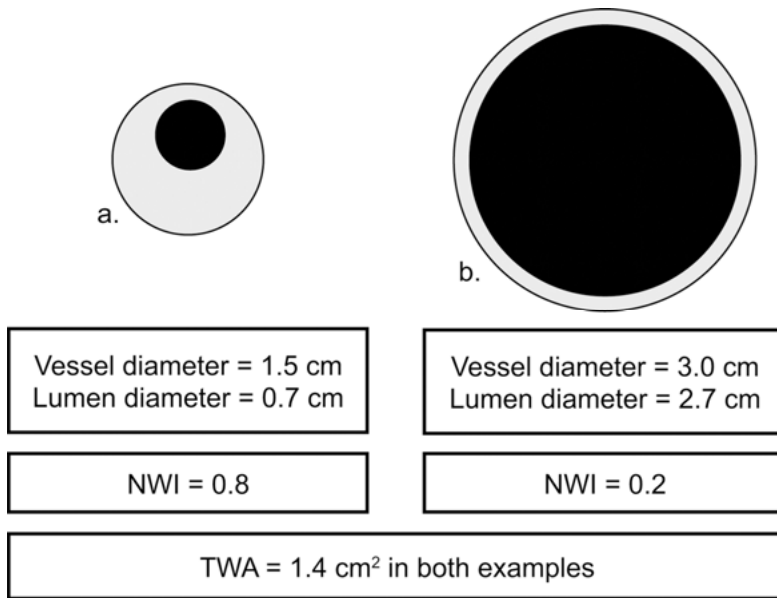


Figure 15. **TWA vs. NWI.** A comparison of the two methods of calculating plaque area where the Normalized Wall Index (NWI), but not the Total Wall Area (TWA), accounts for vessel size. Total Wall Volume (TWV) is achieved by multiplying the TWA from each image with a plaque with the slice thickness and adding those up.

## Analyses with ultrasound imaging

With high-frequency ultrasound the layers of the arterial wall can be visualized with an easily identified difference between the lumen and intima as well as between the media and the adventitia. As it is harder to separate the intima from the media the combined thickness of these layers is measured as the carotid intima media thickness (CIMT)<sup>34</sup> even though the atherosclerotic changes mainly occur in the intima (figure 13).

The use of standardized computer programs when delineating the intima-media complex or plaque size is required to overcome un-precise manual measurements, due to the distances being so tiny they nearly meet pixel-size despite high resolution<sup>35</sup>. In the studies building this thesis such an Artery Measurement Software (AMS)<sup>36</sup> was used.

The US examinations in the studies within this thesis were performed with external B-mode ultrasound imaging using a 10MHz linear transducer (Acuson XP128, Acuson Mountain View, California, USA). One strength in the many examinations was the fact that they were performed by only a few very skilled examiners. An inevitable limitation in a ten year long prospective study is that the machinery in the end will be considered out of date.

## CIMT

As the cervical vessels are easily accessible, CIMT is a frequently used tool to estimate cardiovascular risk.

There have been some debate on how and where in the carotids to do the measurements, some advocate only reporting the thinnest value<sup>37</sup>, some suggest reporting mean values<sup>38</sup>, and some even the highest value<sup>39</sup>. The three parts of the carotid vessel used in CIMT measuring are the common carotid artery (CCA), the bulb, and the internal carotid artery (ICA) (figure 16). Usually the far wall of the common carotid artery is analyzed as imaging of the wall nearest the transducer is proven less accurate or even invalid with ultrasound<sup>40</sup>.

In paper II, III and IV a mean value of three measurements from the far wall of the common carotid artery is used. In paper I results from the far wall of the bulb are also analyzed.

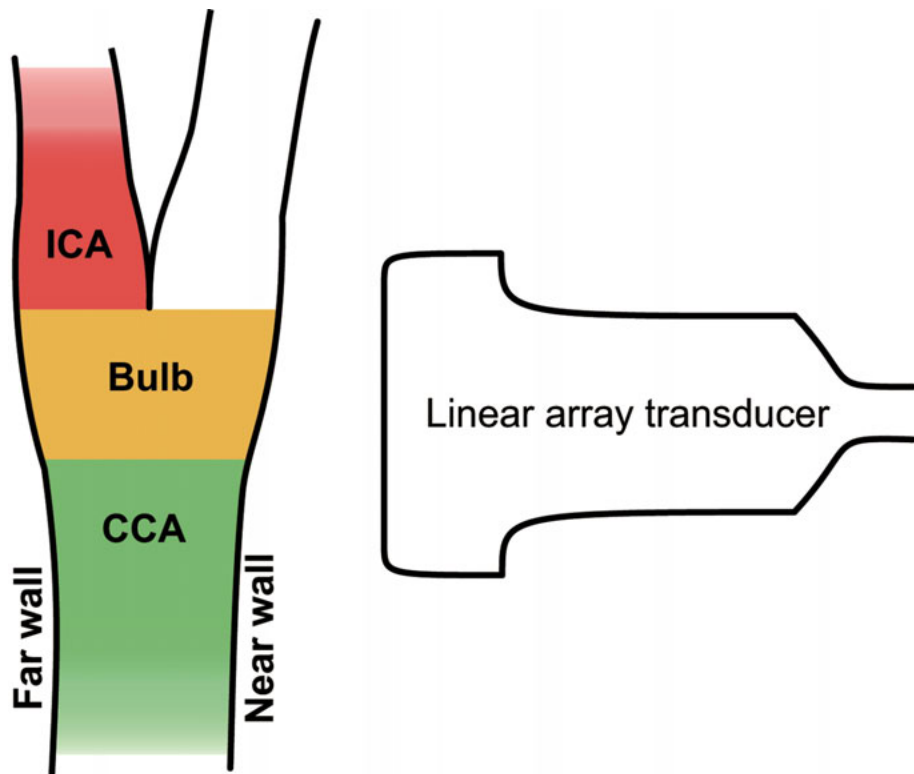


Figure 16. **Carotid artery nomenclature.** The areas of the artery are marked with color; red for the internal carotid artery (ICA), yellow for the bulb, and green for the common carotid artery (CCA). The near and far walls are named according to their relationship to the ultrasound transducer.

## Presence of Plaque

A focal thickness of the IMT can change into a plaque; the definition of when this change occurs varies. Some groups define a plaque as a focal increase in thickness  $>1\text{mm}$  compared to the subjects normal IMT.<sup>37</sup> In our studies a plaque was defined as a focal thickness of the intima-media complex exceeding the surrounding intima-media thickness by at least 50%.

## Plaque Size

In papers I and II plaque size were accounted for. These measurements were received by digitizing and importing the obtained carotid images of good subjective quality into the AMS. A region of interest – ROI – was drawn manually around visible plaques for the measurement of plaque area. Plaque size can also be reported as thickness or, if 3D ultrasound is available, volume.

In paper II, where reproducibility was of issue, the examiner was blinded from the inclusion US and MRI measurements, as well as blinded from the baseline examination when performing the follow-up examination.

## Plaque composition

The Gray Scale Median (GSM) as a way to assess plaque constitution rather than plaque size in order to identify vulnerable plaques was presented in the 1990's.<sup>41</sup> With this technique images from different ultrasound systems, performed by different examiners, each with their individually preferred settings, can be compared.

To achieve this standardized analysis of ultrasound images the grey scale image itself needs to be standardized. This is done by rescaling the grey tones (from black to white) by a scale of 256 steps (0-255), where 0 is as black as the flowing blood and 180-200 is the tone of the adventitia thus rendering 255 bright white. The standardized image is then computer analyzed and the number of pixels of each grey tone is presented in a diagram, the median value of the plaque is then calculated. Darker tones, low on the scale, are seen in lipid rich and more instable plaque whereas calcifications seen in more stable plaque have a brighter appearance and are hence placed higher on the scale.

The GSM only assesses the echogenicity (dark or bright) but does not characterize whether the plaque is homo- or heterogeneous in its structure.

## Other methods of risk evaluation

### Scoring systems

Clinically, risk stratification for atherosclerotic induced events is mainly done by methods that do not use imaging; information on intrinsic factors (age, sex, heredity); observations such as BMI or blood pressure; blood samples (e.g. levels of cholesterol, blood sugar or triglycerides); and previous and concurrent conditions (e.g. diabetes or prior CVE). The results of these risk factors are often inserted into scoring systems.

### FRS

The Framingham Risk Score (FRS) is only one of many scoring systems used to assess cardiovascular risk. It actually contains several scoring systems derived from the Framingham Heart Study that started in 1948 in Framingham, just outside Boston, USA, recruiting 5209 adults (30-62 years). These subjects, and later on their offspring, have been regularly examined and their outcome registered. This information has been used to establish scoring systems adapted for various settings. In the papers in this thesis the 10 year prediction of Coronary Heart Disease Score<sup>42</sup> were used. In this score the following risk factors are analyzed; sex, age, LDL-cholesterol, HDL-cholesterol, blood pressure, diabetes and smoking. A limitation in these systems is that the variables are reported as categorical rather than continuous, since the measured units are given different points, according to preset intervals. These points are then inserted into the scoring system and a risk calculation is received.

FRS is based on a Caucasian population which can be another limitation in some settings but suits our Swedish study populations well.

