Imaging biomarkers for detection of coronary artery disease

den Dekker, Martijn

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Martijn A.M. den Dekker
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Martijn Adrianus Martinus den Dekker

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Promotor

Prof. dr. M. Oudkerk

Copromotor

Dr. R. Vliegenthart

Beoordelingscommissie

Prof. dr. J.E. Wildberger
Prof. dr. W.P.Th.M. Mali
Prof. dr. H.J. Lamb

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Walking is man‘s best medicine
Hippocrates

Contents

1

General introduction

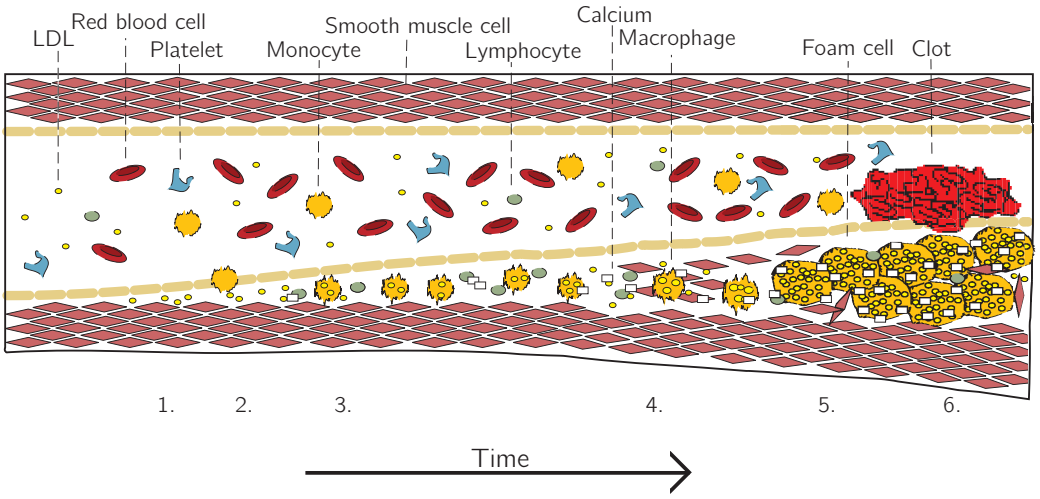
General introduction

Cardiovascular diseases were until recently the leading cause of death in Western countries. It still is the number one cause of death in women. Even though malignancies have a higher mortality rate, 27.2 % of all deaths in the Netherlands in 2012 were caused by cardiovascular events. In this group, death in men is most commonly caused by a myocardial infarction. For women, this is caused by stroke.[1]
In the United States, cardiovascular diseases still have the highest mortality rate in both men and women.[2]

Clinical relationship between ECAD and CAD events

Cardiovascular diseases are caused by atherosclerosis building up in the arteries and narrowing the lumen (stenosis). This may lead to ischemia and infarction. Atherosclerosis is a gradual process, which increases with age. It already starts in childhood with fatty streaks. The process of atherosclerosis development is shown in Figure 1.1. Older people generally have a higher prevalence and severity of atherosclerosis. Besides age, other risk factors of atherosclerosis are high blood pressure, diabetes, male gender and high cholesterol. A familial predisposition, inactivity or obesity can also influence the development of atherosclerosis. Less known risk factors are an increased homocystein or high sensitive C-reactive protein (CRP) in the blood. There are a number of predilection sites for atherosclerosis, of which the most common are the coronary arteries, aorta, femoral arteries and carotid arteries.
The REACH trial revealed that one in every six patients with coronary artery disease (CAD) or extra-cardiac arterial disease (ECAD) also had complaints of atherosclerosis in one or two other arterial territories.[3] From these results it was concluded that atherosclerosis is to be considered and treated as a systemic disease. A regional expression of atherosclerosis or differing severity of disease might also be possible. Risk factors, such as smoking and hypertension can influence for the general expression of atherosclerosis, and on a more local scale, several promotors and inhibitors have been discovered. For example in case of CAD, epicardial fat can play a possible role in the development of atherosclerosis by paracrine effects.[4]
A correlation has been found between low shear stress, turbulence or oscillating flow and plaque development.[5] Therefore, low shear stress, which can cause gene modulations of the endothelial cells, resulting in an increased oxidative stress, may also be a possible promotor of rapid development of atherosclerosis. Areas with a laminar flow have a relatively small chance to develop atherosclerosis.[5]
How many and which other factors can evoke or suppress atherosclerosis is still subject to research. CAD and ECAD patients do not necessarily share a traditional risk factor profile, despite the common underlying disease.[6]

