# A systematic review of histology and imaging methods detecting carotid plaque components associated with ischemic stroke

Master Thesis in Medicine

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# Abstract

**Background:** Rupture of atherosclerotic plaques in the carotid circulation is one of the major causes of ischemic stroke. The recent risk-stratifier for evaluating stroke risk is based on area of lumen narrowing although recent research indicates that plaque components and morphology might be of greater importance. A lot of research is in progress with the aim to find a cost-effective and non-invasive imaging method that adequatly can detect plaque components associated with an increased risk of creating ischemic events.

**Objective**: The aim of this systematic review was to evaluate the scientific evidence for which imaging method of CT and MRI that best identify carotid plaque components associated with an increased risk of creating ischemic events.

**Methods**: Two literature searches (one for each imaging method) was performed in PubMed and Cochrane Central Register of Controlled Trials (CENTRAL). Inclusion criterias was defined and used to select relevant articles. Data were extracted and presented in tables. The quality of the individual articles was assessed. Statistical analyzes could not be performed.

**Findings**: The literature search resulted in 648 articles about CT and 915 about MRI. 6 articles about CT and 8 articles about MRI was included in the analysis. Direct comparisons between studies were not possible because of incomparable methods and outcome measures. Due to this it was not possible to perform any statistical analyze. Instead, a table highlighting the diversity between the articles was performed for each imaging method. However, the results indicated that MRI is a more researched method with better ability to identify plaque components than CT.

**Interpretation**: There is a significant gap in scientific knowledge on this subject. The researchers will need to agree on inclusion criterias, methods to perform imaging, and the use of outcome measures. A standardized list of recommendations of how to perform research on this subject needs to be established in this field. In combination with larger trials and representative populations, the results could be comparable, analyzes could be performed and conclusions be reliable.

**Keywords**

”Carotid stenosis”, ”Vulnerable plaque”, ”Ischemic stroke”, ”Computed Tomography”, ”Magnetic Resonance Imaging”

# Abbrevations

| | |
|---|---|
| AHA | American Heart Association |
| CEA | Carotid endarterectomy |
| CENTRAL | Cochrane Central Register of Controlled Trials |
| CT | Computed tomography |
| CVD | Cardiovascular diseases |
| DSA | Digital subtraction angiography |
| DSCT | Dual source computed tomography |
| FC | Fibrous cap |
| HU | Hounsfield units |
| IPH | Intraplaque hemorrhage |
| LDL | Low density lipoproteins |
| LRNC | Lipid rich necrotic core |
| MDCT | Multi detector computed tomography |
| MRI | Magnetic resonance imaging |
| NPV | Negative predictive value |
| OCT | Optical coherence tomography |
| PET | Positron emission tomography |
| PPV | Positive predictive value |
| QUADAS | Quality Assessment of Diagnostic Accuracy Studies |
| ROC | Reciver operating characteristic |
| SBU | Statens beredning och utvärdering |
| SCM | Sternocleidomastoid muscle |
| SMC | Smooth muscle cells |
| US | Ultrasound |
| WHO | World Health Organization |

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# Background

Cardiovascular diseases (CVDs) are the leading causes of death and decreased life quality in the world. According to the World Health Organization (WHO), CVDs caused 17.3 million deaths in 2008, where heart attacks accounted for 7.3 million deaths (46% of all CVDs) and stroke 6.2 million deaths (34% of all CVDs) (1).

## Stroke pathophysiology

The pathophysiology of stroke includes different mechanisms essential for understandning the disease. The most common mechanism (87% of all strokes) is *ischemic stroke,* due to obstruction of an artery that serves the brain with blood resulting in ischemia of the tissue perfused by the artery (2). The other mechanism is bleeding due to rupture of weakened blood vessels within the brain resulting in an *hemorrhagic stroke* (13% of all strokes) (1, 2). Ischemic strokes is either caused by formation of a blood clot at the site of the damaged part of the vessel, *a thrombus*, or caused by formation of a blood clot in another part of the circulation, *an embolus* that travels with the blood to the brain where it occludes smaller vessels (2). These are usually formed in the vessels of the upper chest or the neck (usually the carotid arteries), or in the atrial chamber of the heart where fibrillation of the heart causes forming of blood clots with the potential of travelling with the blood to the brain.

## Atherosclerosis

The main underlying pathogenesis for cerebrovascular diseases as well as for the diseases in the coronary arteries is atherosclerosis, a process affecting the large arteries. It is not a degenerative consequence of aging, but a slow, progressive disease that is characterized by a chronic inflammatory condition and formation of an atherosclerotic plaque within the artery

wall (3). It is a highly complex process where many conflicting theories exist about several stages in the development. Furthermore, most of the research is conducted on animals with questionable correspondence to the pathology in humans (4).

Although, definite evidence of the details in the pathology is lacking, the major steps in the development of atherosclerotic plaques have been established. The process begin when changes occurs in the matrix of the vessel wall and the single cell layer covering the inner wall of the artery, *the endothelium* (4). This lets LDL (Low density lipoproteins) particles to attach to structures the inner layer of the artery wall, *the intima* (5). The LDL particles are oxidized into proinflammatoric molecules that stimulates inflammation within the artery wall (5). The inflammation recruites monocytes from the blood that engulf the lipids, and thereby they become foam cells (macrophages filled with lipids) (4). Eventually the foam cells die, which contributes to the forming of a necrotic core of the lipids leaking out from the foam cells (3). Cytokines released from inflammatory cells within the arterial wall, recruits smooth muscle cells (SMC) to the intima where they produce extracellular matrix that forms a fibrous cap over the necrotic core of lipids (4). This process continues within the arterial wall thus resulting in plaque formation that within time results in narrowing of the artery lumen.

However, the fibrous cap can eventually weaken and rupture, thus exposing underlying tissue to the flowing blood and thereby initiate formation of a thrumbus or embolus (6). Although atherosclerosis develops over decades, it has the potential to manifest in acute events causing dramatic and sudden clinical events.

## The vulnerable plaque

According to WHO, CVDs are eminently available for preventive intervention with the aim to

reduce cost and health related burden both on individual and population level (1).

There are several riskfactors known to contribute to the development and progress of CVD. The current most well-known risk factor for cerebrovascular events is high-grade luminal narrowing of the internal carotid arteries (7). Preventive actions like carotid endarterectomy (CEA) has shown to be beneficial for patients with symtomatic ipsilateral high-grade (70-99 percent) internal carotid stenosis (8). The golden standard to measure the grade of the stenosis is Digital Subtraction Angiography (DSA)(9), although other methods such as duplex ultrasound is widely used in the clinic due to its simplicity and cost-effectiveness (10). Even though high-grade stenosis is an important risk factor for ischemic events, all patients will not experience clinical manifestations (7). Additionally, patients with low-grade stenosis can experience clinical manifestations. This has lead to a growing interest of other factors than the degree of the stenosis determining the risk of plaque rupture. A new concept of the *vulnerable plaque* has developed, where the emphasis has shifted to focus on plaque composition, morphology and structure as determinants of risk for rupure and clinical events (7, 11-18).

## Histological classification

The golden standard to examine plaque morphology is by histology (18, 19). In 1995, the consensus group of the American Heart Association (AHA) published a report which describes components and pathomechanisms of coronary plaques detected by histology and correlated to clinical symtoms (20). The content of the report is a histological classification that is still widely used to classify plaques based on composition and morphology. Plaques or *lesions* are divided into eight groups (see table 1) graded from precursor lesions (I-III) to advanced lesions (IV-VIII) (20).

**Table 1. Description of plaque types according to the AHA classification scheme.**

| Plaque type | Description | Characterization |
|---|---|---|
| I | Thickening of the intima layer of the vessel | Precursor lesion |
| II | Presence of a fatty streak | Precursor lesion |
| III | Transitional/intermediate lesion (preatheroma) | Precursor lesion |
| **IV** | **Core of extracellular lipid with well-defined region of the intima (*atheroma*)** | **Risk of rupture** |
| **V** | **Fibrous connective tissue overlying the lipid core (*fibroatheroma*)** | **Risk of rupture** |
| **VI** | **Complicated plaques with surface defects, and/or hematoma-hemorrhage, and/or thrombosis (*complicated lesion)*** | **Risk of rupture** |
| VII | Calcified plaque | Stable lesion |
| VIII | Fibrotic plaque without a lipid core and with possible small calcifications | Stable lesion |

Three lesions types (IV-VI) were considered to be relevant in this thesis since these are associated with highest risk of rupture. The type IV lesion, *the atheroma,* is characterized by a severe disorganization in the intima caused by the accumulation of extracellular lipids thus forming a lipid core in the vessel wall. Type V, *the fibroatheroma,* is characterized by a fibrous cap covering the lipid core. It can be of various thickness, sometimes even thicker than the underlying lipid acculumation and consist of a an increased amount of smooth muscle cells and collagen. Type VI, *the complicated lesion,* develops from type VI or V when surface defects like ulceration or fissures occur, causing hemorrhage or thrombus formation within the plaque. In summary, there are three components that distinguish these three groups: the LRNC (Lipid Rich Necrotic Core) for type IV, the FC (Fibrous Cap) for type V and the IPH (Intraplaque Hemorrhage) for type VI.

Even though the AHA report is based on examination of coronary plaques, it is convenient to use the same classification scheme for carotid plaques since the mechanisms behind plaque instability are similar to those in the coronary circulation (13, 20, 21).

## Imaging methods

The shift of focus to the concept of the vulnerable plaque has created a need for new imaging techniques since DSA provides no information of plaque composition (17). There is a need to find non-invasive, fast and cost-effective imaging methods that gives information about plaque composition and morphology. The current most well studied methods are Computed Tomography (CT), Magnetic Resonance Imaging (MRI) and Ultrasound (US). Although other methods, for example Positron Emisson Tomography (PET-scan), Optical Coherence Tomography (OCT) and different molecular imaging methods, are also being studied. This thesis focuses on CT and MRI.

### **Computed Tomography (**CT**)**

CT is a technique using special x-ray beams that are submitted through the patient and detected by sensors. These signals are analyzed by computer that with complex mathematical algorithms enable image reconstruction (22). Different components are differently attenuated, which is measured in Hounsfield Units (HU). Based on the the large amout of measurements of attenuation coefficients, the image can be reconstructed in the computer. The beams and the sensors register while physically moving around the patient which enables reconstruction of 3D images. In order to visualize blood vessels, CT angiography can be used. This technique will scan the patient while a contrast material (usually iodine) is injected through a catheter in a vein (23).

Recently, several new types of CT scanners has developed. Multislice CT scanners (MDCT), which allows multiple slices to be imaged in a shorter period of time. Dual source computed tomography (DSCT) uses two X-ray source/detector systems that rotate simultaneously around the patient. The use of different energy levels gives high temporal resolution and the

time required is almost the half compared with conventional CT (24). Although these methods gives more detailed information on tissue components, the radiation dose is controversial (25).

**Magnetic Resonance Imaging (MRI)**

The human body consist of a large amount of hydrogen atoms. When placed in a magnetic field, these hydrogen atoms are aligned with it. By disturbing the atoms with radiowaves, the polarity changes. Depending on the water content of the tissue, the time for the atoms to return to their original position varies and this time is detected by a sensor. Since different tissues contain different amount of water, they will give different signals (26). The signals are interpreted by a computer that processes and converts these to images of different black-white scales. In the MRI hardware, different types of coils are used as the receivers or transmitters for MRI signals. Depending on the volume of the examined tissue, different coils can be used to improve the resolution (27).