### HeartSCORE

The HeartSCORE scoring system is not mentioned in the papers but in the discussion part of this summary. In this European system, designed to estimate 10-year risk of fatal CVD in subjects between 40-65 years of age, the following risk factors are used; age, sex, systolic blood pressure, total blood cholesterol, and smoking.<sup>43</sup>

### ABI

The Ankle Brachial Index (ABI) is a non-invasive method of evaluating peripheral arterial disease as it estimates presence of stenosis in the lower limbs. This is done by dividing the highest systolic blood pressure in the arms with the highest systolic blood pressure in each leg. A normal quotient lies between 0.9-1.2, results below these numbers indicate peripheral arterial disease.<sup>44</sup>

## Statistical methods

In all papers the level of significance was set at p-values <0.05 if nothing else was stated.

### Paper I

Cox proportional hazard analysis was used to investigate how the different ways of looking at carotid atherosclerosis were related to the outcome (MI and ischemic stroke), when adjusted for sex only and when adjusted for multiple risk factors.

Receiver Operator Characteristics/Area Under the Curve (ROC-AUC) were calculated to compare discrimination between the indices.

Due to the six different ways to evaluate atherosclerosis the p-value for significance was adjusted to 0.0083 according to Bonferroni.

STATA 12 (Stata Inc, College Station, Texas, USA) was used for calculations.

### Paper II

A correlation analysis was performed between baseline and follow-up for MRI and US separately, and also between the baseline results of US and MRI (TWV and NWI separately).

The Coefficient of Variation (CV) was calculated and the effects on sample size determined by the following formula:  $N=k(\sigma/\mu)^2$  where  $k$  is depending on the power and significance level and  $(\sigma/\mu)$  is the CV. Thus, for a given power and significance level the difference in  $N$  is dependent on the  $(CV_1^2/CV_2^2)$ .

### Paper III

Those with TAS=0 were separated from those with TAS≥5, who in their turn were divided by the median TAS-value of 35, resulting in three similarly sized groups and the Chi square test was used to relate these three groups of TAS to MACE.

Logistic regression analysis identified associations between TAS and MACE and the Odds Ratio (OR) was calculated for subjects with TAS>0 in comparison with subjects with TAS=0. This analysis was also performed in an alternative setting where the subjects were divided into three groups according to no changes, significant, or non-significant stenosis.

Receiver Operator Curve (ROC) evaluated the addition of TAS to the FRS and tests of Net Reclassification (NRI) and Integrated Discrimination Improvement (IDI) were done according to the method described by Pencina et al<sup>45</sup> to evaluate the improvement in adding TAS to FRS in the prediction of MACE.

STATA 11 (Stata Inc, College Station, Texas, USA) was used for calculations.

#### **Paper IV**

The ANOVA test was used to analyze differences between groups regarding sex.

The Tobit (censored) regression analysis was used to evaluate the relationships between TAS and CIMT or  $\Delta$ CIMT.

The Kruskal Wallis test was used to evaluate differences in groups regarding TAS, as well as differences between numbers of plaque.

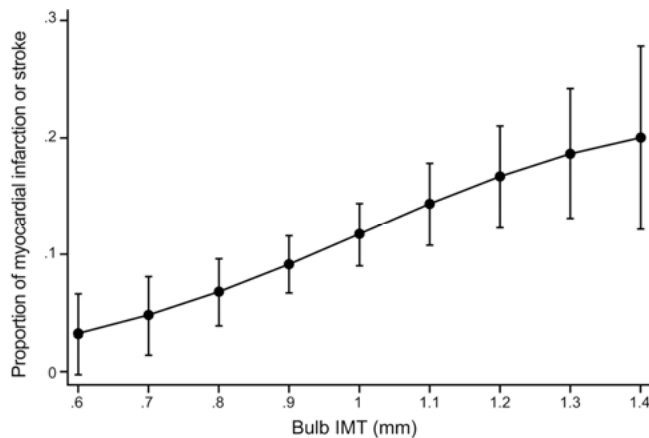
# Results

## Paper I – Six different ways to analyze carotid atherosclerosis and their prediction of MI and stroke.

Eight-hundred and eighty-seven individuals from the PIVUS study with no MI or ischemic stroke prior to baseline were examined at ages 70 and 75 years and followed for 10 years (range 0.18 – 10.9 years) regarding MI and ischemic stroke. With 111 subjects having such an event the incidence rate became 13.8/1000 person years at risk.

Defining a plaque as having an area  $>10\text{mm}^2$  gave the highest hazard ratio (HR) for incident MI or ischemic stroke and was therefore used in the study. The excluded definitions of plaque tested were; thickening of IMT  $>50\%$  of surrounding IMT, area  $>15\text{mm}^2$ , area  $>20\text{mm}^2$  and area  $>30\text{mm}^2$ .

Out of the six indices of atherosclerosis measured by US (IMT in the bulb, IMT in the CCA, presence of plaque, number of plaques, plaque area, and plaque GSM) IMT in the bulb stood out as the strongest predictor of MI and ischemic stroke (HR=6.07, CI 2.36-15.65,  $p<0.0001$ , when adjusted for multiple risk factors).

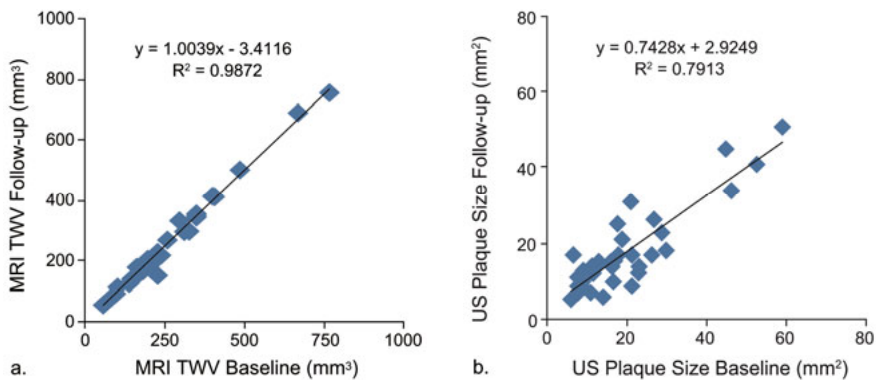


Graph 1 **The relationship between IMT in the bulb and proportion of MI or ischemic stroke.** Predictive margins are used, including a squared term of bulb IMT, to allow for non-linear relationships. Abbreviations: IMT – Intima Media Thickness; MI – Myocardial Infarction.

## Paper II – Measurement of carotid plaque size with MRI and ultrasound.

In 37 non-diabetic male subjects, not on statins, each with at least one carotid plaque with a size not smaller than 2x6 mm, MRI and US was performed on the carotids within 2 weeks of each other at baseline and after three months.

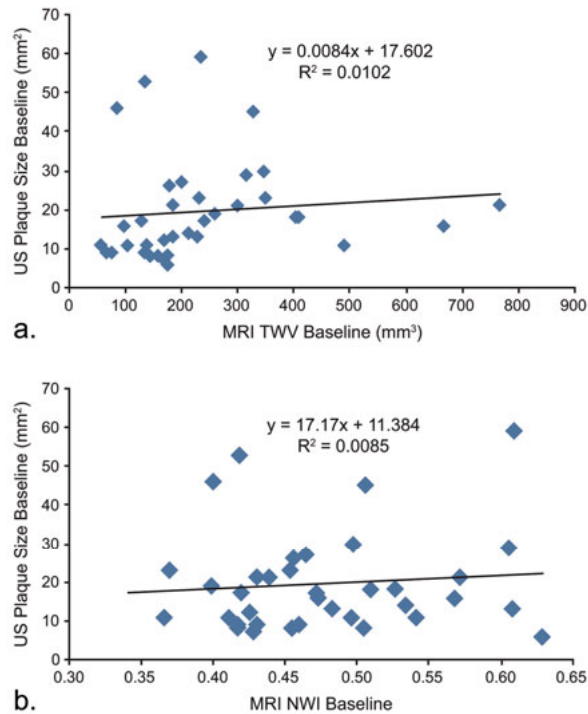
Measuring plaque size as TWV with MRI revealed an excellent level of reproducibility ( $R^2=0.99$ ) as compared with the high level using ultrasound to measure plaque area ( $R^2=0.79$ ) (graph 2).



Graph 2. **Reproducibility levels in repeated examinations of carotid plaques with MRI and US respectively, performed on the same subjects.** Comparison of TWV in carotid plaques identified with MRI at baseline and after 3 months is seen in a). In b) the same plaques are compared at baseline and after 3 months regarding plaque area measured with US. Reproducibility with US is good, and with MRI excellent. Abbreviations: MRI – Magnetic Resonance Imaging; TWV – Total Wall Volume; R – Correlation Coefficient; US – Ultrasound.

To exclude vessel size as a factor, plaque size evaluation by MRI was also done using NWI (figure 15). When comparing US assessment of plaque area with MRI assessment of plaque volume, both with TWV and NWI, in the same plaques no correlation was revealed (graph 3).

The coefficient of variation for MRI (TWV) and US (plaque area) were 4% and 19% respectively, meaning that the sample size needed if an US study comparing sizes of carotid plaques were to be performed would have to be 5 times larger than if MRI was used.