Figure 1.1: Boxplot of skin autofluorescence between patients with increasing degree of atherosclerosis



The last decade research focus has been on identification of as yet asymptomatic patients with an increased to high risk of cardiovascular morbidity and mortality. Several epidemiological studies have proposed different formulas and risk scores to predict cardiovascular morbidity and mortality. In patients with acute chest pain, but without co-morbidity, the majority was free of CAD.[7]

Risk factors can only identify a minor part of patients who will suffer from a coronary event in the future, and the presence of cardiac complaints also is also not clear. On the other hand, ECAD is strongly associated with coronary and cerebral morbidity and mortality.[8] Prospective epidemiological studies in high risk patient groups revealed a 20 - 60 % higher risk of a myocardial infarction and a 2 - 6 times greater chance of cardiac-related death.[9–11] Furthermore, the presence of CAD was found to be 2.5 times higher in patients with ECAD, compared to those without.[8] Patients with peripheral arterial occlusive disease have a greater chance of a myocardial infarction than of a stroke, and these events are more often fatal.[12,13] Even in a patient population with a low incidence of CAD, there is a strong relationship between ECAD and future coronary events.[11]

Imaging for coronary artery disease

Since the development of selective coronary catheter angiography (CAG) by Sones in 1958, this technique has been the method of choice for detection and follow-up of CAD. Several studies in the past have shown that diagnostic CAG has an average

morbidity of 2 % and a mortality of approximately 0.1 %.[14]

New developments in medical imaging modalities have opened the way for non-invasive coronary angiography by multi-detector computed tomography (MDCT) in the last decade.[15,16] Besides the lower complication risk and lower costs, MDCT also has the advantage of vessel wall visualization. In this way, both the composition of the plaque and its impact on the vessel lumen can be detected. A distinction can be made between lipid, fibrous and calcified coronary plaques.[17]

In recent years it has become clear that plaque composition is a better risk-predictor for acute coronary events than stenosis grade. Rupture of, so called, 'vulnerable plaques' account for approximately 70 % of sudden coronary deaths. Although the average absolute risk of severely stenotic plaques may be higher than that of average absolute risk of mildly stenotic plaques, the last category overwhelmingly exceeds the number of plaques with severe stenoses.[18]

In recent years, coronary computed tomography angiography (cCTA) has established itself as an accurate non-invasive alternative to coronary angiography catheterization. cCTA is excellent for detection of CAD, including both plaques and anatomical stenosis. Both the sensitivity and negative predictive value of cCTA for coronary stenosis are nearly 100 %. A challenge in cCTA is the suboptimal specificity. Calcified atherosclerotic plaques can obstruct the visualization of the coronary lumen and quantification of the degree of stenosis. This can lead to overestimation of the degree of stenosis. Another issue, in both cCTA and invasive coronary angiography, is the fact that anatomical stenoses do not necessarily cause ischemia. Evaluation of the effect of anatomical stenosis on myocardial perfusion is generally needed to diagnose hemodynamically significant CAD. The most frequently used method for perfusion imaging is single photon emitting computed tomography (SPECT), whereas the most accurate methods are adenosine perfusion magnetic resonance imaging (APMR) and positron emission tomography (PET). APMR has the advantage of lack of radiation and being more widely available.[19]