# Objectives

A lot of research is in progress to find non-invasive imaging methods that can detect high-risk components within the plaque. By studying the correlation of components detected with imaging methods and histology, the direct correlation between morphology and the ability of the imaging methods to identify different components is evaluated.

The aim of this review was to evaluate the scientific evidence for which imaging method of CT and MRI that best identify carotid plaque components associated with an increased risk of creating ischemic events.

# Significance of study

If non-invasive methods adequately can detect components associated with an increased risk of creating ischemic events, the management of atherosclerotic disease would be facilitated. Cost, time of procedure and health risks related to invasive procedures would be diminished. It might also lead to identification of new components that could not be visualized by previous methods. This review will hopefully contribute to increased knowledge and development of non-invasive imaging methods. In the future, imaging methods could be used to screen populations to identify atherosclerotic plaques with higher risk of creating ischemic events and thereby preventive actions could be taken to prevent disease.

# Method

Information on the subject was collected through hand-search on the internet and in textbooks in neurology and neurosurgery. Two PIRO (a modifed PICO structure including Population, Index test, Reference test and Outcome), one for each imaging method, was determined (see table 2).

**Table 2. Two PIRO questions were set up to define the issue of the thesis.**

| | **PIRO 1** | **PIRO 2** |
|---|---|---|
| **Population** | Patients with carotid plaques | Patients with carotid plaques |
| **Index test** | CT | MRI |
| **Reference test** | Histology (PAD) | Histology (PAD) |
| **Outcome** | Agreement (Specificity, Sensitivity etc.)<br>1) type IV plaque<br>2) type V plaque<br>3) type VI plaque | Agreement (Specificity, Sensitivity etc.)<br>1) type IV plaque<br>2) type V plaque<br>3) type VI plaque |

## Eligibility criteria

Inclusion criterias were set up equal for the two PIRO questions. Only articles in english were determined to be included. Concerning article age limit, it was initially set to include articles published in the last 5 years but was changed to the last 10 years. The reason for this was that a significant amout of relevant literature published in the mid 2000 was found and a relatively small amount in the last 5 years. Study characteristics was set up to patients over 40 years in order to exclude studies of hereditary atherosclerotic diseases. The number of patients was limited initially to >25 patients. This limit had to be ignored for PIRO 1 (CT) during the selection process due to a small amount of literature that matched this criteria (see section study selection). All other articles other than original articles were excluded. Studies performed on coronary arteries and not concurrent with existing PIROs were excluded. Studies performed on ex-vivo plaques, meaning imaging by CT or MRI after plaque extraction, were excluded. Inclusion and exclusion criterias are summerized in table 3.

**Table 3. Inclusion- and exclusion criterias**

| **Inclusion criterias** | **Exclusion criterias** |
|---|---|
| English language articles | Reviews, case reports, editorials etc. |
| Published latest 10 years (2004-2014)* | Studies performed on coronary circulation |
| Patients >40 years | Not concurrent with PIRO |
| Studies with >25 patients** | Ex-vivo characterization |

*** This limit was initally set to 5 years but was changed after the first literature search.**
**** This limit was ignored for PIRO 1 (CT) during the selection process.**

## Literature search

The literature search was performed in two databases, the Cochrane Central Register of Controlled Trials (CENTRAL) and Pubmed. For each studied imaging method, one literature search was performed in each database. Thus, resulting in four separate literature searches. Different keywords was used based on the PIRO structure except keywords for O (outcome)

which were excluded. The search included MeSH-terms (the vocabulary for indexing articles used by a range of medical databases) and free-text words in order to include all recently published articles missing a MeSH index. These terms were combined with the Boolean operator OR for each group of the included PIRO (Population, Indextest and Referencetest) and the final search string was set up by combining the groups with the Boolean operator AND.

## Study selection

Studies were selected according to the predetermined inclusion criterias. First by reading titles, then abstracts and finally full-text articles. When issues came up that had not been considered in advance, thus not mentioned in the inclusion criterias, a modification of these was performed. When sorting out articles for PIRO1 (Computed Tomography) the inclusion criteria for number of patients had to be changed since it showed only a few number of articles matched the criteria. Concerning MRI, a significant amount of studies included over 25 patients thus the limit was respected.

Articles read in full-text were screened for additional references. In addition, two prominent researchers within field was contacted and asked for additional references. These articles were read and sorted by relevance first by title and abstract, followed by full-text. All additional references read in full-text was reported. In order to overview the selection processes, a flow diagram ”Preferred Reporting Items for Systematic Reviews and Meta-Analyses” (PRISMA) was used for each PIRO.

## Data extraction and presentation

When articles were assessed for relevance and suitable for inclusion, data were extracted and presented in tables with individual support from Ola Samuelsson at HTA-centrum (the Health Technology Assessment at Sahlgrenska University Hospital). Headlines used was author/year/country, study design, number of patients/plaques, dropouts, gender, mean/median age, type of patients (symtomatic/asymtomatic), diagnostic test (Index vs Reference), use of contrast agent, outcome variables reported in the study, translated AHA type, time between index and reference test, measurement of agreement and quality of study. For each outcome variable (AHA type IV, V and VI) and PIRO (1 and 2) an inclusion table was performed.

The outcome variables consists of the three AHA groups IV, V and VI which are characterized by specific histological components (see background, histological classification). In a significant proportion of the included articles, the study variables were not presented in AHA groups or components directly correlating to the groups. To enable comparison, these had to be transformed by the author into as comparable components as possible with support from table 1. Therefore outcome variables as reported in the study and the translated type are presented in the inclusion tables.

## Risk of bias in individual studies

The quality of the articles was evaluated with support of the template Quality Assessment of Diagnostic Accuracy Studies (QUADAS) for assessing quality of articles from SBU (Statens Beredning och Utvärdering). The template was modified to suit the actual scientific issue thus resulting in exclusion of three questions from the original template. One question concerning conflicts of interest (see modified template in appendix) was added. The basis for assessment

of the individual questions was set up before the quality was validated. A patient spectrum bias was defined to exist if the study did not include both symptomatic and asymptomatic patients. A disease progression bias was defined to exist if the mean time between the index and reference test exceeded 30 days. The template was applied for each one of the included studies for PIRO 1 and 2 and the quality was determined as high/moderate/low.

### Data analysis

Two aspects were considered in the analysis of the included articles; outcome measures and the methodological approach. Outcome measures were presented in analyze tables for each outcome and PIRO. The variable/variables used to analyze the agreement between imaging and histology and the inter-/intraobserver agreement was presented. The methodological approach was presented in one table for each PIRO highlighting the overall technical differences concerning imaging technique and differences in the analysis of plaque. Headlines used for PIRO 1 was Author/Year/Country, Method to measure density, Method to define density, Landmarks used to match histology and imaging slices, Method of CEA, Extraction of calcium during image processing, Contrast administration and Method of histologic assessment. Headlines used for PIRO 2 was Author/Year/Country, MRI protocool, Contrast administration, Method to detect components, Landmarks used to match histology and imaging slices, Method of CEA and Method of histologic assessment.

## Ethics

This systematic review consists of data retrieved from published scientific articles and therefore did not require consideration of ethical issues. The purpose is to elucidate an up-to-date and new perspective on the subject in order to approach the aim of finding a better method of assessing atherosclerotic disease. This might improve the selection of patients for

preventive actions in the future and therefore decrease the mortality and morbidity of ischemic stroke.

# Results

## Study selection

### PIRO 1 Computed Tomography (CT)

The literature search generated 648 articles (640 in pubmed and 8 in CENTRAL) (search tables for each database are provided in the appendix, table 4 and 5). Two prominent researchers in the field was contacted and asked for additional record which resulted in 3 additional articles. Records were screened for duplicates which resulted in exclusion of 2 articles. Then articles were read by title followed by abstract and all articles not matching the inclusion criterias was excluded which resulted in exclusion of 626 articles. Thereby, 25 articles remained to be read in full-text. All articles read in full-text were screened for references by reading first title then abstract, which resulted in 2 additional references matching the inclusion criterias. (see appendix for additional references, table 6, and flow diagram, figure 1, for selection of articles). 19 of the 25 articles read in full-text were excluded (see appendix, table 7, for reasons of exclusion). 6 articles remained to be included in the analysis.

### PIRO 2 Magnetic Resonance Imaging (MRI)

The literature search resulted in 915 articles (892 in pubmed and 23 in CENTRAL) (search tables for each database are provided in the appendix, table 8 and 9). Records were screened for duplicates which resulted in exclusion of 5 articles. Then articles were read by title followed by abstract and all articles not matching the inclusion criterias were excluded which resulted in exclusion of 888 articles. Thus, 25 articles remained to be read in full-text. All

articles read in full-text were screened for references by reading first title then abstract, which resulted in 3 additional references matching the inclusion criterias. (see appendix, table 10, for additional references and flow diagram, figure 2, for selection of articles). 17 of the 25 articles read in full-text were excluded (see appendix, table 11, for reasons of exclusion). 8 articles remained to be included in the analysis.

## Quality assessment of included studies

### PIRO 1 Computed Tomography (CT)

The quality was overall acceptable for all 6 included studies. One article (17) was assessed as low quality and 5 as moderate quality (contact author for full information about quality assessment of each article). All studies declared reasons for droputs. 3 studies did not include both symptomatic and asymtomatic patients, thus only 3 studies included a representative population. In 4 studies, pathologists were blinded for the results of the indextest (CT) while the radiologists were blinded for the reference test (histology) in 2 studies. No study reported non- interpretable results. The risk of conflicts of interest affecting the result was determined as non-existent in 4 studies while it was unclear in 2 studies.

### PIRO 2 Magnetic Resonance Imaging (MRI)

The quality was overall good for all 8 included studies. One article (28) was assessed as being of low quality, 5 as moderate and 2 articles (29, 30) as high (contact author for full information about quality assessment of each article). All studies declared reasons for droputs. 5 studies did not include both symptomatic and asymtomatic patients, thus only 3 studies included a representative population. In 2 studies, pathologists were blinded for the results of the indextest (MRI) while the radiologists were blinded for the reference test (histology) in 4 studies. No study reported non-interpretable results. The risk of conflicts of

interest affecting the result was determined as non-existent in 5 studies while it was unclear in 3 studies.

## Study characteristics

### PIRO 1 Computed Tomography (CT)

Inclusion tables for the three outcome variables (AHA type IV, V and VI) are provided in the appendix (table 12, 13 and 14). The studies showed overall many differences regarding several aspects. However all studies were cross-sectional. Contrast agent was used in all studies, but varied between Iodine, Iodine+Saline chaser and Iopamidol. In two studies specific agent was not reported. Number of patients varied between 8 and 51. Number of dropouts varied between 0 (in a study with 8 patients(15)) and 39 (in a study with 51 patients(14)). All studies included a significant larger number of men than women. The mean age varied between 62 and 71.4 years. 3, out of 6, studies included both asymtomatic and symtomatic patients. MDCT was studied in all studies except one (17) where DSCT was studied. The outcome was reported in AHA types in 3 studies although one had studied type IV and V as the same outcome (17). The outcome in the other 3 studies was translated into suitable AHA types by the author. The time between the index test and reference test varied between 2 days (17) and 3 months (16).