**Graph 3. Correlation between US measurements of area and MRI measurements of volume in the same carotid plaques.** No correlation was seen between carotid plaque area assessed by US and MRI assessment of plaque volume, neither given as TWV (a) nor NWI (b). Abbreviations: US – Ultrasound; R – Correlation Coefficient; MRI – Magnetic Resonance Imaging; TWV – Total Wall Volume; NWI – Normalized Wall Index.

### Paper III – WBMRA with TAS predicts MACE

When analyzing the WBMRA and calculating TAS in 305 70 year old subjects one third of the subjects were found to have normal vessels with zero points in the scoring system. In order to achieve three equally sized groups the remaining two thirds were split in half by the median value of 35 points.

Twenty-five subjects suffered a MACE during the follow-up period of nearly five years and experiencing a MACE was significantly more common in the groups with higher TAS compared with subjects without atherosclerotic findings ( $p=0.003$ ); 1 out of 99 subjects in the group with TAS=0; 10 out of 106 subjects in the group with TAS 5-35; and 14 out of 100 subjects in the group with TAS>35.

The addition of TAS to FRS or common risk factors separately increased the ability to predict MACE. In fact, when adjusting for sex and the FRS the odds ratio of predicting a MACE was increased more than eightfold (table 2).

	Models adjusted for sex and FRS		
	OR	95%CI	p-value
Trend test for the 3 TAS-groups	1.89	1.01-3.55	0.046
TAS >0p vs. 0p	8.80	1.15-67.29	0.036
TAS 1-35p vs. 0p	8.94	1.10-72.45	0.040
TAS >35p vs. 0p	8.67	1.08-69.42	0.042
CIMT (mm)	4.80	0.32-71.78	0.26
ABI	1.44	0.07-28.71	0.81
Presence of plaque	1.45	0.50-1.38	0.50

Table 2. **A logistic regression model of the relationship between four groupings of TAS as independent variables for risk of MACE (n=25) during 4.8 years of follow-up.** Adjustments are made for sex and the FRS and atherosclerosis was investigated as a continuous variable (OR per SD). Sample size: 305. Abbreviations: FRS – the Framingham Risk Score; OR – Odds Ratio; CI – Confidence Interval; TAS – Total Atherosclerotic Score; CIMT – Carotid Intima Media Thickness; ABI – Ankle Brachial Index; MACE – Major Adverse Cardiovascular Event; SD - Standard Deviation.

When analyzing reclassification of risk of MACE by adding TAS to the FRS 7 of 14 subjects with a subsequent MACE were shifted from the low or intermediate risk groups to the high risk group. In reverse 66 of 164 subjects without a MACE were reclassified from the intermediate or high risk groups to the low risk group as seen in table 3.

			FRS + TAS			Total
			<6%	6-10%	≥10%	
MACE	only FRS	<6%	3		3	6
		6-10%	1	3	4	8
		≥10%			11	11
		Total	4	3	18	25
no MACE	only FRS	<6%	100	10	6	116
		6-10%	52	17	33	102
		≥10%	14	6	42	62
		Total	166	33	81	280

Table 3. **Net reclassification after the follow-up period (4.8 years).** Net reclassification improvement = 0.32 (p=0.0072). The upper half of the table shows how the 25 subject receiving a MACE were categorized according to only FRS or with the addition of TAS: FRS alone put 11 subjects as being at high risk whereas FRS + TAS classified 18 as being at high risk, meaning that 7 subjects were correctly reclassified with the addition of TAS. Abbreviations: FRS – the Framingham Risk Score; TAS – Total Atherosclerotic Score; MACE – Major Adverse Cardiovascular Event.

In an alternative setting the subjects were divided into three groups depending on degree of stenosis; 99 subjects with no abnormalities, 113 subjects with a no significant stenosis (1-49% luminal reduction), and 92 subjects with at least one significant stenosis (at least 50% luminal reduction). In both groups with pathology 12 subjects experienced a MACE and both groups were significantly associated with future MACE.

## Paper IV – CIMT but not $\Delta$ CIMT relate to TAS

A total of 272 subjects from the PIVUS study were examined with WBMRA with evaluation of TAS at 70 years of age, as well as had their CIMT measured by US at the ages of 70 and 75 years. CIMT at age 70 was correlated with TAS both when adjusted for sex ( $p=0.0001$ ), and when adjusted for multiple risk factors (antihypertensive medication, systolic blood pressure, statins and LDL cholesterol) ( $p=0.0221$ ). However, the change in CIMT over five years ( $\Delta$ CIMT) did not reach a significant correlation with TAS (table 4).

	TAS vs. CIMT and $\Delta$ CIMT					
	Adjusted for sex			Adjusted for multiple factors		
	Beta	95%CI	p-value	Beta	95%CI	p-value
CIMT	0.61	0.371-0.903	0.0001	0.358	0.053-0.663	0.0221
$\Delta$ CIMT	-0.342	-0.765-0.081	0.1144	-0.168	-0.566-0.231	0.41

Table 4. **TAS assessed with WBMRA vs. CIMT and  $\Delta$ CIMT assessed with US.** CIMT was significantly correlated with TAS when adjusted for sex as well as multiple factors.  $\Delta$ CIMT over five years did not correlate significantly with TAS. Abbreviations: TAS – Total Atherosclerotic Score; CIMT – Carotid Intima Media Thickness;  $\Delta$ CIMT – change in CIMT over five years (from age 70-75); multiple factors include antihypertensive medication, systolic blood pressure, statins and LDL-cholesterol.



# Discussion

## Imaging of atherosclerosis

Screening for atherosclerosis in general practice is done by screening for risk factors that is supposed to foretell the risk for CVE. None of the traditional risk factors actually visualize or measure the atherosclerotic changes but rather assess known contributors in the pathogenesis of the condition. The addition of imaging methods to depict the actual atherosclerotic changes in the vessels has the potential to improve the risk stratification and to raise awareness of the need for preventive action and boost motivation for treatments on an individual level.

## CIMT of the bulb, the CCA or the ICA

CIMT has been established as a predictor of cardiovascular outcome in numerous studies.<sup>46</sup> Different areas of the carotids (figure 15) are put in focus; the common carotids<sup>47,48</sup>, the bulbs<sup>49</sup>, the internal carotids<sup>46</sup> and combinations<sup>50</sup>. This variety of areas analyzed makes comparison between studies difficult. In 2006 the Mannheim consensus put forward standardized recommendations on how to assess atherosclerotic changes of the carotid arteries in an effort to overcome this heterogeneity. They argued for using common carotid IMT alone as this is easily reproduced.<sup>35</sup>

A later meta-analysis objected to this statement as IMT in the CCA is suggested to be without clinical importance.<sup>51</sup> Furthermore, it is proposed that increased CIMT in the bulb is strongly linked with atherosclerosis whereas increased CIMT in the CCA is instead linked with hypertension.<sup>52</sup> This is in concordance with the findings in several papers, including paper I in this thesis, which state that CIMT measured in the bulb is more strongly related to CVE than CIMT measurements from the CCA.<sup>49,53</sup> This might be explained by the suggestion that the intima media thickening in these two areas are of different origins. A common notion is that the normal age induced thickening of the inner vessel layers<sup>38</sup> is merely speeded in atherosclerosis, eventually converting the intima media complex into a plaque.<sup>50</sup> This is questioned by histological findings showing that normal thickening of the vessel wall differs from the development of the atherosclerotic plaque.<sup>54</sup> The anatomical location of the pathological plaque is closely related to areas with

low shear stress<sup>28</sup> such as the carotid bulb and the proximal part of the ICA. Therefore measurement of CIMT in the bulb might more likely be a measurement of atherosclerosis.

## Plaque analysis

Adding information on presence of plaque to CIMT analyses improves prediction of cardiovascular outcome as the plaque is a definitive manifestation of atherosclerosis.<sup>53,55,56</sup> Meta-analyses have indicated that adding further information of the plaque (e.g. quantity and size) improves correlation with CVE in comparison to mere presence of plaque.<sup>57</sup>

In paper I an effort was made to assess the plaque composition as GSM was taken into account. There was however no significant relation between GSM and CVE which might be explained by the frequent use of statins and antihypertensive medication in the cohort.<sup>58,59</sup> More likely it is explained by the fact that GSM does not take variation within the plaque into account but presents the median. Other recent studies disfavor GSM as a suitable method to assess plaque vulnerability<sup>60</sup> and there are other ways to evaluate plaque composition as discussed in the next section.

## MRI or US in plaque analysis

Since B-mode US is two-dimensional only area, not volume, could be estimated. With US the plaque area is measured longitudinally to the vessel as opposed to MRI where plaque area is measured from images transversal to the vessel.

Furthermore, the stacking of sequential MR images makes it possible to render volume measurements and be sure to incorporate the whole vessel in the examination. The fanning way of obtaining US images in combination with the fact that near wall measurements are less accurate than far wall measurements<sup>40</sup> explains discrepancies in plaque evaluation between the two modalities, as was seen in paper II (figure 17).

The disadvantage in plaque size analyses with 2D US might be overcome by the use of 3D US, a fairly new method that is gaining ground. Some studies have shown good correlation to MRI measurements<sup>61,62</sup> whereas others have not<sup>63</sup>.