APMR is used to identify wall perfusion abnormalities of the left ventricle indicative of myocardial ischemia. Perfusion abnormalities of the myocardium due to CAD occur early in the ischemic cascade, before the onset of symptoms, ECG-abnormalities and wall motion disturbances. This makes the visualization of myocardial perfusion relevant in a functional sense, and suitable for detecting early functional effects of coronary atherosclerosis. The increase in flow through the coronary arteries relies mostly on vasodilatation. Compensatory vasodilatation distal to a stenosis is able to maintain flow during rest, but during (pharmacologically induced) stress conditions this capacity is exceeded. Adenosine induces vasodilatation of the coronary arteries. Stenotic coronary arteries lack the capacity to further dilate, which creates a relative area of hypoperfusion in the supplied myocardium compared to segments supplied by normal coronary arteries.

Nuclear perfusion imaging techniques have been widely studied, and proven feasible for perfusion imaging, but are limited by their spatial resolution, radiation burden, the use of radioactive tracers and susceptibility to attenuation artifacts. MR has the

ability to overcome these problems. First-pass perfusion MR imaging has also been proven contributory in identifying segments with impaired perfusion and distinguishing segments with different degrees of obstruction. MR perfusion imaging with the use of adenosine is routinely performed, the technique has been well described, and the clinical indications published in a consensus panel report.[20] First-pass perfusion MR imaging has shown that it can identify regional reductions in full-thickness myocardial blood flow during global coronary vasodilatation over a wider range than SPECT imaging. APMR has been shown to be an accurate and safe diagnostic modality to assess myocardial ischemia and viability in patients with proven or suspected CAD.[21]
The problem of correlation between anatomical and functional (hemodynamically significant) information does not only exist for non-invasive imaging. Fractional flow reserve (FFR) is the only way to detect hemodynamic impact of a stenosis using invasive coronary angiography: It measures the coronary blood pressures before and after a stenosis. In case of a hemodynamically significant stenosis, a decrease in coronary blood flow is seen, which gives an estimate for the severity of ischemia.
Recent developments in CT suggest that CT may provide an alternative method to assess myocardial ischemia at some point in the future.[22,23]

Screening for coronary artery disease in extra-cardiac arterial disease

Worldwide, cardiologists have set guidelines for the treatment of patients with cardiac symptoms and related abnormalities. Flowcharts have been developed to help physicians in the diagnostic process. Patient characteristics and the results of non-invasive testing determine the need for therapy and/or further invasive testing. The finding that a large proportion of patients in whom therapies are indicated do not receive those therapies in actual clinical practice is discouraging.[24]

Until now CAG has been used to judge the degree of stenosis and determine the need for revascularization. The role of cardiac CT and APMR imaging in the decision process is not yet established. For instance, no randomized clinical trials have been performed to establish the value of cCTA in detecting left main stenosis eligible for surgery. Revascularization (coronary artery bypass graft surgery (CABG) or percutaneous coronary intervention (PCI)) may be considered when medical therapy appears to be insufficient in patients with coronary artery abnormalities. The results of the Asymptomatic Cardiac Ischemia Pilot (ACIP) study indicate that high-risk patients with asymptomatic ischemia and significant coronary artery abnormalities, who undergo revascularization with CABG or PCI, may have a better outcome compared to those only receiving medical therapy.[25]
CABG is advised in case of left main stenosis or 3-vessel disease. Overall, the improvement in survival with CABG compared to drug treatment is 4.3 months at 10-year follow-up. The effect on survival is larger when the a priori risk is higher.