### PIRO 2 Magnetic Resonance Imaging (MRI)

Inclusion tables for the three outcome variables (AHA type IV, V and VI) are provided in the appendix (table 15, 16 and 17). The studies showed overall many differences regarding several aspects. However all studies were cross-sectional. Contrast agent (Gadolinum) was used in one study (31). Number of patients varied between 27 and 70. Number of dropouts

varied between 0 (in a study with 35 patients (32)) and 38 (in a study with 70 patients (33)). All studies included a significant larger number of men than women. The mean age varied between 67.7 and 71 years. 4 studies included both asymtomatic and symtomatic patients, 2 included only symtomatic patients (28, 32) and 2 had not specified prevalence of symptoms (30, 31). 1,5 T MRI was used in all studies but the use of coil types varied. The outcome was reported in AHA types in one study (32) but translated to AHA types in the remaining 8 studies. The time between index test and reference test was 7 days in five studies, 20.5 in one study (32) and not reported in 2 studies (28, 34).

## Agreement analysis

### Outcome measures

#### PIRO 1 Computed Tomography (CT)

Analyze tables for each outcome variable are provided in the appendix (table 18, 19 and 20). The overall agreement of outcome measures showed a significant heterogenity. Density was presented in Hounsfield Units (HU) in all but one study (14) where it was presented with ΔHU (difference in HU between early and late phase contrast). The cut-off value for differentiation between components showed little conformance since it was measured between a variety of components and with variable methods. A variety of outcome measures was used, 2 studies analyzed the agreement with coefficient of determination ($R^2$) (15, 16), one with Pearson correlation coefficient (14), 2 with Cohen's kappa (15, 17) and 2 with sensitivity and specificity (35, 36). Only one study presented interobserver variability (between different radiologists) (16) while no study presented intraobserver variability (multiple assessments of one radiologist).

**PIRO 2 Magnetic Resonance Imaging (MRI)**

Analyze tables for each outcome variable are provided in the appendix (table 21, 22 and 23). The overall agreement of outcome measures shows a fairly significant heterogenity. Cut-off values were presented for 2 studies (33, 37). In one study (31) plaque enhancement (after contrast administration) was compared with plaque composition by comparing MRI images with histological slices obtained after extraction and presented with fractional plasma volume, $V_p$ and transfer constant, $K_{trans}$. In another study (33) the signal intensity was defined from comparison with a reference material. Different weighted images were not specified in all studies. In one study (37) the outcome measures were defined separately for each sequence. A variety of outcome measures were used, 2 studies analyzed agreement with Pearson correlation coefficient (r) (30, 31), one with a Bland-Altman plot (28), 2 with Cohen's kappa (29, 30) and 4 with sensitivity/specificity (one also with Positive Predictive Value (PPV) and Negative Predictive Value (NPV)). 5 studies presented interobserver variability and 3 studies presented intraobserver variability.

**Methological approach**

**PIRO 1 Computed Tomography (CT)**

Tables of diversity are provided in the appendix (table 24). The overall agreement of methodology varied significantly. The method to measure density varied significantly from manual to computerized processing of images and in 2 studies both methods were used (15, 16). Furthermore, the method for analyzing density manually varied (only 2 studies used the same method (35, 36)). Concerning the studies using computerized software, one study (16) used a custom made plug-in software (ImageJJ) while the other (15) used an algorithm specifically developed for this study. Regarding methods to define density, one study used predefined Hounsfield values to define different components (17) while 4 studies measured it

after imaging. 4 studies reported cut-off values to distinguish between components, two by performing ROC (Reciver operating characteristic) analyzes (35, 36) one based on the predetermined values (17) and one by determining the halfway attenuation between mean densities for each components (15). In 4 studies, the carotid bifurcation was used as a landmark to match histologic and imaging slices. 2 studies did not report this parameter (14, 17). No study reported the method of how the CEA was performed. Extraction of calcium was performed in one study (14). Standardized protocols for contrast administration was used in 3 studies. 2 studies used a test bolus technique (15, 17), one assessed peak enhancement after graphical presentation at the aortic arch to optimize the timing of images to contrast enhancement (17). Histological assessment of components was performed manually in all studies.

**PIRO 2 Magnetic Resonance Imaging (MRI)**

Tables of diversity are provided in the appendix (table 25). The overall agreement of methodology varied significantly. 3 studies used standardized protocols to perform MRI although only one of these had specified type of protocol (30). One study (31) quantified contrast agent dynamics by using a generalized kinetic model for dynamic contrast enhanced MRI imaging. The remaining 4 studies did not specify how the imaging was performed. Only one study (31) used contrast agent (gadolinium-based). The method to measure density varied significantly from manual to computerized processing of images and in 2 studies both methods were used (28, 37). Although 5 studies used the sternocleidomastoid (SCM) muscle as reference tissue for determining components, one study (32) did not report method to detect components. 5 studies used the carotid bifurcation and the shape of the lumen as landmarks to assure correlation between imaging- and histology slices. 3 studies did not report landmarks. 2 studies specified how the CEA was performed (32, 33). Histological assessment of components was performed either manually, with computerized methods, or

with a combination of these. In addition, all studies used different manual methods as well as different software for computerized analyze when analyzing components.

### Synthesis of results

No GRADE-analyze or meta-analysis could be performed due to major differences between studies regarding methods. This review also found an inconsequent use of outcome measures. This inherent heterogenity made statistical analyze impossible to perform. Although there are indications (as described above in study characteristics, quality assessment of included studies) that MRI is a more explored method to image atherosclerotic plaque components. This will be discussed further in discussion and conclusion.

## Discussion and conclusion

In this review, 6 articles about the agreement between CT and histology and 8 articles about MRI and histology was included in the analysis. However, the results were highly variable and direct comparisons between studies were not possible because of incomparable methods and outcome measures, thus no meta-analysis or statistical analyze was performed. Although, results indicate MRI being a more explored method with better ability to identify carotid plaque components, which corresponds well with recent knowledge (38, 39).

Overall, a significant amount of essential data was not presented or specified in some studies of both CT and MRI, as for example blinding, prevalence of symtoms and dropouts. Furthermore, a small number of patients were generally included, which obstruct the implementation of strong statistical analyses with tight confidence intervals. These factors weaken the overall scientific reliability in the field, which indicates that larger trials of better

quality are needed.

## Methodological considerations and suggestions for future studies

### Designing the scientific issue

In the process of defining the question at the beginning of this review, a lot of literature in the field was studied in order to set up the right question. It generated a very low number of studies examining both CT and MRI against histology. Therefore, the issue of this review was divided into two separate questions, one for each index test (CT and MRI) but with the same reference test (histology) in order to get sufficient material to analyze. The aim was to compare the imaging methods, but since the literature searches were performed separately for each issue, the studied populations varied between studies of CT and MRI which reduced the comparability between the two imaging methods. Furthermore, a low amount of literature was found validating imaging methods with histology but the literature search generated a significant amount of literature correlating vulnerable components detected by imaging directly to stroke incidence. Excessive effort should in the future studies be invested to study previous research and understand the field to facililtate designing of the issue. A well-designed issue enable the finding of more relevant answers and facilitates the work process.

### Literature search

The literature search resulted in a larger number of articles about MRI than CT even though the limit for number of patients was reduced for CT. This indicates that MRI is a more well explored method since many more articles have been published in the field. As an indicator of the adequacy of the literature search, a large number of articles when screening reference-lists for articles read in full-text were already identified in the primary search results. This

indicates that the literature search probably included most of the relevant articles on the subject.

**Quality of individual articles**

Concerning assessment of quality in individual articles, blinding and time between index test and reference test were two factors affecting the quality. The assessment generated better results for MRI (2 studies assessed as high quality) than CT (no studies assessed as high quality) with a higher prevalence of blindning for both index test and reference test, thus indicating a stronger scientific approach in studies of MRI. Regarding time between index test and reference test, results varied strongly for CT (2 days to 3 months) but less for MRI (2 days to 20.5 days). Since atherosclerosis has a dynamic development and prevalence of different plaque components i.e. IPH can vary over short time, it is of great importance that time between imaging and extraction of plaque during CEA is as short as possible. In all included articles, it was not specified if time between tests were between imaging and CEA or imaging and microscopic examination by a pathologist. It is unlikely to assume CEA and histology were performed at the same point, although only information of when CEA was performed is relevant since plaque components are fixed in formalin after extraction and thereafter the risk of change in plaque components is non-existent. Due to practical reasons it is often not possible to perform the CEA directly after imaging, although it is of great importance that this time is kept as short as possible. Also, more detailed information of time aspects should be reported.

**Eligibility criteria**

Inclusion criterias for both CT and MRI varied significantly between included studies. The criteria for a representative population in this study was set up to include both asymtomatic

and symtomatic patients, although results showed only a limited number of studies examining both these groups. There is an existing gap in scientific knowledge concerning components in plaques in patients with asymtomatic stenosis and patients with lumen narrowing < 70 % (40). If further research examined asymtomatic patients and patients with low grade stenosis in the same manner as symtomatic patients and patients with high-grade stenosis, a lot of knowledge would be obtained on this issue.

**Factors considered in the analyzing process**

If the method to extract plaques during CEA is performed without a uniform preparation and reporting system, it might result in artifacts of plaque features such as ruptured fibrous cap or hemorrhage (32). However, in the articles included in this review, no studies with CT and only 2 studies with MRI (32, 33) reported the technique for plaque extraction. When comparing imaging slices with histology the correlation is of importance to ensure that the same area is analyzed. Reported landmarks used as reference were more similar in studies of MRI than CT, which indicate that studies of MRI used more equal methods than studies of CT.

Regarding the extraction of calcium, it is considered a factor affecting the enhancement of other components of the plaque when studying CT (41, 42). Although, only one study extracted calcium before analyzing the images (14). To obtain representative images, extraction of calcium should therefore be obtained.

Another considerable factor is which type of CT (MDCT and DSCT was used in the analyzed articles) and which radiation dose is being used. As mentioned in the background, this subject is a highly debated issue. There are evidence that DSCT produces comparable or lower radiation doses than conventinal CT (43). On the other hand, the radiation dose of both

MDCT and DSCT has to improve before it can proceed to clinical trials (44, 45) due to etchical dilemmas. Although, DSCT offers advantages over conventional MDCT with a reduction in radiation dose (45). MRI uses no radiation to obtain images which is a significant advantage for this type of imaging. If some of these methods will be used as a screening tool for high-risk atherosclerotic plaques in the future, a low radiation dose is of major importance to enable repeated examinations during a long time period.

Furthermore, a large amout of preclinical studies examining plaques ex vivo (imaging performed after CEA) was found in the literature search and reference lists. There are several aspects differing ex vivo from in vivo plaques such as affection of formalin fixation (46), use of contrast (42) (which is not available in ex vivo studies) and plaque shrinkage due to changes in content when extracted from the body (30). In this study, studies performed ex vivo were excluded (see section Method, eligibility criteria) since data from these studies cannot be compared directly with studies performed on humans. However, there is a lot of knowledge in this field that should be considered when performing clinical studies.

## Definition of plaque components

An important factor to enable comparison between studies is how the components are defined in both imaging and histology. Some studies used the AHA criterias while other used different terms for describing the content of the plaques. Since translation of described contents had to be performed (as described in the section method), it may have affected the results of how plaques were classified. As an example, in studies reporting fibrous tissue, it was translated to fibrous cap, AHA type V, which might affect the results since the reported fibrous tissue was not defined if it was covering the LRNC or acting as an underlying

component.