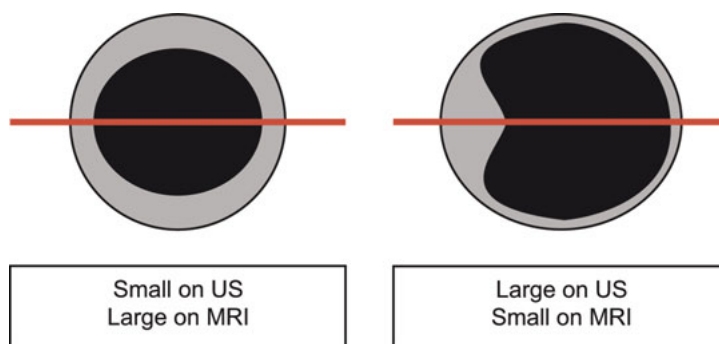


Figure 17. **US vs. MRI in plaque analyses.** Plaques can appear different on MRI and US due to its spread in the vessel wall. The red line represents the US beam and the parts of the walls intersected by the red line are visible in a longitudinal US image. The left hand image portrays a circumferential plaque that involves all parts of the vessel wall in this transversal image but with US the thickness of the wall parts seen is not very substantial. The right hand image illustrates a plaque that seems large on US but is revealed to be limited in spread on an MRI. Abbreviations; US – Ultrasound; MRI – Magnetic Resonance Imaging.

Not only size matters since the composition of a plaque gives information of its vulnerability where cap thickness and different components such as calcifications, hemorrhages, lipid cores and inflammation are of importance. Analyzing plaque composition with MRI, especially with enhancement by contrast agents, has an advantage over US in the ability to separate the different components. But also in this area US methodologies are improved by the addition of US specific contrast agents that enables identification of neo-vascularization.<sup>64</sup> In addition, the contrast agents used for US are safer than the contrast agents used in MRI.<sup>65,66</sup>

Another advantage of MRI, over US in plaque analyses, is the high level of reproducibility, seen several other studies<sup>67-69</sup> as well as in paper II where the reproducibility level of repeated carotid MRI was near perfect. Even though training programs in combination with strictly uniform examination protocols increases reproducibility levels in US analyses of carotid plaques,<sup>70</sup> these levels decrease if different sites are compared.<sup>71,72</sup>

In the aspect of costs and availability, both in machinery and examiners, US is the cheaper and more available method of the two.

## WBMRA or carotid MRI

Choosing between WBMRA and carotid MRI is in essence a choice between seeing the big picture or seeing the details. Even though the two methods in some ways contrast each other (e.g. WBMRA depicts the lumen whereas carotid MRI depicts the vessel wall, and WBMRA examines a large area in a short amount of time whereas carotid MRI examines a few square cm during

a much longer time period) contrast agents are needed in both cases and they can both predict CVE. Even though paper IV, as well as other studies<sup>73</sup>, has suggested carotid atherosclerosis to correlate with peripheral atherosclerotic changes, WBMRA has the advantage of actually visualizing all major vessels including those in the lower limbs.

## Contradictive results

Some of the results may appear contradictive – in paper III CIMT did not predict MACE, whereas TAS did, in paper IV however, CIMT does correlate to TAS. This can be explained by some differences in the analyses: In paper IV 272 subjects were included and the models presented in table 4 were adjusted for either sex or multiple factors (antihypertensive medication, systolic blood pressure, statins and LDL-cholesterol). In paper III 305 subjects were included and the model in table 2 was adjusted for sex and the FRS. When adjusted for sex only the OR became 11.84 (95%CI 0.96-145.2), nearly significant with a p-value of 0.053. The risk factors used in the FRS are, as previously described, sex, age, LDL-cholesterol, HDL-cholesterol, blood pressure, diabetes and smoking, and they are given as categorical values as opposed to the continuous values given in paper IV.

This variation in subjects, type of values and number of risk factors has obviously tipped the p-values to either side of the “significance border”. This is in a way consistent with the current circumstances in the literature where CIMT is often presented as border significant as a predictor of CVE.<sup>74</sup>

## Imaging of atherosclerosis by MRI and US and the criteria for mass screening

In order to investigate whether MRI and/or US can be used in mass screening of atherosclerosis, the knowledge gained have to be tested against the principles described in the background section.

### **The disease**

Atherosclerotic CVD is a leading health problem. In the WHO Global Health Estimates (June 2014) ischemic heart disease and stroke are the number one and two leading causes of death globally as well as in Europe. In Sweden these conditions are the number one and three in leading causes of death (table 5) even though both have declined between the years of 1995-2011.<sup>75</sup>

Cause of death (%)	Globally	Europe	Sweden
<b>Cardiovascular Diseases</b>	31.4	38.8	36.8
- Ischemic Heart Disease	13.2	24.5	15.1
- Stroke	11.9	14.0	7.9

Table 5. **Deaths by cardiovascular disease.** The percentage of deaths caused by cardiovascular disease (with ischemic heart disease and stroke specified) globally, in Europe and in Sweden during 2012.

The prevalence of atherosclerosis in general is not an exact figure but estimated by the prevalence of medication and study results. In paper II 2/3 of the 70 year old subjects had at least one vessel wall irregularity as a sign of atherosclerosis. In a meta-study compiling 14 world-wide studies on atherosclerosis with a total of 45828 subjects (52.6% women, mean age 58, age range 35-75), 12% were on statins and 24% were on hypertensive lowering medication.<sup>51</sup> Another study investigated 262 healthy heart donors with intravascular coronary US at a mean age of 33 years. Atherosclerotic lesions were seen in 52% of the subjects and in 17% among subjects <20 years of age.<sup>76</sup>

The natural history of atherosclerosis is well examined but new findings are still made continuously and it is questionable if we will ever fully understand the complex nature of the condition. The knowledge up to date makes atherosclerosis comprehensible in a large population, but the condition shows great variation between individuals due to the inherent and extrinsic variables, both in time of onset and speed of progression. The fact that some of the atherosclerotic findings (e.g. IMT) coincide with findings in normal ageing complicates the analyses. Another complicating matter is the variation in length of the subclinical phase (figure 1) as well as the severity of the first clinical symptom – from mild angina to fatal stroke or MI. But with the continuing research in this field and the addition of imaging in risk evaluation the knowledge and predictability of atherosclerosis will presumably keep on growing.

### **The test**

Although other imaging methods of assessing atherosclerotic burden and risk for CVE exist focus in this discussion will be put on whether the addition of WBMRA, MRI and/or US to traditional risk factors can improve the risk stratification.

Despite its lumenographic approach without actually depicting the vessel walls WBMRA has been proposed as a dependable method of identifying atherosclerosis by different studies,<sup>77-79</sup> and the manner of which the addition of TAS improved the prediction of outcome in paper III is encouraging. The reasonably short examination time (approximately 15 min of total patient

handling time) makes this method favorable in a screening perspective. However, the time for evaluation of images and calculating TAS will at least double that time, and both the examination and evaluation calls for specialized medical personnel.

Focal MRI depiction of the vessel walls is a more time consuming method, but since atherosclerotic changes in the carotids have been proven to correlate with general atherosclerosis<sup>80</sup> it appears reasonable to focus on this area alone. Some studies have revealed correlation between MRI identified plaques and CVE.<sup>81,82</sup> Paper II focused on the reproducibility of the method revealing excellent results. All these findings corroborate the potential of this method as a screening tool. Further advantages of MRI are the lack of ionizing radiation and its ability to differentiate between various soft tissues.

US has no contraindications per se although some subjects are more difficult to examine due to anatomical variations (e.g. short neck or tortuous vessels). With high frequencies US has at least equal resolution as MRI although some material (e.g. calcifications) hinder the sound waves. Additionally, paper II revealed encouraging reproducibility in the US analyses, although not as high as with MRI, in part owing to consistency in choice of a single experienced investigator within the study.

Since neither the sensitivity nor the specificity of either imaging method reaches a perfect 100% there will be false negatives and false positives. False positive findings can result in psychological effects such as increased anxiety, physical effects due to further testing with potential harm and complications, and economical consequences due to the time needed for further tests. This is well reported in the aspect of breast cancer screening by the use of mammography where the call back rate can be tenfold the rate of detected cancer<sup>83</sup> and the cumulative risk for false positive screening results during 20 years can vary between 8-21%<sup>84</sup>. When screening for atherosclerosis with imaging a false positive finding would equal a pathological finding that does not cause an event. And even though both WBMRA and carotid evaluation, examined with US in this thesis as well as with MRI in other studies<sup>81,85</sup>, improve the predictive value for CVE over mere traditional factors, there will probably always remain some false positives that would have to be treated as true positives with consequential treatment and possible side effects (psychological, physical or economical).

False negative findings can result in false reassurance leading to delayed detection with the possibility of a condition more difficult to treat at finding, loss of faith in the medical profession once the fault is discovered, legal implications such as legislation, and potentially increased costs to society due to these effects.<sup>86</sup> These consequences could very well arise in the wake of a falsely negative screening result. The results in paper III did however indicate that WBMRA is effective in ruling out the healthy individuals and it

would be interesting to evaluate the prospective value of this method in a younger and larger population.

A screening test should not cause discomfort to the subject, in this aspect US has an advantage over MRI, although most people cope very well with the requirements of lying absolutely still for the duration of an MRI examination. Some individuals are prohibited to undergo MRI, namely those with pacemakers and other incompatible metal objects. Claustrophobia can cause a hindrance and poor kidney function will exclude the use of contrast agents due to the risk of consequent morbidity. US examinations of the carotids are usually very well accepted.

The screening test should be reliable and comparable between all screening sites, in this aspect MRI has an advantage over US.