The poorer the left ventricular function the larger is the potential benefit of surgery. In patients with left main disease, the survival benefit of CABG compared to medical therapy is 19.3 months at 10-year follow-up. Therefore, the benefit of surgery over drug treatment for patients with significant left main stenosis (>50 %) is little argued.[26] CABG has an expected 30-day mortality of <1 % when performed in elective patients <65 years of age, who have no severe LV dysfunction or congestive heart failure. Technical modifications of traditional CABG have been developed in the last several years in an attempt to decrease the morbidity of the operation, either by using limited incisions or by eliminating the use of cardiopulmonary bypass.[26]

PCI may be considered when a high likelihood of success and a low risk of morbidity or mortality exists, e.g., non-diabetic patients with asymptomatic ischemia with one or more significant lesions in one or two coronary arteries. Little effort has been directed toward comparing medical therapy with PCI.[27] PCI has low risks and a high initial success rate. Re-stenosis decreases long-term clinical success, but studies with improved technologies, e.g. drug eluting stents, are promising.[28]

The treatment of silent myocardial ischemia in absence of severe coronary abnormalities is not studied extensively. They are all conducted in small groups of patients with coronary abnormalities. The presence of extensive ischemia on dobutamine stress SPECT imaging, whether silent or not, indicates an increased risk of cardiovascular events, and as with exercise testing, the threshold at which ischemia occurs carries important prognostic information.[29]

As discussed previously, stress imaging seems to allow for improved risk stratification. With a lack of evidence on the best treatment, silent ischemia in the absence of coronary abnormalities is mainly an indication for an increase of anti-ischemic and risk factor modifying medication. In a study by Kuijpers et al. dobutamine stress MR testing had high accuracy in assigning risk levels for future cardiovascular events.[30] However, the patients in this study were all suspected of CAD and not cardiac asymptomatic.

In conclusion, at present CABG or PCI may be considered as first line therapy in case of severe abnormalities in the coronary artery tree, even in asymptomatic patients with a high risk of cardiac events. In addition, silent myocardial ischemia has been shown to increase CAD risk and evidence indicates that in certain groups of these patients CABG or PCI treatment may reduce the risk. Alternatively, optimizing anti-ischemic drug treatment appears to affect CAD risk beneficially.

Purpose and outline

In summary, symptomatic ECAD is a common disease associated with a considerable increased risk of future coronary events and with a high prevalence of coronary atherosclerotic disease. Patients with aneurysmal or stenotic artery abnormalities may have similar risks. Therefore, in this thesis it is studied whether coronary atherosclerosis in these patients has a similarly high prevalence. Improvement of the prognosis of these patient groups is needed. Screening for CAD has become a realistic possibility with recent developments in cCTA and MR stress testing. Non-invasive cardiac imaging joined with a dedicated treatment algorithm based on the imaging findings, may beneficially affect the prognosis patients with aneurysmal or stenotic artery abnormalities, by reducing the incidence of cardiovascular events.

One of the weaknesses of cCTA has always been coronary calcium. A large amount of calcium causes blooming artifacts, which hinder coronary lumen evaluation. However, CT has evolved enormously since its introduction, while calcium score cut-off has remained the same. To assess whether diagnostic accuracy of cCTA for coronary stenosis with modern CT scanners is adequate in patients with a high calcium score, a meta-analysis is performed in **Chapter 2**.
To evaluate the necessity of cardiac imaging in cardiac asymptomatic patients at high risk, a cohort of cardiac asymptomatic patients with known ECAD underwent non-invasive imaging by cCTA and APMR. In **Chapter 3**, the results of this study are reported and the prevalence of severe, asymptomatic CAD in these patients is described.
Currently, no established non-invasive imaging modality can depict both anatomically and functionally CAD in a single examination. In **Chapter 4** a novel method to assess hemodynamic significance of stenoses based on common cCTA data is introduced, the so-called corrected contrast opacification evaluation.
cCTA yields additional information that may have diagnostic and prognostic value beyond the evaluation of coronary stenosis. One such measure, the amount of epicardial fat, is investigated in **Chapter 5**. Lastly, in **Chapter 6** a novel non-invasive skin-derived marker of inflammation and atherosclerosis is investigated in relation with the degree of atherosclerosis on cCTA. Possibly, this new marker could aid in the coronary risk assessment.