The process to define components in CT and MRI images, was performed either manually, by automated computerized algorithms or with a combination of these. There are several advantages of using computerized methods, among these time saving and improvement of interrater variability (47). Recent research indicate it might also improve reproducibility and be of interest in longitudinal studies of progression of atherosclerotic disease (15). Although, the included studies in this review used completely different algorithms, which makes comparison between studies impossible. If the research in the field could agree on the use of a common software, comparison of detected components would be made possible.

In order to define components, some studies about CT had predefined HU values for different components while some studies visually estimated components before defining ranges of HU values. This leads to large differences in how components are defined, which further weakens comparison between studies. In studies of MRI, a reference tissue was defined (in the majority of studies, the sternocleidomastoid muscle (SCM) was used). Provided SCM is an acceptable reference with small variations between patients, the results for MRI are more comparable than CT.

## Gap in scientific knowledge

There seem to exist a significant gap in scientific knowledge within this area. A lot of research has been published correlating vulnerable components directly with clinical symtoms. Since histology is considered to be the golden standard of determining components (18, 19), this area should be further explored to establish the state of the scientific knowledge.

A tool for approaching this aim is to agree on the basis for how to perform studies of imaging and histology. In 2005, Lovett et al published a review (48) of different imaging methods examining carotid plaques compared with histology. The results were highly variable, even between studies using the same criterias for the imaging method. Therefore, direct comparisons between studies were not possible because of incomparable methods and interpretation of the results. Based on these results Lovett et al, proposed a list of recommendations for how studies of carotid plaque imaging versus histology should be performed and reported. It consists of 8 items concerning sample size, the presence of symtoms, time interval beween index test and reference test, detailed information of plaque processing, blinding, histological methods and report of certain components.

Although these gudielines were published 9 years ago, the results in this thesis indicates that recent studies are not following these recommendations to any significant extent. The researchers need to agree on inclusion criterias, methods to perform imaging and the use of outcome measures. An updated recommendation, performed by a well-established and trusted organization, needs to be implemented in this field. In combination with larger trials and representative populations, the results would be comparable, analyzes could be performed and conclusions would be reliable. Not until then can non-invasive imaging methods be used in the clinic to prevent progression of atherosclerotic disease.

## Limitations

Because of the importance of an appropriate study selection, at least two reviewers should be involved in order to ensure a high quality of the review (49). Since this review was performed by one reviewer, this might have affected the results of the selection process and

interpretation of the results. Continuous contact with the Library of Gothenburg University and Health Technology Assessment Department (HTA-centrum) at the Sahlgrenska University Hospital was maintained to ensure a systematic and transparent approach during the process. It is desirable for future reviews to be conducted by two or more reviewers to enhance the quality of the review. Furthermore, a deeper technical knowledge of radiological methods and performance of systematic reviews would significantly facilitate the work.

A well designed scientific issue is of great importance when working with a systematic review (50, 51). A well validated question leads to a low risk of need to change criterias and methods along the process. In this review, two inclusion criteras had to be changed after the literature search (see section Method, egliability criterias) which is not desirable. This sligthly diminish the transparent and systematic approach of this review since the risk of subjective conclusions after performing the literature search cannot be excluded. Therefore, future studies need to enhance the focus on the initial stages of designing a review in order to obtain the systematic approach thoughout the whole process.

In this review, only two databases were used (pubmed and CENTRAL). Even though these are considered to be comprehensive in the medical field, more databases, both medical and technical should be considered, as this would probably increase the size of the material which might lead to the finding of more articles of relevance.

# Populärvetenskaplig sammanfattning

Åderförkalkning är ett mycket vanligt tillstånd som ökar med åldern och beror på inlagringar i våra blodkärl, även kallat plack, som bland annat består av fett, bindväv och blodkroppar. En konsekvens av åderförkalkning är att blodproppar bildas, vilket leder till syrebrist till olika organ beroende på var åderförkalkningen sitter. Stroke är en av de vanligaste och allvarligaste konsekvenserna av detta och innebär att syretillförseln till hjärnan är påverkad. Halskärlsartärerna är en vanlig plats där åderförkalkningen sitter som orsakar stroke.

Metoden man idag använder för att undersöka om man har plack som har ökad risk att orsaka stroke bygger på att man mäter hur stor del av kärlets innerdiameter som utgörs av plack. Detta är avgörande när man bestämmer om man ska utföra förebyggande åtgärder som exempelvis att operera bort ett plack. Ny forskning pekar dock på att förekomsten av olika komponenter i placket har större betydelse för risken att drabbas av stroke. Om man kan hitta en undersökningsmetod som inte är riskfylld för patienten, som med tillräcklig säkerhet kan ge information om komponenter med ökad risk att orsaka stroke, kan förebyggande insatser leda till minskat lidande och dödlighet i stroke.

I denna studie sammanställdes artiklar från studier som undersökt hur väl Datortomografi (DT) och Magnetröntgen (MR) kan avbilda komponenter som man i tidigare forskning har kopplat till ökad risk för stroke som konsekvens av åderförkalkning. För att inkluderas i studien ska komponenterna jämföras med vävnadsprov, vilket innebär att placken efter bildtagning opererats bort och analyserats i mikroskop. Sammanställningen är gjord på ett systematiskt och strukturerat sätt där varje steg i analysen har beskrivits för att säkerställa kvalitén.

Resultaten pekar på att underlaget är för magert för att kunna dra väl underbyggda slutsater om vilken metod av MR och DT som bäst avbildar plackkomponenter med ökad risk att orsaka

sjukdomsbörda. Studierna använder metoder för bildtagning, analyserar vävnad samt sina resultat på så skilda sätt att de inte är jämförbara. Tidigare forskning har publicerats där rekommendationer angivits för hur forskningen inom detta område bör genomföras, men denna studie förstärker behovet av ett uppdaterat, enhetligt och tydligt styrdokument som bör följas av kommande forskning för att kunna sammanställa och dra slutsatser inom detta medicinska område. Det finns dock en del data som indikerar att MR är en mer studerad metod än CT och i något högre grad kan påvisa komponenter med ökad risk att orsaka sjukdom.

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# Appendix

**Table 4. Literature search in database Pubmed for PIRO 1 (CT). Date of search: 140911.**

| PIRO issue | Type of search | Nr | Search terms | Items found |
|---|---|---|---|---|
| P- Patient | MeSH | #1 | "Carotid Stenosis"[Mesh] OR "Plaque, Atherosclerotic"[Mesh] | 14494 |
| P- Patient | Free-text | #2 | "Carotid plaque" OR "carotid stenosis" OR "carotid atherosclerosis" | 16551 |
| I- Index | MeSH | #3 | "Tomography, X-Ray Computed"[Mesh] | 302367 |
| I- Index | Free-text | #4 | "Computed tomography" OR CT | 385219 |
| R- Reference | MeSH | #5 | "Pathology"[Mesh] OR "Histology"[Mesh] OR "anatomy and histology" [Subheading] | 4095138 |
| R- Reference | Free-text | #6 | histolog* OR patholog* OR morpholog* | 3498867 |
| | Combination MeSH and free-text | #9 | #1 OR #2 | 18889 |
| | ” | #10 | #3 OR #4 | 528363 |
| | ” | #11 | #5 OR #6 | 4853647 |
| | ” | #12 | #9 AND #10 AND #11 | 908 |
| | ” | **#13** | **#12 AND limitations: english, humans, 10 years** | **640** |

**Table 5. Literature search in database CENTRAL for PIRO 1 (CT). Date of search: 140913.**

| PIRO issue | Type of search | Nr | Search terms | Items found |
|---|---|---|---|---|
| P- Patient | MeSH | #1 | "Carotid Stenosis"[Mesh] OR "Plaque, Atherosclerotic"[Mesh] | 655 |
| P- Patient | Free-text | #2 | "carotid plaque" OR "carotid stenosis" OR "carotid atherosclerosis" | 1185 |
| I- Index | MeSH | #3 | "Tomography, X-Ray Computed"[Mesh] | 4023 |
| I- Index | Free-text | #4 | "computed tomography" OR CT | 42070 |
| R- Reference | MeSH | #5 | (("Pathology"[Mesh]) OR "Histology"[Mesh]) OR "anatomy and histology" [Subheading] | 3641 |
| R- Reference | Free-text | #6 | histolog* OR patholog* OR morpholog* | 57180 |
| | Combination MeSH and free-text | #9 | #1 OR #2 | 1240 |
| | ” | #10 | #3 OR #4 | 43006 |
| | ” | #11 | #5 OR #6 | 57574 |
| | ” | #12 | #9 AND #10 AND #11 | 29 |
| | | **#13** | **#12 and limit: date latest 10 years and trials** | **8** |

**Table 6. Additional references found for PIRO 1 (CT).**

| # | Found via | Authors, Year, Title |
|---|---|---|
| 1 | Mats Danielsson, Professor in physics, Kungliga tekniska högskolan, Stockholm. | Boussel, L., Coulon, P., Thran, A. et al.<br>2014<br>”Photon counting spectral CT component analysis of coronary artery atherosclerotic plaque samples” |
| 2 | Luca Saba,<br>Università degli studi di Cagliari,<br>Department of Biomedical Science,<br>Italy, Radiology. | Saba, L., Lai, M. L., Montisci, R. et al.<br>2012<br>”Association between carotid plaque enhancement shown by multidetector CT angiography and histologically validated microvessel density” |
| 3 | Luca Saba,<br>Università degli studi di Cagliari,<br>Department of Biomedical Science,<br>Italy, Radiology. | Saba, L., Tamponi, E., Raz, E. et al.<br>2014<br>”Correlation between fissured fibrous cap and contrast enhancement: Preliminary results with the use of CTA and histologic validation” |
| 4 | Reference list of<br>Saam, T.<br>et al, 2013. | Appel, A. A., Chou, C. Y., Greisler, H. P. Et al.<br>2012<br>”Analyzer-based phase-contrast x-ray imaging of carotid plaque microstructure” |
| 5 | Reference list of<br>Vukadinovic, D.<br>Et al, 2012 | de Weert, T. T., de Monye, C., Meijering, E. Et al<br>2008<br>”Assessment of atherosclerotic carotid plaque volume with multidetector computed tomography angiography” |