With methods focusing on specific areas such as the carotids, findings of pseudodiseases are uncommon. With WBMRA, though, most parts of the body are within the field of view making it possible to discover other potential or manifest conditions.<sup>87</sup> How to handle such incidental findings must be decided before the implementation of a SP.

Finally a screening test should preferably be inexpensive. In this aspect a US machine is by far cheaper than an MR machine, and the US examinations are not as costly as the MR ones.

### **The population**

Deciding the appropriate age for when a population should enter a SP for atherosclerosis is complicated by the long detectable preclinical phase. Even though early detection might make preventive measures more effective there is a risk of psychological effects due to the awareness of the condition over a substantial period of life and if medication is administered this will add a cost to society as well as putting the subjects at risk for side effects. Still, the SP should be administered early enough to identify those who might otherwise have succumbed to sudden cardiovascular death during their working years. In a SP in the UK people between the ages of 40-74 years were deemed appropriate to be screened<sup>88</sup>, and as the SMPA recommends the use of the HeartSCORE in the primary health care individuals between the ages of 40-65 years are consequently in focus<sup>16</sup>.

Even though men are more likely to be affected by atherosclerotic changes the prevalence for atherosclerosis and its effects is still high in both men and women, which makes it necessary to screen both sexes.

To make a SP worthwhile the population must adhere to the program, the treatment and possible follow-up exams. It has been established that discontinuation of long-time and life-time medication is higher in routine care settings than within clinical trials<sup>89,90</sup> as an example of the difference between the two populations, probably due to higher motivation in the trial setting. To motivate a screening population to participate it has to be educated on the purpose, the risk, the procedure, etc. which makes information to the popula-

tion extremely important. Due to the extreme heterogeneity of a whole country's population, both in the aspect of degree of education and language, this can be problematic and affect participation in the SP.<sup>23</sup>

Instead of nationwide screening, selective screening could be performed. In this kind of screening focus is put on specific populations with higher risk, such as diabetics, smokers or, as with opportunistic screening, those who visit health centers. These populations would probably have a higher prevalence of atherosclerotic changes which could justify the costs. However, ethically it might not be justifiable as some of those asymptomatic individuals with sudden severe CVE would still be missed.

### **The treatment**

There is no cure for atherosclerosis. The treatment is instead aimed at dealing with life threatening symptoms, preventing new symptoms and/or slowing down further progression of the condition. These measures are defined secondary if there is an existing symptomatic atherosclerotic condition and primary if the individuals are asymptomatic.

All treatments are associated with the risk of side effects. This risk is easier to accept if the cause of treatment is symptomatic. In the event of a SP, opportunistic or not, of an asymptomatic population the preventive measures are always primary and therefore side effects are accepted to a lesser degree.

Pharmacological treatments are used to address high cholesterol levels, risk of thrombosis and elevated blood pressure in order to decrease risk of CVE.

Statins lower cholesterol levels effectively by interfering with the cholesterol production in the liver. They are shown to stabilize the atherosclerotic plaques and even commence regression of the condition.<sup>91</sup> Since the advent in the 1990's<sup>92</sup> statins have become the drugs of choice in lowering lipid levels throughout the world as their preventive effect is well documented. In Sweden 10% of the population is taking statins and among 65 years old men the prevalence of statin use is 30%.<sup>93</sup> Some side effects are known to cause subjects to discontinue medication, such as myalgia, elevated liver enzymes and onset of diabetes. Myalgia seems to be more common in clinical practice (20%) than in randomized clinical trials (5%)<sup>94</sup> but most patients tolerate this side effect if well motivated<sup>89</sup>. Since most statins are available as generics the cost is low and recently the drug has become recommended in some studies already at the age of 40, in low-risk individuals.<sup>95</sup>

Antiplatelet agents decrease the ability of platelets to clot and are often prescribed with statins in secondary prevention. As primary preventive treatment they are recommended in the USA but not in Europe due to increased risk of bleeding.<sup>16</sup>

Hypertension is an important risk factor that must be considered when aiming to reduce the risk for CVD. It is important to rule out secondary hypertension as this condition requires further investigation and specific treat-

ment. The quantity of drugs available for treatment of primary hypertension and their different side effects are too complex to address in the scope of this thesis.

Smoke cessation is difficult to achieve. The best results are seen when medical intervention is used in combination with cognitive therapy.<sup>96</sup> Even though smoking is one of the major factors in atherosclerotic progression, cessation does not necessarily result in regression in the condition but rather halts the development. Overall, reduced risk of CVD, lung diseases and several forms of cancer outweigh the fact that smoke cessation can be linked with weight gain<sup>97</sup>.

Many of the risk factors (e.g. obesity, high cholesterol levels, elevated blood pressure, etc.) can be addressed and reversed with life style changes, a treatment with few side effects.<sup>98,99</sup> However simple in theory lifestyle changes have been proven very difficult to implement in practice.

The prevalence for asymptomatic but still significant carotid artery stenosis that might require surgical intervention is very low and in these rare cases it is currently believed that medical treatment is preferred due to higher risks with surgery.<sup>100</sup>

### **Availability**

With availability being a key issue in mass screening, MRI as a method becomes problematic. In Sweden MR-cameras can be found in most but not all cities. This is however not enough as some rural inhabitants have more than 300 km to the nearest MR-camera.

US is more easily available and with proper training this technique could be used in most health centers making it accessible to all.

What usually matters in the end is cost effectiveness. To date no nationwide SP for atherosclerosis, with or without imaging, has been proposed but the SMPA recommends opportunistic screening based on the HeartSCORE.

It could be argued that if a SP was to be established more individuals fulfilling the criteria for medication would be identified, this would add further costs to society due to the present system of government subventions for medication in Sweden. On the other hand these individuals would probably require less health care due to the decrease in events.

### **Evaluation & continuation**

Atherosclerosis is inherent to us all, although with very varying progression. The generally slow progression of atherosclerosis, as demonstrated in paper IV, makes it possible for relatively long intervals between assessments, but argues against one-off screening. In the UK a SP for opportunistic vascular risk assessment was presented in 2008 with the aim of assessing all citizens aged 40-74 years every fifth year by questionnaires, specific physiological parameters and blood tests.<sup>88</sup> The program was implemented in 2009 aiming for full roll out from April 2012 and hoping to enroll 3 million people by that

date. In 2013 “only” 1.1 million had received the NHS Health Checks. It has been argued that this is due to difficulties within the local authorities in meeting logistic and/or financial demands. It is estimated the program will become cost-effective in 15 years.<sup>101</sup>

Wilson & Jungner argued against one-off SPs as they would only pick up those affected with the sought after disease at that very moment.<sup>18</sup> Despite this, some active SPs are presented as one-off screenings at a certain age. For instance, in screening for AAA it is only those with an aortic diameter below a certain cut-off value are excluded from further screening. In the event of pathological findings the subjects are either included in a post-screening surveillance program or operated on. The level of cut-off may be lowered to increase the amount of individuals included in the following surveillance according to a recent study.<sup>101</sup>

The need for continuous evaluation is apparent and should comprise analyses of number of participants, the ability of the SP to detect atherosclerosis, results and effects (positive and negative) of the SP.<sup>20</sup>

## Do MRI and US fulfill the requirements for mass screening for atherosclerosis?

Before answering the question of imaging in screening one must decide on whether atherosclerosis should be screened for at all. At present, there is insufficient scientific evidence to support mass screening for atherosclerosis. For such a program to become feasible evidence would be needed from long-term prospective, randomized studies covering the relevant population, as well as health-economical evaluations. Clinical trials presented are not entirely translatable to a general population as these cohorts are associated with an element of selection. In a selected population the prevalence of the condition in question is usually higher than in the general population making the results, and thereby the implications drawn from them, somewhat skewed.

The opportunistic screening approach has similar issues. Even though it is believed that “most middle-aged persons contact healthcare facilities at least once during a two-year period”<sup>16</sup> those individuals will probably have more health issues than those not examined. Here one might argue that the ones caught in the screening net are the ones more likely affected by atherosclerotic disease. However this does not necessarily mean they are the ones that have the most to gain from preventive treatment.

If mass screening became a reality in the future MRI would probably not be the imaging method of choice since some of the criteria for mass screening, as set up by the WHO and SNBHW, cannot be met (lack of availability

in rural areas, its contraindications and the need for specialized medical personnel making it too expensive).

US with focus on the carotids is more easily available, less expensive and easier to perform. This makes it a more plausible tool for mass screening. In such an event presence of plaques might be the index of choice as CIMT measurements are not as strongly correlated to CVE. CIMT measurements are not recommended on an individual level at follow-ups due to the minute measurements close to the limit of ultrasonic resolution.<sup>102</sup> However, adding imaging with carotid US would only become relevant if the findings made a difference. If medication is already recommended, even in low-risk individuals, based on traditional risk factor analyses, imaging of plaques would not alter treatment. Similarly, in the last years medical treatment has increasingly been favored over surgical intervention in asymptomatic individuals with carotid plaques.<sup>103</sup>

Despite the fact that no method has yet overcome the difficulty of identifying the true positives among individuals at intermediate risk of CVE, opportunistic screening is in use in many countries.