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2

Diagnostic performance of coronary CT angiography for stenosis detection according to calcium score: systematic review and meta-analysis

M.A.M. den Dekker
K. de Smet
G.H. de Bock
R.A. Tio
M. Oudkerk
R. Vliegenthart

Abstract

Objectives A systematic review and meta-analysis to assess sensitivity and specificity of coronary CT angiography (cCTA) for significant stenosis at different degrees of coronary calcification.

Methods A literature search was performed including studies describing test characteristics of cCTA for significant stenosis, performed with at least 16-MDCT and according to calcium score (CS). Invasive coronary angiography was the reference standard. Pooled sensitivity and specificity of cCTA by CS categories and CT equipment were calculated.

Results Of 14,121 articles, 51 reported on the impact of calcium scoring on diagnostic performance of cCTA and could be included in the systematic review. Twenty-seven of these studies (5,203 participants) were suitable for meta-analysis. On patient-basis, sensitivity of cCTA for significant stenosis was 95.8, 95.6, 97.6 and 99.0 % for CS 0-100, 101-400, 401-1,000 and >1,000, respectively. Specificity was 91.2, 88.2, 50.6 and 84.0 %, respectively. Specificity of cCTA was significantly lower for CS 401-1,000 due to lack of patients without significant stenosis. Sensitivity and specificity of 16-MDCT were significantly lower compared to more modern CT systems.

Conclusion Even in cases of severe coronary calcification, sensitivity and specificity of cCTA for significant stenosis are high. With 64-MDCT and newer CT systems, a CS cut-off for performing cCTA no longer seems indicated.

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2.1 Introduction

Computed tomography (CT) is increasingly used for non-invasive imaging of the coronary arteries. With the rising number of detectors in multidetector CT (MDCT) and the introduction of dual-source CT (DSCT), the accuracy of coronary CT angiography (cCTA) for detection of obstructive stenosis compared to invasive coronary angiography has improved,[1–3] with sensitivity increasing from 81 % for 16-MDCT to 94-100 % for newest CT systems at maintained high specificity of 92-95 %.[4–6]

The specificity of cCTA may be affected by coronary calcification, since severe calcification limits lumen assessment due to blooming artifacts.[7] In case of a high calcium score (CS), cCTA can yield false positive results, which is one of the main reasons why current guidelines still consider a high CS a contra-indication for performing cCTA.[8,9] For this reason, some study groups have limited cCTA to patients with CS below an arbitrary cut-off, with invasive coronary angiography being used for diagnostic purposes in those with higher CS.[3,8,10–13] In other studies, no CS cut-off was applied.[1,2,4–7,14–51] The question arises, especially with the improved technology of latest CT systems, whether a CS cut-off is needed to obtain good diagnostic accuracy in cCTA.

We performed a systematic review and meta-analysis to: (1) review the CS cut-off values reported in literature; (2) assess sensitivity and specificity of cCTA by MDCT and DSCT for significant stenosis ($\geq$50 %) at different degrees of coronary calcification.

2.2 Materials and Methods

Data sources

Pubmed and Embase were searched for studies published between January 2001 and June 2011, using the following search terms: "Coronary Angiography"[MeSH] OR "Coronary Artery Disease"[MeSH] OR "Coronary Stenosis"[MeSH] OR coronary[TIAB]) AND "Tomography, X-Ray Computed"[MeSH] OR CT[TIAB] OR MDCT[TIAB] OR DSCT[TIAB] OR "computed tomography"[TIAB] (Limits: Publication Date from 2001/1/1). We combined MeSH terms with free text searches to assure the maximum number of suitable articles. In Embase the same search was performed, but MeSH terms were translated into Emtree terms. As a starting date, 2001, the year 16-MDCT was introduced, was chosen as 16-MDCT is still frequently used for cCTA[48] and minimum recommended for calcium scoring.[52] The meta-analysis was executed and reported according to the Meta-Analysis of Observational Studies in Epidemiology (MOOSE)[53] and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statements.[54]