**Figure 1. Flow diagram for selection process for PIRO 1 (CT).**

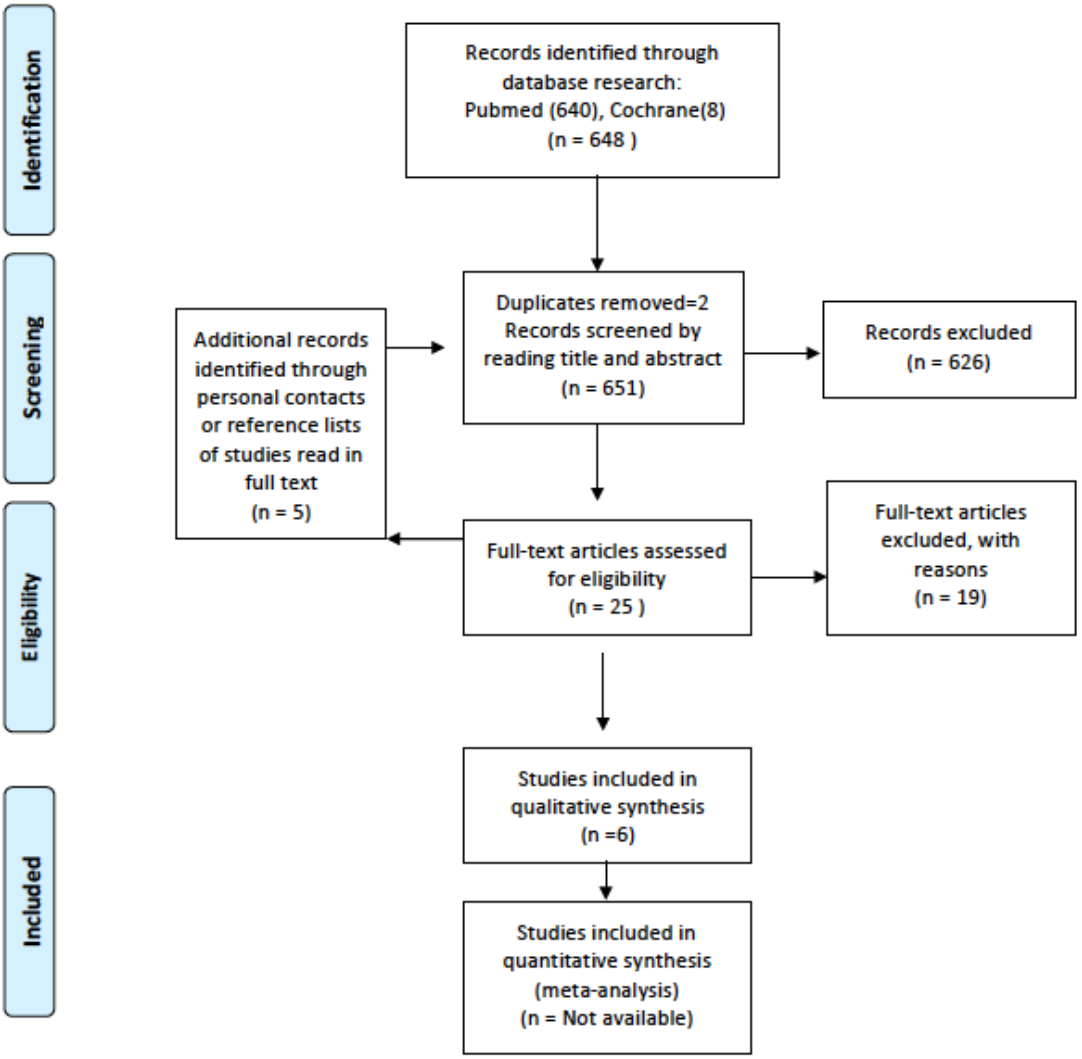

**Table 7. List of excluded articles for PIRO 1 (CT).**

| # | Study<br>Authors, publication year | Reason of exclusion |
|---|---|---|
| 1 | Wintermark, M.<br>Arora, S.<br>Tong, E. Et al. 2008 | Reference not according to PIRO |
| 2 | Haraguchi, K.<br>Houkin, K.<br>Koyanagi, I.et al. 2008 | Reference not according to PIRO |
| 3 | Mauriello, A.<br>Sangiorgi, G. M.<br>Virmani, R. et al. 2010 | Not detecting components of plaques |
| 4 | Groen, H. C.<br>van Walsum, T.<br>Rozie, S. et al. 2010 | Not detecting components of plaques |
| 5 | Korn, A.<br>Bender, B.<br>Thomas, C. et al. 2011 | Reference not according to PIRO (Reference not according to PIRO) |
| 6 | Obaid, D. R.<br>Calvert, P. A.<br>Gopalan, D. et al. 2013 | Only coronary arteries studied. |
| 7 | Pecoraro, F.<br>Dinoto, E.<br>Mirabella, D.et al. 2013 | Not detecting components of plaques |
| 8 | Jaff, M. R. 2008 | Review |
| 9 | Vukadinovic, D.<br>Rozie, S.<br>van Gils, M. Et al. 2012 | Reference not according to PIRO |
| 10 | de Weert, T. T.<br>de Monye, C.<br>Meijering, E. Et al 2008 | Reference not according to PIRO |
| 11 | Saba et al 2014 | Not detecting relevant histologic components |
| 12 | Saba et al 2012 | Not detecting relevant histologic components |
| 13 | Zaignon, R. Et al 2012 | Ex vivo plaques |
| 14 | Saam, T. Et al 2013 | Ex vivo plaques |
| 15 | Hetterich, H. Et al 2013 | Ex vivo plaques |
| 16 | Appel, A.et al 2012 | Ex vivo plaques |
| 17 | Appel, A. Et al 2012 | Ex vivo plaques |
| 18 | De Weert T T et al, 2005 | Ex vivo plaques |
| 19 | Hetterich, H. Et al 2014 | Ex vivo plaques |

**Table 8. Literature search in database Pubmed for PIRO 2 (MRI). Date of search: 140911.**

| PIRO issue | Type of search | Nr | Search terms | Items found |
|---|---|---|---|---|
| P- Patient | MeSH | #1 | "Carotid Stenosis"[Mesh] OR "Plaque, Atherosclerotic"[Mesh] | 14494 |
| P- Patient | Free-text | #2 | "Carotid plaque" OR "carotid stenosis" OR "carotid atherosclerosis" | 16551 |
| I- Index | MeSH | #3 | "Magnetic Resonance Imaging"[Mesh] | 308100 |
| I- Index | Free-text | #4 | "Magnetic resonance imaging" OR MRI | 398197 |
| R- Reference | MeSH | #5 | "Pathology"[Mesh] OR "Histology"[Mesh] OR "anatomy and histology" [Subheading] | 4095138 |
| R- Reference | Free-text | #6 | Histolog* OR patholog* OR morpholog* | 3498867 |
| | Combination MeSH and free-text | #9 | #1 OR #2 | 18889 |
| | ” | #10 | #3 OR #4 | 398197 |
| | ” | #11 | #5 OR #6 | 4853647 |
| | ” | #12 | #9 AND #10 AND #11 | 1478 |
| | ” | **#13** | **#12 AND limitations: english, humans, 10 years** | **892** |

**Table 9. Literature search in database CENTRAL for PIRO 2 (MRI) Date of search: 140913.**

| PIRO issue | Type of search | Nr | Search terms | Items found |
|---|---|---|---|---|
| P- Patient | MeSH | #1 | "Carotid Stenosis"[Mesh] OR "Plaque, Atherosclerotic"[Mesh] | 655 |
| P- Patient | Free-text | #2 | "carotid plaque" OR "carotid stenosis" OR "carotid atherosclerosis" | 1185 |
| I- Index | MeSH | #3 | "Magnetic Resonance Imaging"[Mesh] | 5666 |
| I- Index | Free-text | #4 | "Magnetic resonance imaging" OR MRI | 9699 |
| R- Reference | MeSH | #5 | (("Pathology"[Mesh]) OR "Histology"[Mesh]) OR "anatomy and histology" [Subheading] | 3641 |
| R- Reference | Free-text | #6 | histolog* OR patholog* OR morpholog* | 57180 |
| | Combination MeSH and free-text | #9 | #1 OR #2 | 1240 |
| | ” | #10 | #3 OR #4 | 10003 |
| | ” | #11 | #5 OR #6 | 57574 |
| | ” | #12 | #9 AND #10 AND #11 | 56 |
| | | **#13** | **#12 and limit: date latest 10 years and trials** | **23** |

**Table 10. Additional references found for PIRO 2 (MRI).**

| # | Found via | Authors, Year, Title |
|---|---|---|
| 1 | Reference list of Biasiolli, L. 2013 | Chu B, 2004<br>”Hemorrhage in the atherosclerotic carotid plaque: a high-resolution MRI study” |
| 2 | Reference list of Cai, J., 2005 | Trivedi, R. A., 2004<br>”MRI-derived measurements of fibrous-cap and lipid-core thickness: the potential for identifying vulnerable carotid plaques in vivo” |
| 3 | Reference list of Cai, J., 2005 | Trivedi, R. A., 2004<br>”Multi-sequence in vivo MRI can quantify fibrous cap and lipid core components in human carotid atherosclerotic plaques” |

**Figure 2. Flow diagram for selection process for PIRO 2 (MRI).**

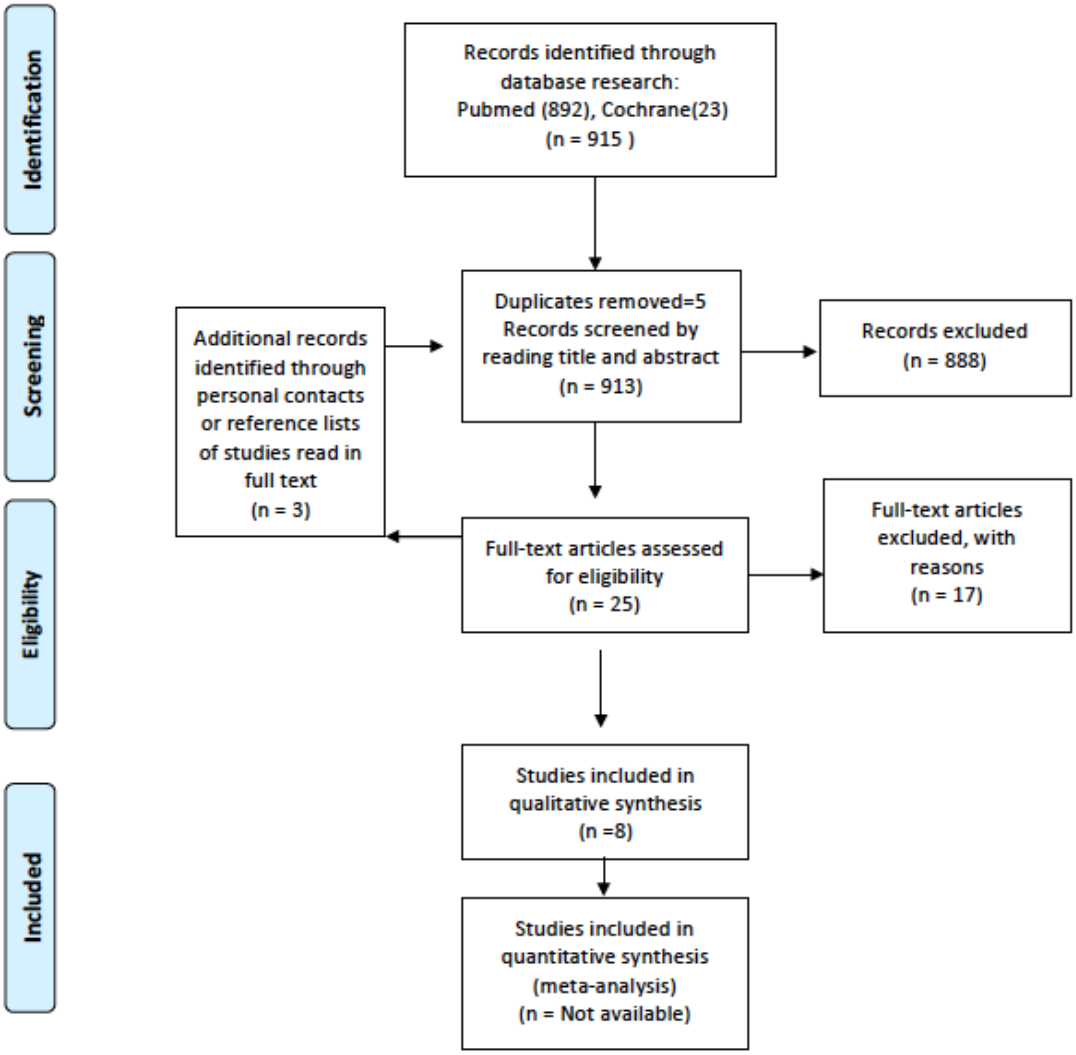

**Table 11. List of excluded articles for PIRO 2 (MRI).**

| # | Study<br>Authors, publication year | Reason of exclusion |
|---|---|---|
| 1 | Arai D et al, 2011 | Not detecting components of plaques |
| 2 | Chan C.F., 2010 | Not detecting components of plaques |
| 3 | Chu B et al, 2005 | Case report |
| 4 | Demarco et al, 2010 | Not comparing with histology |
| 5 | Gao et al, 2009 | Not detecting components of plaques |
| 6 | Hnad P.J, 2009 | Comment |
| 7 | Hinton-Yates, D. P. 2007 | Ex vivo plaques |
| 8 | Ota H et al 2009 | Not comparing wtih histology |
| 9 | Ronen R.R et al 2007 | Ex vivo plaques |
| 10 | Anumula S, 2005 | Not comparing wtih histology |
| 11 | Saloner D, 2007 | Review |
| 12 | Boekhorst, B. C., 2012 | Ex vivo plaques |
| 13 | Zhao X et al 2010 | Not comparing wtih histology |
| 14 | Clarke, S. E. 2006 | Ex vivo plaques |
| 15 | Fabiano, S. 2008 | Ex vivo plaques |
| 16 | Trivedi RA 2004 | Duplicate |
| 17 | Hishikawa, T. 2010 | Reference not according to PIRO |