Even if carotid US or MRI/WBMRA would be added to the traditional risk factors in a SP and the number of intermediates would be reduced (as in the middle line in figure 18) they would not disappear totally since atherosclerosis is such a complex condition with numerous risk factors, normal ageing being one. There might never be a perfect screening test as in the top line in figure 18.



Figure 18. **Illustration of screening tests for atherosclerosis.** The bottom line illustrates how traditional risk factors identify high-risk individuals (red) and healthy individuals (green) but have trouble classifying those in between (yellow). If these individuals of intermediate risk were to be further screened by the addition of MRI and US, among other methods, the risk stratification might be improved as in the middle line. Some individuals with intermediate risk will probably always remain as no perfect screening test for atherosclerosis, where those with no future cardiovascular event (CVE) can be separated from those with a future CVE, as in the top line, exists.

## Do MRI and US fulfill the requirements for selective screening for atherosclerosis?

As of now, MRI and US would fit better in a selective SP as part of a step-by-step program. The first step would be to use a scoring system like the FRS or HeartSCORE to sort out healthy individuals, either in a true mass SP or through opportunistic screening. Those with intermediate risk could then be further assessed with carotid US to improve identification of individuals in need of preventive treatment. Such a program could be started at the age of 40 years as prevention of sudden deaths in the working years is the main justification for the economical cost to society. A screening interval of five years should be sufficient.

Both the Multi-Ethnic Study of Atherosclerosis (MESA) starting in 2002 and the Screening for Heart Attack Prevention and Education (SHAPE) initiative starting in 2006 have suggested adding imaging to traditional risk factors to further stratify cardiovascular risk.<sup>104,105</sup> The MESA-study found coronary artery calcium (CAC) assessed by computed tomography (CT) to be of value and CAC was found to have a strong negative predictive value, as seen with TAS by WBMRA, where normal findings nearly excluded future CVE.<sup>106</sup> The SHAPE task force reports presence of plaque to be a valuable indicator for CVE, as does paper I in this thesis, and recommends against screening of those above the age of 75 years as they should all receive unconditional atherosclerotic treatment.<sup>107</sup>

With the High-Risk Plaque Initiative including the BioImage Study numerous imaging methods (US, CT, MRI and PET) will be tested with regard to their predictive value for CVE. This study also aims at creating a screened population comparable to the general population. The results are not in yet.<sup>108</sup>

In Sweden another large study, the Swedish cardiopulmonary bioimage study (SCAPIS), where 30000 men and women aged 50 to 65 years will be screened with CT, US and MRI and followed for several years, has just commenced.<sup>109</sup> Hopefully all these studies will help us better predict the outcome of atherosclerosis, and answer the question of whom to treat and whom not to treat.

## Do MRI and US fulfill the requirements for screening for atherosclerosis in research settings?

In a research setting both modalities are suitable for atherosclerotic assessment. In these settings MRI is suitable both for analyzing specific atherosclerotic changes and for giving a general estimation of the total atherosclerotic burden by WBMRA. The superb reproducibility of carotid MRI seen in

paper II suggests that the method is suitable for research situations and claiming smaller samples than if US was used. US might, however, become even more reproducible with the addition of contrast agents and 3D-technique. This has not been discussed in this work.

## Other ways of dealing with the atherosclerotic burden in the general population

Screening different populations for elevated risk factors or atherosclerotic changes are not the only way of dealing with the affects of atherosclerosis on the society as well as the afflicted individuals. As suggested in the 1<sup>st</sup> SHAPE guideline primary prevention of atherosclerotic risk factors should be the base in preventing CVE.<sup>107</sup> This could mean educating the population about preventive measures, facilitating physical activities and discourage negative behavior. Such campaigns should also focus on encouraging healthy behavior in early ages and increasing physical education in the school system.



# Conclusions

CIMT measurements from the bulb are better predictors of MI and ischemic stroke than CIMT analyzed in the CCA. (Paper 1)

The excellent level of reproducibility when analyzing plaque volume with MRI greatly reduces the required sample size in studies of the carotids, as compared to repeated calculations of plaque area with US. Plaque volume assessment with MRI and plaque area assessment with US are not interchangeable measures. (Paper II)

Analysis of atherosclerotic burden measured as TAS with WBMRA predicts MACE, independently of major cardiovascular risk factors. The risk for MACE is eight-fold if  $TAS > 0$  compared to FRS only. (Paper III)

CIMT at age 70, but not  $\Delta$ CIMT over five years (70-75 years), in the common carotid artery is related to stenoses throughout the arterial tree, as assessed by WBMRA, supporting CIMT as a general marker for atherosclerosis. (Paper IV)

For now, mass screening for atherosclerosis with WBMRA, carotid MRI or carotid US in addition to traditional risk factors is not feasible.

In a selected population (e.g. those with intermediate risk for CVE) imaging of atherosclerosis is a plausible addition to risk stratification with carotid US as the most likely method of choice.

Imaging of atherosclerosis with WBMRA and carotid MRI are well suited methods for screening in research settings. The costs of the method are outweighed by the reduced sample size needed due to great reproducibility.



# Acknowledgments

Starting my work on this thesis I was very reluctant towards doing research at all. It felt like a must and I just wanted to be a physician interacting with patients. But during the work I have come to realize that anything you focus on can become interesting – even MRI and atherosclerosis. I've learnt a lot, not just about the subject but about research, ethics, statistics and myself.

Writing a thesis is not a one person job, it takes a small village. I owe thanks to so many for giving me these insights and time, for helping me and believing in me in numerous ways. Here is my village; I hope I have remembered you all...

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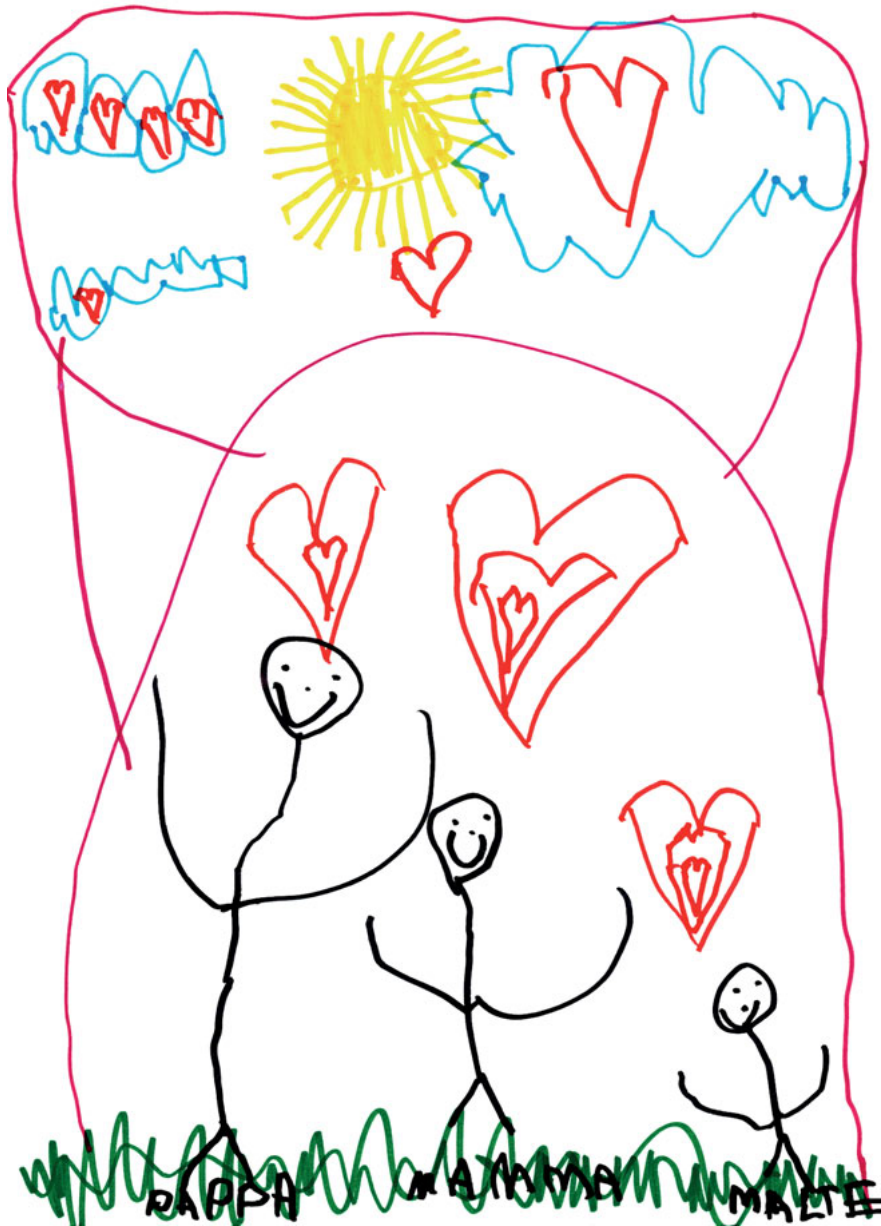
To my brother *Jakob* and his family, *Karin, Movitz, Lo* and *Valdemar*; thank you for support in so many ways; medical discussions, proofreading and choosing the appropriate fonts for the cover.

To my friends near and far; *Josefin M, Jenny L, Renée A, Yrsa K, Anna-Pya S, Inger R* and *Raquel T* with families, I may not be the most frequent phoner/mailler but every now and then when I get my act together you are always there – thank you!