Study selection

Two reviewers independently assessed articles for suitability. Disagreements were resolved by a third reviewer. The articles were first screened on title and abstract. When considered suitable or when in doubt, the full text was reviewed. Reference lists of suitable articles were searched for additional studies. Studies were included in the systematic review if they: (1) used or suggested a CS cut-off for performing cCTA; or (2) addressed diagnostic accuracy of cCTA according to CS categories. Articles were excluded if they: (1) were laboratory or phantom studies; (2) concerned a review or case report; (3) included examinations of stented or bypassed coronary arteries; or (4) used <16-MDCT. There was no language restriction for the search, but during selection language was restricted to English. Articles in the systematic review were subsequently included in the meta-analysis if they: (1) reported test characteristics of cCTA by CS categories; (2) used invasive coronary angiography as reference standard; and (3) reported patient characteristics.

Data extraction

Using a standardized form, two reviewers extracted author, year of publication, study design, type and brand of CT system, study population size, mean or median patient age, body mass index, heart rate during CT data acquisition, use of beta-blockers or nitro-glycerine during CT data acquisition, mean or median CS with range, number of examined segments and non-interpretive or excluded segments with reasons, sensitivity, specificity, positive and negative predictive value (PPV and NPV), and if available, accuracy (total and per CS) per patient and per segment. A third reviewer verified the assembled data in case of discrepancies.

Quality assessment

Methodological quality and potential sources of bias in the meta-analysis articles were assessed with 14 standard items of the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool.[54,55] For each article, a quality score was accumulated by assigning 1 point to each QUADAS item that was fulfilled, 0.5 point to unclear items, and 0 points to un-fulfilled items. A score $\geq$11 points was considered high-quality and a score <11 points was considered low-quality.[56] Patient spectrum was defined as patients at intermediate risk of coronary artery disease or primarily referred for cCTA. Time period between tests was defined as 1 month. Two reviewers evaluated independently, with disagreements resolved by the third reviewer.

Statistical analysis

For the systematic review we summarized the data. For the meta-analysis, studies were divided in categories based on whether they reported the results per segment or per patient (or both). We predefined CS categories of 0-100, 101-400, 401-1,000 and >1,000. Study results were matched to these categories. If CS categories in the studies did not match predefined categories, we compared median CS with $25^{th}/75^{th}$ percentile of the reported categories to the predefined categories. If median CS was not available, we used mean CS with standard deviation. The results of reported categories were then included in the predefined category in which 80 % of the patients fell. In a subsequent analysis the studies were stratified by CT system type. Sensitivity, specificity, PPV, NPV, positive and negative likelihood ratios, and diagnostic odds ratio were calculated from the true positive, false positive, false negative and true negative. If counts were unavailable, we calculated these from available diagnostic test characteristics using an internet tool.[57] From reconstructed data, test characteristics were back-calculated for verification. We used a two-level mixed logistic regression model, taking into account random effects. For all calculations, 95 % confidence intervals (CIs) were obtained using the *F* distribution method to compute the exact confidence limits for the binomial proportion. The sensitivity and specificity per CS category were pooled weighted to the study sample size. A forest plot was generated to visualize this information. A summary receiver operating characteristics (sROC) curve was constructed to assess the diagnostic performance.[58] Analyses were repeated after exclusion of low-quality studies and after exclusion of studies that excluded over 10 % of segments. The normality of data on non-assessable and false positive segments was tested with the Kolmogorov-Smirnov test. The difference between 16-MDCT and newer CT systems regarding non-assessable and false positive segments was then compared with a weighted Mann-Whitney *U* test.