**Table 12. Characteristics of included studies for PIRO 1 (CT), outcome variable AHA plaque type IV.**

| Article reference number, Author, Year | Study Design | Patients(n) Plaque(n) | Dropouts | Gender (Male(M)/ Female(F)) | Mean/median age (years) | Type of patients (Symtomatic(S) / Assymtomatic(A) | Diagnostic tests: Index vs Reference | Contrast agent | Outcome variables reported in the study | Trans-lated AHA type | Time between index and reference test | Measurement of agreement | Quality of study |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **#17 Das, M. 2009** | Cross sectional | 30 patients NR | 0 | 26 M 4 F | Mean: 70 (52-85) | 30 S 0 A | DSCT vs Histology | Yes (Iodine and saline-chaser) | AHA IV/V | IV/V | 2 days | Cohens cappa | Low |
| **#16 De Weert, T. 2006** | Cross sectional | 15 patients 15 plaques | 1 | 6 M 9 F | Mean: 70,3 (62-84) | 15 S 0 A | MDCT vs Histology | Yes (NS) | LC+ hemorrhage + Necrotic debris | IV* | 3 months | Linear regression analysis Bland Altman plot | Moderate |
| **#15 Wintermark, M. 2008** | Cross sectional | 8 patients 8 plaques | 0 | 8 M 0 F | Mean: 62 (55-69) | 8 S 0 A | MDCT vs Histology | Yes (Iodine) | LRNC | IV* | 1-5 days | Cohens kappa | Moderate |

**NR= Not Reported. NS= Not Specified. DSCT= Dual source Computed Tomography. MDCT= Multidetector Computed Tomography. LC= Lipid Core. LRNC= Lipid Rich Necrotic Core. Outcome component reported as described in the article. Asterix (*)= outcome components translated to AHA type according to the AHA (American Heart Association) classification report.**

**Table 13. Characteristics of included studies for PIRO 1 (CT), outcome variable AHA plaque type V.**

| Article reference number, Author, Year | Study Design | Patients(n) Plaque(n) | Dropouts | Gender (Male(M)/ Female(F)) | Mean/median age (years) | Type of patients (Symtomatic(S) / Assymtomatic(A) | Diagnostic tests: Index vs Reference | Contrast agent | Outcome variables reported in the study | Trans-lated AHA type | Time between index and reference test | Measurement of agreement | Quality of study |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **# 36 Ajduk, M. 2013** | Cross sectional | 50 patients 50 plaques | 0 | 36 M 14 F | Median: 69 (48-87) | 20 S 30 A | MDCT vs Histology | Yes (Iopamidol) | AHA V | V | 1 week | Sensitivity, specificity | Moderate |
| **#17 Das, M., 2009** | Cross sectional | 30 patients NR | 0 | 26 M 4 F | Mean: 70 (52-85) | 30 S 0 A | DSCT vs Histology | Yes (Iodine and saline-chaser) | AHA IV/V | IV/V | 2 days | Cohens kappa | Low |
| **#16 De Weert, T. 2006** | Cross sectional | 15 patients 15 plaques | 1 | 6 M 9 F | Mean: 70,3 (62-84) | 15 S 0 A | MDCT vs Histology | Yes (NS) | FT area+tunica media | V* | 3 months | Linear regression analysis Bland Altman plot | Moderate |
| **#14 Horie, N. 2012** | Cross sectional | 51 patients 59 plaques | 39 | 46 M 5 F | Symtomatic mean: 71,84 (+/- 8,5) Assymtomatic mean: 67,8 (+/- 9,1) | 33 S 18 A | MDCT vs Histology | Yes (NS) | FT | V* | 2 weeks | Linear regression analysis Pearson rank correlation test | Moderate |
| **#15 Wintermark, M. 2008** | Cross sectional | 8 patients 8 plaques | 0 | 8 M 0 F | Mean: 62 (55-69) | 8 S 0 A | MDCT vs Histology | Yes (Iodine) | Fibrous cap thickness | V* | 1-5 days | Linear regression analysis | Moderate |

**NR= Not Reported. NS= Not Specified. DSCT= Dual Source Computed Tomography. MDCT= Multidetector Computed Tomography. LC= Lipid core. LRNC= Lipid Rich Necrotic Core. FT= Fibrous Tissue. Outcome component reported as described in the article. Asterix (*)= outcome components translated to AHA type according to the AHA (American Heart Association) classification report.**

**Table 14. Characteristics of included studies for PIRO 1 (CT), outcome variable AHA plaque type VI.**

| Article reference number, Author, Year | Study Design | Patients(n) Plaque(n) | Dropouts | Gender (Male(M)/ Female(F)) | Mean/median age (years) | Type of patients (Symtomatic(S) / Assymtomatic(A) | Diagnostic tests: Index vs Reference | Contrast agent | Outcome variables reported in the study | Trans-lated AHA type | Time between index and reference test | Measurement of agreement | Quality of study |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **#37 Ajduk, M. 2008** | Cross sectional | 31 patients 31 plaques | 0 | 21 M 10 F | Median: 70 (51-87) | 6 S 25 A | MDCT vs Histology | Yes (Iopamidol) | AHA VIb | VI | 1 week | Sensitivity, specificity | Moderate |
| **#36 Ajduk, M. 2013** | Cross sectional | 50 patients 50 plaques | 0 | 36 M 14 F | Median: 69 (48-87) | 20 S 30 A | MDCT vs Histology | Yes (Iopamidol) | AHA VIb | VI | 1 week | Sensitivity, specificity | Moderate |
| **#17 Das, M. 2009** | Cross sctional | 30 patients NR | 0 | 26 M 4 F | Mean: 70 (52-85) | 30S 0A | DSCT vs Histology | Yes (Iodine and saline-chaser) | AHA VI | VI | 2 days | Cohens kappa | Low |
| **#14 Horie, N. 2012** | Cross sectional | 51 patients 59 plaques | 39 | 46 M 5 F | Symtomatic mean: 71,84 (+/- 8,5) Assymtomatic mean: 67,8 (+/- 9,1) | 33 S 18 A | MDCT vs Histology | Yes (NS) | LRNC+ hemorrage | VI* | 2 weeks | Linear regression analysis Pearson rank correlation test | Moderate |
| **#15 Wintermark, M. 2008** | Cross sectional | 8 patients 8 plaques | 0 | 8 M 0 F | Mean: 62 (55-69) | 8 S 0 A | MDCT vs Histology | Yes Iodine | IPH | VI* | 1-5 dagar | Cohens cappa | Moderate |

**NR= Not Reported. NS= Not Specified. DSCT= Dual Source Computed Tomography. MDCT= Multidetector Computed Tomography. LC= Lipid Core. LRNC= Lipid Rich Necrotic Core. Outcome component reported as described in the article. Asterix (*)= outcome components translated to AHA type according to the AHA (American Heart Association) classification report.**

**Table 15. Characteristics of included studies for PIRO 2 (MRI), outcome variable AHA plaque type IV.**

| Article reference number, Author, Year | Study Design | Patients(n) Plaque(n) | Dropouts | Gender (Male(M)/ Female(F)) | Mean/median age (years) | Type of patients (Symtomatic(S) /Assymtomatic(A) | Diagnostic tests: Index vs Reference | Contrast agent | Outcome variables reported in the study | Trans-lated AHA type | Time between index and reference test | Measurement of agreement | Quality of study |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **# 32 Kerwin, WS. 2006** | Cross sectional | 30<br>NR | 3 | 29 M<br>1 F | Mean: 67.7 ± 10.7<br>Range: 48–83 | NS | 1.5-T MR without cardiac gating vs histology | Yes, (Gadolinum) | NC | IV* | 1 week | Pearson correlation coeffiscient | Moderate |
| **# 31 Saam T. 2005** | Cross sectional | 40<br>NR | 9 | 38 M<br>2 F | Mean: 68<br>SD 9 | NS | 1.5 T MR phased array surface vs histology | - | LRNC | IV* | 1 week | Sensitivity specificity, cohens cappa, pearsons rank test | High |
| **# 29 Trivedi R. 2004** | Cross sectional | 40<br>NR | 18 | 28 M<br>12 F | mean age 71, range 45–88 years) | 40 S<br>/<br>0 A | 1.5 T MR custumized 4 channel phased array coil vs hisology | - | FC+LC | IV*/V* | NR | Bland Altman plot | Low |
| **# 34 Yoshida K. 2007** | Crossctional | 70<br>NR | 38 | 61 M<br>9 F | Median: 69;<br>Range: 53– 80 | 48<br>/<br>22 | 1.5 T MR with 8 cm surface coil vs Histology | - | Soft plaques | IV* | 2 weeks | Sensitivity, specitivity | Moderate |
| **# 35 Young V E. 2009** | Crossctional | 28<br>NR | 9 | 21 M<br>7 F | Mean: 73<br>Range 54–87 | 14<br>/<br>12 | 1.5 T MR bilateral 4 channel phased array carotid coil vs histology | - | LRNC | IV* | NR | Sensitivity, specitivity | Moderate |

**NR= Not Reported. NS= Not Specified. NC= Necrotic Core. LC= Lipid Core. LRNC= Lipid Rich Necrotic Core. IPH= Intra Plaque Hemorrhage. Outcome component reported as described in the article. Asterix (*)= outcome components translated to AHA type according to the AHA (American Heart Association) classification report.**