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I saved the best for last: *Anders*, my husband, and *Malte*, my son; thank you both for bringing me love and laughter everyday (and a few grey hairs...). You keep me grounded and remind me of the real world beyond patients and papers. I love you!





## Summary in Swedish (graphic illustrations)

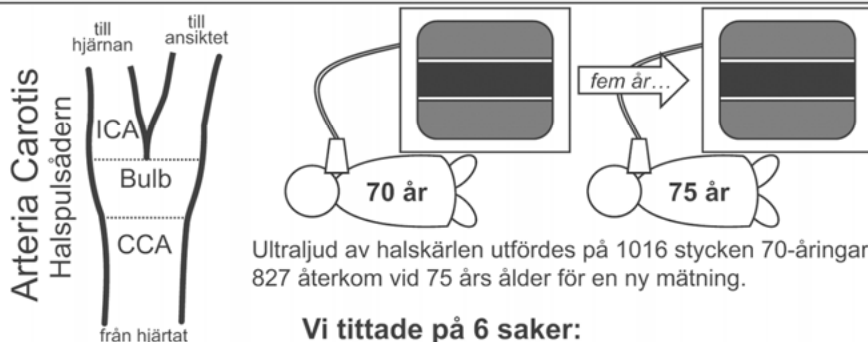
Den svenska sammanfattningen av avhandlingen ”Screening av åderförkalkning med magnetkameraundersökning och ultraljud” sker över de följande sidorna i grafisk form för att lätta upp den annars så kompakta texten. Först följer varje artikel sammanfattad på en sida vardera, därefter en summering av kappans diskussion.

# Arbete 1

## A Comparison between Different Indices of Carotid Artery Atherosclerosis at Ultrasound Regarding Risk of Incident Myocardial Infarction and Stroke

Inskickat manuskript

Man kan med hjälp av ultraljud mäta graden av åderförkalkning i halskärlen på olika sätt. Dels genom att mäta tjockleken av de två innersta vägglagren (CIMT), dels genom att titta på förekomsten och storleken av kärnplack, och dels genom att titta på gräskalan i plaquet (verkar det stabilt eller ömtåligt). I det här arbetet tittar vi på vilken av dessa metoder som bäst förutspår hjärtinfarkt och stroke och var i kärlet det är lämpligast att göra mätningarna.



### Vi tittade på 6 saker:

Hur tjock är kärnväggen i CCA?	Hur tjock är kärnväggen i bulben?	Finns det några plaque?	Hur många plaque finns det?	Hur stora är plaquen (area)?	Hur ser plaquen ut (gräskala)?
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Efter 10 år (när individerna var 80 år gamla) genomsöktes journalerna för att se vilka som fått hjärtinfarkt och vilka som fått stroke.

**CIMT-mått  
Plaque-värden  
Utfall**



Resultat:

★ Risken för hjärtinfarkt eller stroke är starkast kopplat till kärnväggens tjocklek i bulben

## Slutsats:

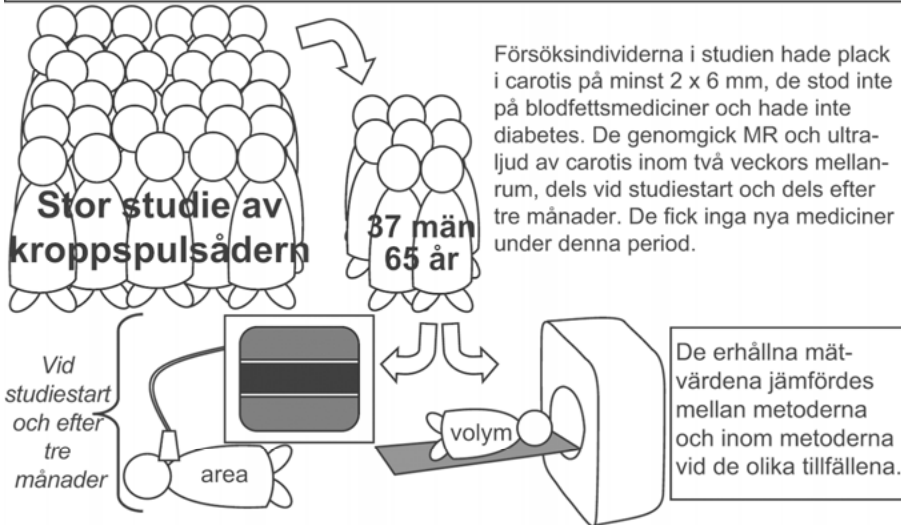
Trots att en del förespråkar att man ska mäta CIMT i CCA, bl.a. för att det är enklare och lättare att upprepa på samma sätt trots olika undersökare, är CIMT-mätningar i bulben bättre på att förutspå risken för hjärtinfarkt och stroke. Kärnväggstjockning i bulben ökar risken för utfall mer än 6 gånger.

## Arbete 2

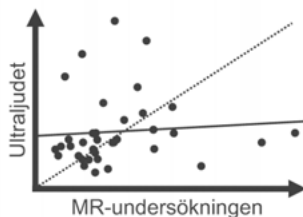
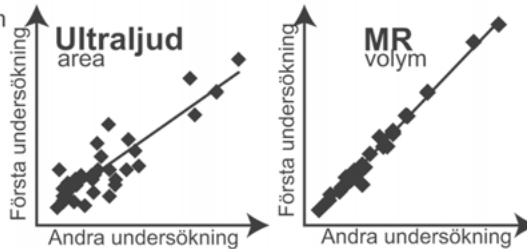
# Measuring carotid plaque size in asymptomatic individuals: comparison and reproducibility of MRI and B-mode ultrasound

Manuskript

För att utvärdera resultat i medicinska studier är det viktigt att dels kunna göra en god bedömning av det område i kroppen man förväntar sig resultat från, och dels kunna upprepa denna bedömning före och efter behandling. I det här arbetet jämförs MR-mätning av plaquevolym och ultraljudsmätning av plaquearea i halspulsådern (carotis), hur bra det går att upprepa metoderna, samt om metoderna är jämförbara.



Linjerna i diagrammen motsvarar var punkterna skulle hamna om mätningarna gav exakt samma utfall vid båda undersökningstillfällena. Punkterna i MR-kurvan ligger närmare linjen, därför är denna metod säkrare när det gäller att upprepa en undersökning av plack i halspulsådern.



När man jämför mätsätten ses inget bra samband (vid ett perfekt samband hade prickarna följt den streckade linjen, men nu blir resultatlinjen väldigt flack).

## Lärdomar:

1. Om man vill göra en studie av carotis utan allt för många individer är plaquevolym med MR att föredra framför plaquearea med ultraljud.

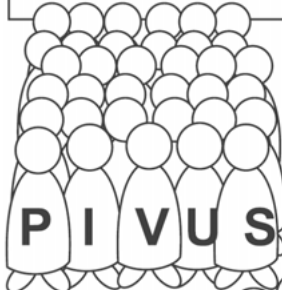
2. Ultraljud respektive MR av carotis är inte jämförbara metoder.

# Arbete 3

## Total Atherosclerotic Burden by Whole Body Magnetic Resonance Angiography Predicts Major Adverse Cardiovascular Events

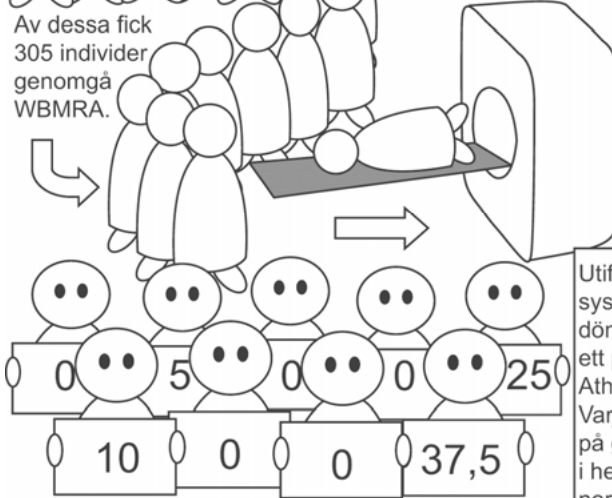
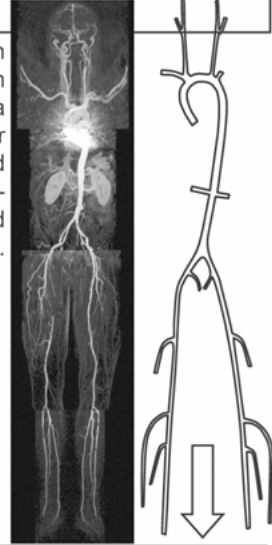
Atherosclerosis 2013 May; 228(1):148-52

Många dör i hjärt/kärlsjukdomar. Det är viktigt att identifiera de som behöver förebyggande behandling, men samtidigt utesluta de som inte ska medicineras, vilket görs på många olika sätt. Kan MR av kroppens kärl (WBMRA) underlätta att hitta de som behöver behandling?



Av dessa fick 305 individer genomgå WBMRA.

I den epidemiologiska studien PIVUS (Prospective Investigation of the Vasculature in Uppsala Seniors) har 1016 st 70-åringar undersökts noggrant med blodprover, blodtryck, ultraljud och enkäter med hälsorelaterade frågor.