Publication bias was assessed with the Begg and Mazumdar rank correlation and Egger's regression test. Heterogeneity and inconsistency was tested with the Cochran Q test and I^2 statistic for sensitivity and specificity separately.[59,60] Possible sources of heterogeneity were predefined and checked in subgroup analyses based on CS, average age, gender, CT system employed, study size, study design, slice thickness, tube current, and iodine contrast.[61,62] Statistical analyses were performed using Stata SE 11.2 (StataCorp, College Station, USA), METANDI package[63] and Meta-DiSc, version 1.4 (Hospital Universitario Ramón y Cajal, Madrid, Spain).

2.3 Results

Systematic review

The results of the literature search including reasons for exclusion of articles are shown in Figure 2.1. In the primary search, 14,121 articles were retrieved. On the basis of title and abstract, 13,784 articles could be excluded. After reviewing 437 full-text articles, 51 were included in the systematic review. Of these, 32 were performed in Europe, 10 in Asia, 7 in the United States and 2 in Australia and Brazil. The number of included patients ranged from 19 to 1,500. Overall mean patient age was 60.7 years (range 48-70.8 years). Percentage men ranged from 42.4 to 87.5 %. Mean CS varied from 96 to 1,589 and median CS from 15 to 1,146.

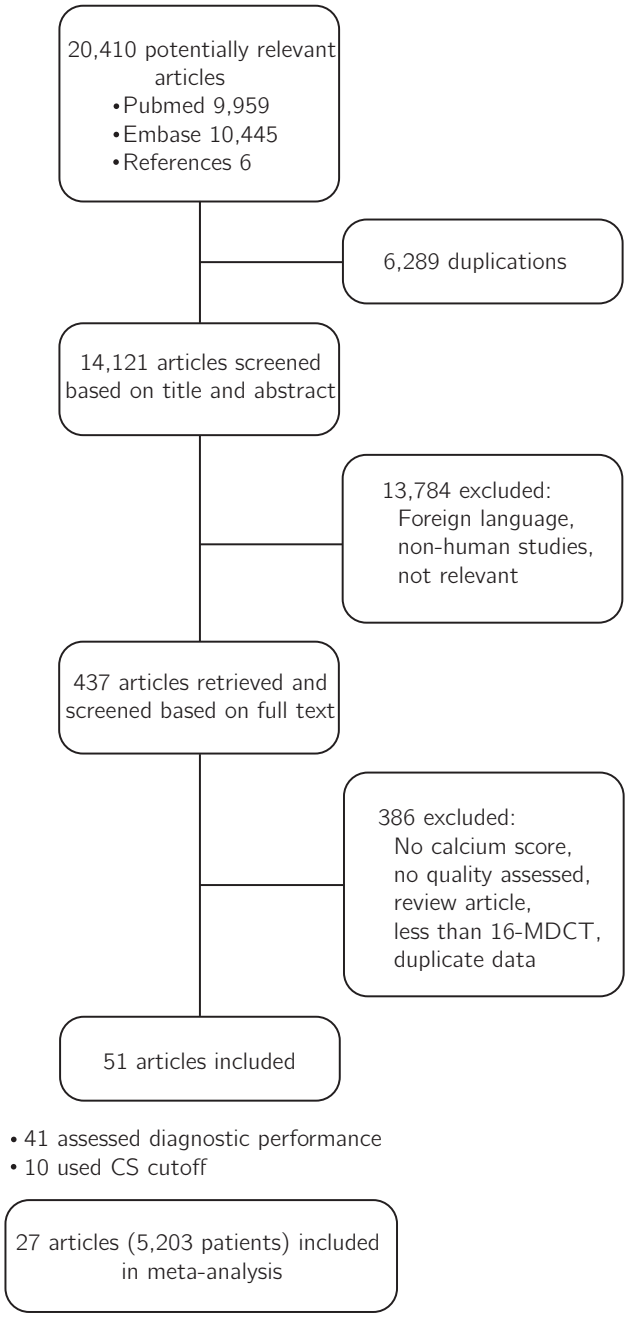
Twelve studies demonstrated good diagnostic accuracy of cCTA, even in case of high CS. Of these, 16-MDCT was used in three studies,[16–18] 64-MDCT in four,[14,19–21] DSCT in two,[22,23] 320-MDCT in one.[64] The last compared 64-MDCT to DSCT.[24] Thirty-four studies suggested a cut-off. The recommendations of 24 studies were based on the decrease in diagnostic accuracy of cCTA and the risk of non-diagnostic examinations,[2–6,8,10,12,15,24–38] 6 were based on the probability of coronary artery disease (CAD)[39–44] and 3 on the ROC.[45–47] Of the 34 studies, 7 suggested a CS cut-off of around 100 (range 40-142). Three suggested a cut-off of about 300 (range 297-350), 16 suggested a cut-off of 400. Eight studies opted for a cut-off of >400: one for 600, one for 800 and six for 1,000. The suggested CS cut-off differect by CT system type. Five studies found a decrease in accuracy or quality of cCTA, without proposing a CS cut-off.[7,48–51]