**Table 16. Characteristics of included studies for PIRO 2 (MRI), outcome variable AHA plaque type V.**

| Article reference number, Author, Year | Study Design | Patients(n) Plaque(n) | Dropouts | Gender (Male(M)/ Female(F)) | Mean/median age (years) | Type of patients (Symtomatic(S)/Asymtomatic(A) | Diagnostic tests: Index vs Reference | Contrast agent | Outcome variables reported in the study | Trans-lated AHA type | Time between index and reference test | Measurement of agreement | Quality of study |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **# 32 Kerwin, WS. 2006** | Cross sectional | 30<br>NR | 3 | 29 M<br>1 F | Mean: 67.7 ± 10.7<br>Range: 48–83 | NS | 1.5-T MR without cardiac gating vs histology | Yes, (Gadolinum) | FT | V* | 1 week | Pearson correlation coefficients | Moderate |
| **# 31 Saam T. 2005** | Cross sectional | 40<br>NR | 9 | 38 M<br>2 F | Mean: 68<br>SD: 9 | NS | 1.5T MR and phased-array surface vs histology | - | Dense fibrous tissue | V* | 1 week | Pearson correlation coeffiscient | High |
| **# 29 Trivedi R. 2004** | Cross sectional | 40<br>NR | 18 | 28 M<br>12 F | mean age 71, range 45–88 years) | 40 S<br>/<br>0 A | 1.5 T MR custumized 4 channel phased array coil vs hisology | - | FC+LC | IV*/V* | NR | Bland Altman plot | Low |

**NR= Not Reported. NS= Not Specified. FT= Fibrous Tissue. FC= Fibrous Cap.Outcome component reported as described in the article. Asterix (*)= outcome components translated to AHA type according to the AHA (American Heart Association) classification report.**

**Table 17. Characteristics of included studies for PIRO 2 (MRI), outcome variable AHA plaque type VI.**

| Article reference number, Author, Year | Study Design | Patients(n) Plaque(n) | Drop-outs | Gender (Male(M)/ Female(F) | Mean/median age (years) | Type of patients (Symtom-atic(S)/ Asymtom atic(A) | Diagnostic tests: Index vs Reference | Contrast agent | Outcome variables reported in the study | Trans-lated AHA type | Time between index and reference test | Measure-ment of agreement | Quality of study |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **# 33 Altaf N. 2013** | Cross sectional | 35<br>NR | 0 | 24 M<br>11 F | Mean: 69 +/- 10 | 35<br>/<br>0 | Coronal T1-weighted magnetization-prepared 3D gradient echo sequence MRI vs Histology | - | AHA VI | VI | Mean: 20,5 days (8-33) | Sensitivity, Specificity, PPV, NPV | Moderate |
| **# 30 Chu B. 2004** | Cross sectional | 27<br>NR | 3 | 21 M<br>6 F | Years span: 55-82 | 13<br>/<br>14 | 1.5-T MRI with phased-array surface coil vs histology | - | IPH | VI* | 1 week | Sensitivity, Specificity, Cohens kappa | High |
| **# 32 Kerwin, WS. 2006** | Cross sectional | 30<br>NR | 3 | 29 M<br>1 F | Mean: 67.7 ± 10.7<br>Range: 48–83 | NS | 1.5-T MR without cardiac gating vs histology | Yes, (Gadolinu m) | Hemorrhage | VI* | 1 week | Pearson correlation coefficients | Moderate |
| **# 31 Saam T 2005** | Cross sectional | 40<br>NR | 9 | 38 M<br>2 F | Mean: 68<br>SD: 9 | NS | 1.5T MR and phased-array surface vs histology | - | Hemorrhage | VI* | 1 week | Sensitivity, Specificity, Cohens kappa Pearson correlation coeffiscient | High |
| **# 38 Saito A . 2012** | Cross sectional | 31<br>NR | 10 | 30 M<br>1 F | Mean: 69.6<br>Range: 54–80 | 25<br>/<br>6 | 1.5- T MR with 8-channel neurovascular coil vs histology | - | Lipid/necrosi s+ hemorrhage | IV* | 1 week | Sensitivity, specitivity, | Moderate |

**NR= Not Reported. NS= Not Specified. LC= Lipid Core. IPH= Intra Plaque Hemorrhage. Outcome component reported as described in the article. Asterix (*)= outcome components translated to AHA type according to the AHA (American Heart Association) classification report.**

**Table 18. Analyze table for PIRO 1 (CT), outcome variable AHA plaque type IV.**

| Article reference number, Author, Year | Type of CT | Outcome component | AHA type | Density (Houndsfield values) | Cutoff HU-value | Coefficient of determination | Persons rank test | Cohens cappa | Sensi-tivity | Speci-ficity | Inter(a)/ Intra(b) variability | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **#17 Das, M. 2009** | DSCT with contrast | AHA IV/V | IV/V | Fatty-(<50HU) Mixed (50-119 HU) Calcified (>120HU) | - | - | - | 0,86 | - | - | NR | HU values predefined |
| **#16 De Weert, T. 2006** | MDCT with contrast | Lipid+ hemorrhage + necrotic debris = LC | IV* | LC (-20-60 HU) | LRNC- FT: 60 HU | $R^2$: 0,24 P<0.002 | - | - | - | - | a) 20%<br>b) NR | Coefficients of variation in % for interobserver variability. |
| **#15 Wintermark, M. 2008** | MDCT with contrast | LRNC | IV* | LRNC: 33.6 SD:20.0 CI:-7.4-72.5 | LRNC- FT: 39,5 | - | - | 1) Small LC: K= 0.495; *P* <0.492<br>2) Large LC K= 0.796; *P* < 0.001 | - | - | NR | - |

**MDCT= Multidetector Computed tomography. DSCT= Dual Source Computed Tomography. FT= fibrous tissue, LC= lipid core, LRNC= Lipid rich necrotic core. NR= Not reported. ROI= Region of interest. Asterix (*) means outcome component translated to AHA type according to the AHA (American Heart Association) classification report.**

**Table 19. Analyze tables for PIRO 1 (CT), outcome variable AHA plaque type V.**

| Article reference number, Author, Year | Type of CT | Outcome component | AHA type | Density (Houndsfield values) | Cutoff HU-value | Coefficient of determination | Persons rank test | Cohens cappa | Sensi-tivity | Speci-ficity | Inter(a)/ Intra(b) variability | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **# 36 Ajduk, M. 2013** | MDCT with contrast | AHA V | V | Median range: 44.9 (-23.6-150) | Cut off: 38.9 | - | - | - | 63.2% (p=0.035) | 80.6% (p=0.035) | NR | ROC analyse was used to determine cut-off HU-value |
| **#17 Das, M. 2009** | DSCT with contrast | AHA IV/V | IV/V | Fatty-(<50HU) Mixed (50-119 HU) Calcified (>120HU) | - | - | - | 0,86 | - | - | NR | HU values predefined |
| **#16 De Weert, T. 2006** | MDCT with contrast | FT+tunica media | V* | FT (60-140 HU) | LRNC- FT: 60 HU | $R^2$: 0,76 P<0.001 | - | - | - | - | a) 10%<br>b) NR | Coefficients of variation in % for interobserver variability. |
| **#14 Horie, N. 2012** | MDCT with contrast | FT | V* | Δ*HU* Symtomatic: 5.6 HU +/- 10.2 Asymtomatic: 13.5 HU +/-14.5 (p=0.02) | NS | - | Δ*HU* *r*=0.67 *P*=0.001 | - | - | - | NR | Δ*HU*= early phase contrast/ late phase contrast |
| **#15 Wintermark, M. 2008** | MDCT with contrast | Fibrous cap thickness | V* | FT: 46.4 SD. 19.9 CI: (-6.6-86.2) | LRNC- FT: 39,5<br>FT- IPH: 72.0 | $R^2$= 0.77 P<0.001 | - | - | - | - | NR | - |

**MDCT= Multidetector Computed tomography. DSCT= Dual Source Computed Tomography. IPH= Intra plaque hemmorrhage, FT= fibrous tissue, LC= lipid core, LRNC= Lipid rich necrotic core. NR= Not reported. ROI= Region of interest. Asterix (*) means outcome components translated to AHA type according to the AHA (American Heart Association) classification report.**

**Table 20. Analyze table for PIRO 1 (CT), outcome variable AHA plaque type VI.**

| Article reference number, Author, Year | Type of CT | Outcome component | AHA type | Density (Houndsfield values) | Cutoff HU-value | Coefficient of determination | Persons rank test | Cohens cappa | Sensi-tivity | Speci-ficity | Inter(a)/ Intra(b) variability | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **#37 Ajduk, M. 2008** | MDCT with contrast | AHA VIb | VI | Plaques with IPH Vib: 22 (range -17-31) Plaques without IPH: 59 (range-6-150) | Cut off: IPH: 31 | - | - | - | 100% | 64,7% | NR | ROC analyse was used to determine cut-off HU-value |
| **# 36 Ajduk, M. 2013** | MDCT with contrast | AHA VIb | VI | Plaques with IPH Vib: 14.7 (range -17.6-31.8) Plaques without IPH: 54.3 (range-23.6- 150) | Cut off: IPH: 33,8 | - | - | - | 100% | 70.4% | NR | ROC analyse was used to determine cut-off HU-value |
| **#17 Das, M. 2009** | DSCT with contrast | AHA VI | VI | Fatty-(<50HU) Mixed (50-119 HU) Calcified (>120HU) | - | - | - | 0,81 | - | - | NR | HU values predefined |
| **#14 Horie, N. 2012** | MDCT with contrast | LRNC + IPH | VI* | Δ*HU* Symtomatic: 5.6 HU +/- 10.2 Asymtomatic: 13.5 HU +/-14.5 (p=0.02) | NS | - | Δ*HU* r=-0,7 *P*=0.001 | - | - | - | NR | Δ*HU*= early phase contrast/ late phase contrast |
| **#15 Wintermark , M. 2008** | MDCT with contrast | IPH | VI* | Hemorrhage: 97.5 SD. 22.0 CI: (53.5-141.6) | FT- IPH: 72.0 | - | - | 1) Large IPH: K= 0.712; *P<0.*102 2) Ulceration K= 0.855 | - | - | NR | - |

**MDCT= Multidetector Computed tomography, DSCT= Dual Source Computed Tomography. IPH= Intra plaque hemmorrhage, FT= fibrous tissue, LRNC= Lipid rich necrotic core, NR= Not reported. Asterix (*) means outcome components translated to AHA type according to the AHA (American Heart Association) classification report.**

**Table 21. Analyze table for PIRO 2 (MRI), outcome variable AHA plaque type IV.**

| Article reference number, Author, Year | Type of MR sequence | Outcome component | Trans-lated AHA type | Cut off value | Pearson correlation coeffiscient | Bland Altman Plot | Cohens cappa statistics | Sensi-tivity | Speci-ficity | PPV | NPV | Inter(a)/ intra(b) observer variability | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **# 32 Kerwin, WS. 2006** | 1.5-T MR transverse 2D spoiled gradient-recalled- echo sequence | NC | IV* | - | Vp: r=-0,26 p=NS<br>K-trans: r=-0,05 p=NS | | - | - | - | - | - | - | Vp= plasma volume<br>K-trans= transfer constant |
| **# 31 Saam T. 2005** | 1.5 T MR with phased-array surface coil | LRNC | IV* | - | r= 0.75 p<0.001 | - | 0.73 | 95% | 76% | - | - | a) 0.89<br>b) 0.92 | - |
| **# 29 Trivedi R. 2004** | 1.5-T with customized 4 channel phased array coil | FC+LC | IV*/V* | - | - | 95% of values within 2 SD from the mean diff erence in ratios (0.02±0.04) | - | - | - | - | - | a) 0.87<br>b) NR | - |
| **# 34 Yoshida K. 2007** | 1.5 T MR with 8-cm diameter surface coil | Soft plaque | VI* | 1.25 (mean of roSI) | - | | - | 79.4% | 84.4% | - | - | - | rSI (relative signal intensity)= SI component/SI reference.<br>roSI= SI whole plaque/SI ref.<br>T1 weighted images. |
| **# 35 Young V E. 2009** | 1.5-T MR with bilateral four-channel phased-array carotid coil | LRNC | IV* | - | - | | - | 86% | 40% | - | - | a) 0.60<br>b) NR | Cohens kappa: lipid staining on histology correlating with a lower ADC (apparent diffusion coefficient) value. |