Utifrån MR-undersökningen systematiserades och bedömdes kärlsystemet m. hj. a. ett poängsystem, TAS (Total Atherosclerotic Score). Varje individ erhöll ett värde på graden av åderförkalkning i hela kroppen, 2/3 hade normala kärl.

Sedan fick det gå nästan fem år...



...varpå det vid journalgenomgång visade sig att

# 25 av de 305

drabbats av MACE (hjärtinfarkt, stroke och/eller genomgått bypass eller ballongsprängning av hjärtats kranskärl).

### PIVUS-info Utfall TAS-värden



Resultat: ☆ ☆

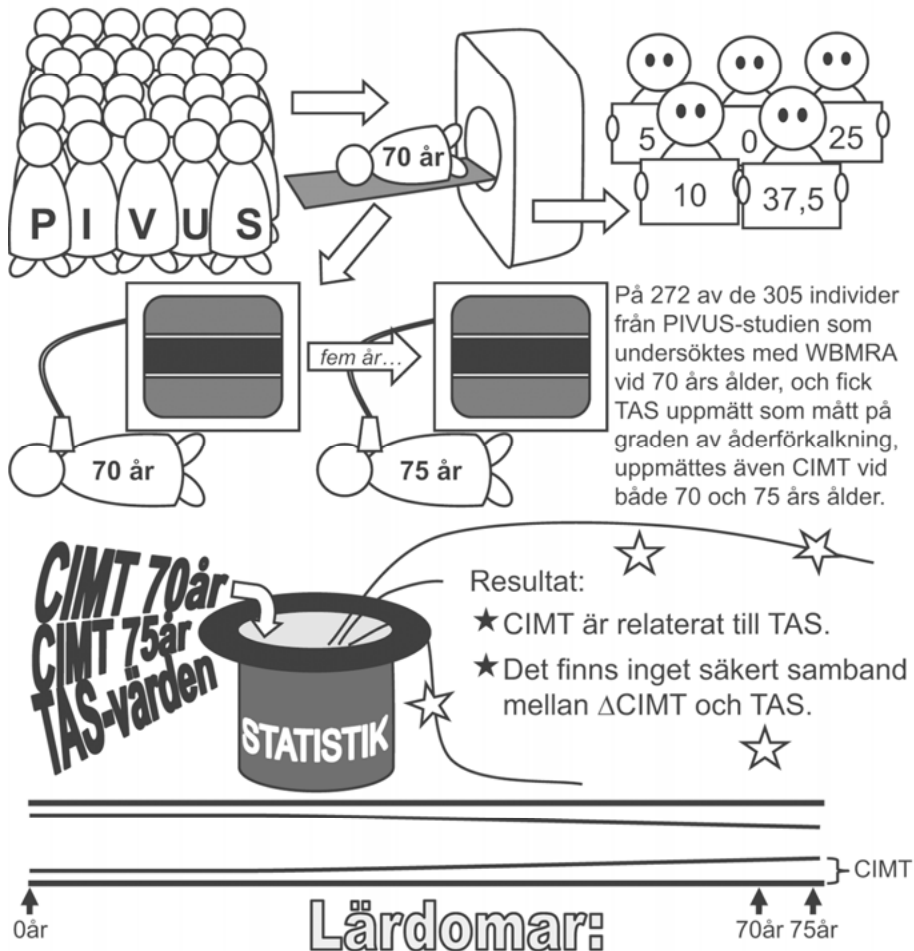
- ★ TAS förbättrar Framingham Risk Score i riskbedömningen av MACE.
- ★ Minsta åderförkalkning i kroppen ökar risken för MACE 8 gånger jämfört med normala kärl.

# Arbete 4

## On the Relation between Carotid Intima-Media Thickness and Global Atherosclerosis

Clinical Physiology and Functional Imaging 2014, 34:457-462

I halsen finns två stora kärl som heter carotis och leder blod från hjärtat till hjärnan. Kärlväggen består av tre lager. Genom att med ultraljud mäta den sammanlagda tjockleken på två av dessa lager får man fram ett värde som kallas Carotis Intima Media Tjocklek (CIMT). Har detta mått vid 70 års ålder, och förändringen i måttet mellan 70-75 år ( $\Delta$ CIMT), samband med den totala åderförkalkningen i kroppen?



1. Då åderförkalkning är en så långsamt tilltagande sjukdom har CIMT vid 70 år mycket större samband med den spridda sjukdomen än den lilla förändring som sker mellan 70 och 75 år.
2. Då CIMT visar sig överensstämma bra med kroppens generella åderförkalkning är det fortsatt en bra metod att använda för att utvärdera åderförkalkning.

# KAPPAN

Kappan är den del av avhandlingen som sammanfattar alla delarbeten och diskuterar metoder och resultat i ett större sammanhang. Här visas också den röda tråden som håller samman alla delarbeten. Jag har valt att fokusera diskussionen på screening.



## De fyra stora ämnena i denna avhandling:

### Aderförkalkning



- Uppstår tidigt men ger symptom senare i livet
- Beror på icke påverkbara (ärflighet etc.) och påverkbara (rökning, vikt, etc.) faktorer
- Orsakar många dödsfall/år
- Kan bromsas med mediciner och livsstils-förändringar men kan inte botas.

### Magnetkameraundersökningar (MR)



- Använder ingen röntgenstrålning
- Kan i en sammanhängande undersökning med kontrastmedel visa hela kroppens stora kärl
- Kan i detalj visa beståndsdelarna i kärnväggen i halskärlen
- Är ganska dyr, tar ganska lång tid och vissa individer kan inte genomgå undersökningen.

### Ultraljud



- Använder ingen röntgenstrålning
- Har mycket bra upplösning på ytligt belägna kärl, men sämre upplösning på djupet
- Är undersökarberoende
- Är billigt och väldigt tillgängligt.

### Screening



- Screening innebär att en population undersöks i syfte att hitta sjukdomar innan de gett symptom
- Det är väldigt viktigt att etiska hänsyn tas
- Sjukdomsbehandlingen måste leda till förlängt liv eller kraftigt minskat lidande
- Vid screening måste nyttan i populationen väga upp kostnaden för samhället.

## Uppfyller MR och Ultraljud kriterierna för screening i en stor population (t.ex. Sverige)?

1968 togs ett flertal kriterier fram åt WHO av Wilson och Jungner. Kriterierna ska vara uppfyllda för att screening ska anses försvarligt att genomföra. År 2008 kompletterade WHO kriterierna och år 2015 tog svenska Socialstyrelsen fram 15 egna kriterier baserade på de ursprungliga.

1979 beskrev Beauchamp och Childress fyra etiska principer som man, sedan dess, ansett vara viktiga att uppfylla i medicinska sammanhang och som man också måste ta hänsyn till när varje screening-kriterium värderas.

På nästa sida värderas om screening-kriterierna kan uppfyllas när det gäller att identifiera äderförkalkning med MR eller ultraljud.



De fyra etiska principerna som måste uppfyllas. Om två står mot varandra måste man bestämma vilken som är viktigast.

• **Tillståndet ska utgöra ett viktigt hälsoproblem**



Åderförkalkning är orsak till c:a en tredjedel av alla dödsfall (i världen, Europa och Sverige)

• **Det måste finnas en tillgänglig behandling som accepteras av populationen**



Man kan inte bota åderförkalkning utan behandlar de tillstånd som åkomsten leder till. Om man upptäcker riskfaktorer behandlas de med mediciner eller livsstilsförändringar.

• **Möjligheter för diagnosticering och behandling måste vara tillgänglig för alla**



Behandling är tillgänglig för alla i Sverige. Det finns god tillgänglighet till ultraljud, men inte fullt så god till MR.

• **Tillståndet måste kunna identifieras innan det ger symptom**



Detta är möjligt med MR och ultraljud, dock får inte alla med åderförkalkning symptom.

• **Det måste finnas ett tillgängligt test/lämplig undersökningsform**



De fyra arbeten som ingår i denna avhandling har visat att både MR och ultraljud kan vara lämpliga i kombination med kända riskfaktorer (blodfetter, blodtryck, rökning, m.m.)

• **Testet/undersökningen måste accepteras av befolkningen**



Alla kan inte genomgå MR med kontrastmedelsinjektion. En stor majoritet av befolkningen kan genomgå ultraljud.

• **Tillståndets naturliförlopp ska vara känt**



Detta är redan välkänt men forskningen hittar fortfarande ny information.

• **Man ska veta säkert vilka som behöver behandling**



Detta kriterium uppfylls hos de säkert friska och de kraftigt drabbade, men det finns fortfarande en stor grupp där behovet av behandling är oklart

• **Kostnaden för screeningprogrammet måste vägas upp av hälsovinster**



Mycket svårvärderat i detta sammanhang, sannolikt är det inte ekonomiskt försvarbart.

• **Screeningprogrammet ska vara återkommande**



Detta talar mot MR men för ultraljud med tanke på kostnad och tillgänglighet.

### *Slutsats:*

Då alla "trafikljus" inte lyser grönt är screening av åderförkalkning med MR eller ultraljud i en generell population ännu inte aktuell.

Däremot kan man tänka sig MR, eller mer sannolikt ultraljud, som en tilläggs-screening för de som inte friskförklarats med de vanliga riskfaktorena.

Redan nu kan metoderna med fördel användas inom forskningen.

Det är då viktigt att välja lämplig metod från början och veta att olika mätmetoder inte alltid är jämförbara.



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