**NC= Necrotic core. LRNC= Lipid rich necrotic core. FC= Fibrous cap. NS= Not specified. PPV= Positive predictive value. NPV= Negative predictive value. NR= Not reported. Asterix (*) means outcome components translated to AHA type according to the AHA (American Heart Association) classification report.**

**Table 22. Analyze table for PIRO 2 (MRI), outcome variable AHA plaque type V.**

| Article reference number, Author, Year | Type of MR sequence | Outcome component | Trans-lated AHA type | Cut off value | Pearson correlation coeffiscient | Bland Altman Plot | Cohens cappa statistics | Sensi-tivity | Speci-ficity | PPV | NPV | Inter(a)/ intra(b) observer variability | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **# 32 Kerwin, WS. 2006** | 1.5-T MR transverse 2D spoiled gradient-recalled- echo sequence | FT | V* | - | Vp: r=0.18 p=NS K-trans: r=-0.02 p=NS | | - | - | - | - | - | - | Vp= plasma volume K-trans= transfer constant |
| **# 31 Saam T. 2005** | 1.5 T MR with phased-array surface coil | Dense fibrous tissue | V* | - | r=0.55 p=0.001 | - | - | - | - | - | - | - | - |
| **# 29 Trivedi R. 2004** | 1.5-T with customized four-channel phased array coil | FC+LC | IV*/V* | - | - | 95% of values within 2 SD from mean diff erence in ratios (0.02±0.04) | - | - | - | | - | a) 0.87<br>b) NR | - |

**= Fibrous tissue. FC= Fibrous cap. LC= Lipid core. PPV= Positive predictive value. NPV= Negative predictive value. Asterix (*) means outcome components translated to AHA type according to the AHA (American Heart Association) classification report.**

**Table 23. Analyze table for PIRO 2 (MRI), outcome variable AHA plaque type VI.**

| Article reference number, Author, Year | Type of MR sequence | Outcome component | Trans-lated AHA type | Cut off value | Pearson correlation coeffiscient | Bland Altman Plot | Cohens cappa statistics | Sensi-tivity | Speci-ficity | PPV | NPV | Inter(a)/ intra(b) observer variability | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **# 33 Altaf N. 2013** | Coronal 3D gradient echo sequence MR | AHA VI | VI | - | - | - | - | 82% | 79% | 90% | 64% | Cohens K a) 0.88 b) 0.81 | - |
| **# 30 Chu B. 2004** | 1.5-T MR with phased-array surface coil | IPH | VI* | - | - | - | Reader 1: 0.74 Reader 2: 0.52 | 90% | 74% | - | - | Cohens K a) 0.4 b) NR | - |
| **# 32 Kerwin, WS. 2006** | 1.5-T MR transverse 2D spoiled gradient-recalled- echo sequence | Hemorrhage | VI* | - | Vp: r=-0.01 p=NS K-trans: r=0.26 p=NS | - | - | - | - | - | - | - | Vp= plasma volume K-trans= transfer constant |
| **# 31 Saam T. 2005** | 1.5 T MR with phased-array surface coil | Hemorrhage | VI* | - | r= 0.66 p<0.001 | - | 0.71 | 87% | 84% | - | - | a) 0.74 b) 0.73 | - |
| **# 38 Saito A. 2012** | 1.5- T MR with eight-channel neurovascular coil | Lipid/ necrosis with Hemorrhage | VI* | SE: 1.19 BB: 1.11 MPRAGE: 1.08 SI-MRA: 1.10 | - | - | - | SE: 100% BB: 75% MPRAGE: 88% SI-MRA: 100% | SE: 100% BB: 100% MPRAGE: 100% SI-MRA: 80% | - | - | a) NR b) 0.98–0.99 (median, 0.99) and 0.95– 0.99 (median, 0.98), | Analyzed four kinds of T1W images Analyzed lipid+necrosis+ hemorrage vs FT |

**IPH= Intra Plaque Hemorrhage. NR= Not reported. SE= non-gated spin echo sequence. BB= cardiac-gated black-blood sequence. MPRAGE= fast-SE magnetization-prepared rapid acquisition with gradient echo sequence. SI-MRA= source image of three-dimensional time-of-flight MR angiography sequence. PPV= Positive predictive value. NPV= Negative predictive value. Asterix (*) means outcome components translated to AHA type according to the AHA (American Heart Association) classification report.**

**Table 24. Diversity between methods for studies of PIRO 1 (CT).**

| Article reference number, Author, Year | Method to measure density | Method to define density | Landmarks used to match histology and imaging slices | Method of CEA | Extraction of calcium | Contrast administration | Method of histological assessment |
|---|---|---|---|---|---|---|---|
| **#37 Ajduk, M. 2008** | Manually by measuring density three times on the visually least dense area at the level of maximal stenosis | Median tissue density of type VIb vs other plaques.<br>Cut-off value for type Vib was determined by ROC analysis. | Distance between carotid bifurcation and level of maximal stenosis. | NR | No | A standardized optimized, contrast-enhanced protocol was used with intermediate reconstruction: 120 kVp, 120 mAs, collimation 16 x 0.75 mm, pitch 1. Iopamidol was administrated 370 mg iodine/mL, 4 mL/sec, 70 mm3, 325 psi. | Manual |
| **# 36 Ajduk, M. 2013** | Manually by measuring density three times on the visually least dense area at the level of maximal stenosis | Median tissue density of type VIb vs other plaques.<br>Cut-off value for type Vib and V was determined by ROC analysis. | Distance between carotid bifurcation and level of maximal stenosis. | NR | No | A standardized optimized, contrast-enhanced protocol was used with intermediate reconstruction: 120 kVp, 120 mAs, collimation 16 x 0.75 mm, pitch 1. Iopamidol was administrated 370 mg iodine/mL, 4 mL/sec, 70 mm3, 325 psi. | Manual |
| **#17 Das, M. 2009** | Manually by measuring Hounsfield densities for each part of the plaque by using a pixel lens. | Cut-off values was predefined:<br>< 50 HU = fatty<br>50-119 HU= mixed<br>>120 HU=calcified | NR | NR | No | A test bolus was performed to assess the optimal bolus timing prior to the scan. The optimal scan delay was assessed after graphical resentation of peak enhancement at the aortic arch. | Manual |
| **#16 De Weert, T. 2006** | A combination of manual and cumputerised processing:<br>A ROI was manually drawn on histological slices and corresponding CT slices. A custommade plug-in for the software (ImageJ) determined different component areas within the ROI. | Hounsfield values was measured for LC and FT in all plaques and based on the distribution, cutoff values was determined between LC and FT. | Shape of the lumen and vessel wall, the location of the bifurcation, and the presence of calcifications | NR | No | A standardized optimized contrast-enhanced protocol was used with 120 kVp, 180 mAs, collimation 16$0.75 mm, table feed 12 mm/rotation, pitch 1. | Manual |
| **#14 Horie, N. 2012** | Manually by free-hand rating and measuring segmentation of the ROI in each plaque. The mean HU in the ROI was measured early and delayed contrast phase. | Studying dynamic plaque enhancement.<br>No cut-off value for differentiation of specific components. | NR | NR | Yes (Severe calcified components covering the vessel wall was excluded from the ROI) | Two-phase contrast-enhanced CT was performed in the early- and delayed phase (2 minutes interval). An automatic bolus-tracking program was used to start acquisition after contrast injection. Iohexol, was administered with injection rate of 3.5 ml/s, for a total volume of 70 ml followed by a saline chaser of 40 ml. | Manual |
| **#15 Wintermark, M. 2008** | A computer algoritm, developed by the authors, segmented contours of the artery, calculated HU thresholds and colored the components after composition. The color overlays was manually reviewed. | Average HU in each pixel compared with percentage of each component in corresponding histologic squares.<br>Cut-off determined by the halfway attenuation between the mean densities for each component. | Overall morphologic features and the distance from the carotid bifurcation | NR | No | Optimal timing of acquisition was achieved with test bolus technique. 70 mL Iohexol was injected with a injector rate of 4 mL/s. | Manual |

**CEA= Carotid endarterectomy. ROI= Region of interest. HU= Hounsfield Units. LC= Lipid core. FT= fibrous tissue. NR= Not reported. NS= Not specified.**

**Table 25. Diversity between methods for studies of PIRO 2 (MRI).**

| Article reference number, Author, Year | MRI protocool | Contrast administration | Method to detect components | Landmarks used to match histology and imaging slices | Method of CEA | Method of histologic assessment |
|---|---|---|---|---|---|---|
| **# 33 Altaf N. 2013** | A predetermined protocol optimized to detect hemorrhage (NS) | - | Not described | NS | Standard endartecectomy (plaque incised and subsequently removed) | Semiquantitative analysis as recommended by Lovett JK, et al 2005 |
| **# 30 Chu B. 2004** | A standardized protocol (NS) | - | Manually defining intensity of tissue with the adjacent SCM muscle as reference. | Carotid bifurcation and morphological features of lumen, vessel wall and calcifications | NS | Manually |
| **# 32 Kerwin, WS. 2006** | A generalized kinetic model for dynamic contrast enhanced MR imaging was used to quantify contrast agent dynamics | Gadolinium-based contrast agent per kg, injection rate: 2 mL/sec | Manually by comparing intensity of contrast in the jugular vein to intensity within the plaque. | Carotid bifurcation and overall shape of the lumen and plaque | NS | Manual drawing technique |
| **# 31 Saam T. 2005** | A stardardized protocol (Yuan C, et al 2001) optimized for examining bilateral carotid arteries | - | A custom designed imaging analysis tool (QVAS) was used to perform area measurements. SCM was used a reference for intensity. | Carotid bifurcation, lumen size and shape, wall size and shape, plaque configuration and calcifications | NS | Manually |
| **# 38 Saito A. 2012** | NR | - | The ROI was manually defined but analyzed with a software (zioTerm2009). SCM was used a reference for intensity. | NS | NS | The ROI was manually defined but analyzed with a software (ImageJ Ver. 1.44) |
| **# 29 Trivedi R. 2004** | NR | - | Qualitative characterization was performed manually. Quantitative measurements Was performed by using (BIR, Mayo Clinic) software. | Carotid bifurcation, size and shape of the lumen and relative position of the external carotid artery intima | NS | By using software (Leica Microsystems) on computerized images captured with a 1.6-magnification lens |
| **# 34 Yoshida K. 2007** | NR | - | Manually. SCM was used a reference for intensity on T1-weighted and the submandibular gland on T2-weighted images. | Carotid bifurcation and size and shape of the lumen and plaque | Without placement of an incision whenever possible | Manually by a operator-defined ROI |
| **# 35 Young V E. 2009** | NR | - | Both the CMR Tools software (Cardiovascular Imaging Solutions Ltd) and a software developed by the authors providing intensity values for each pixel. SCM was used a reference for intensity. | NS | NS | Manually |

**SCM= Sternocleidomastoideus muscle. NR= Not reported. NS= Not specified. ROI= Region of interest.**