Seven studies applied a CS cut-off above which no cCTA was performed. Mentioned CS cut-offs were 400,[13] 500,[11] 600,[3] 800,[12] and 1,000.[8,10] One study[64] applied a minimum CS cut-off of 600.

Meta-analysis: study and patient characteristics

Twenty-seven studies were included in the meta-analysis. In total, 5,203 patients were included. Characteristics per study are presented in Table 2.1. On average, 193 patients were included per study (range 30-1,500). The mean CS was 500 Agatston Units (range 0-8,420), median 220 (interquartile range 133-330). The median age was 62 years (interquartile range 59-63) and 67 % were male. Of included patients, 767 (14.7 %) had diabetes, 2,372 (45.6 %) hypertension, 2,617 (50.3 %) hyperlipidemia and 906 (17.4 %) obesity. Smoking was present in 1,658 (31.9 %) patients. One hundred and seventy-six (3.4 %) patients were asymptomatic, 680 (13.1 %) had atypical chest pain, 1,866 (35.9 %) had typical angina, 769 (14.8 %) had unstable angina or non-ST-wave myocardial infarction and 1,406 (27.0 %) were suspected of CAD for other reasons. CT was performed preoperatively in 306 (5.9 %) patients.

Figure 2.1: Flow chart of the literature search



On invasive coronary angiography, 2,075 patients (39.4 %) had at least one stenosis with $\geq$50 % lumen diameter reduction, with significant CAD in one, two and three vessels in 15, 10 and 5 % of patients, respectively. Furthermore, 1.5 % of patients had non-quantified multivessel disease and 8 % of patients had significant CAD,

single- or multivessel. On CT, significant stenosis was present in 36.8 % of patients with CS 0-100, 58.4 % with CS 101-400, 86.2 % with CS 401-1,000 and 67.1 % with CS >1,000. Twenty-one studies with a total of 4,504 patients reported patient-based results, and 23 studies with a total of 56,256 segments showed segment-based results. In these studies, an extra 1,539 segments were excluded. In most studies, the main reasons were motion artifacts or small vessel size. Only five studies specifically mentioned the exclusion of segments due to severe calcification.[4,26,28,29,40] In patient-based (segment-based) analyses, 3 (6) studies used 16-MDCT, 12 (9) 64-MDCT, 1 (1) 320-MDCT and 5 (7) DSCT.

Meta-analysis: study quality

Quality assessment is shown in Figure 2.2. Overall, the quality of included articles was high (mean score, 11.8; 3 studies with score <11). Many studies were performed double-blinded, so they fulfilled the clinical review bias item (clinical review bias avoided). In 96 % of the studies, interpreters of cCTA were blinded to the results of invasive coronary angiography (test review bias avoided) and vice versa in 74 % (diagnostic review bias avoided).

Figure 2.2: Study quality summaries of articles included in the meta-analysis, assessed by the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool

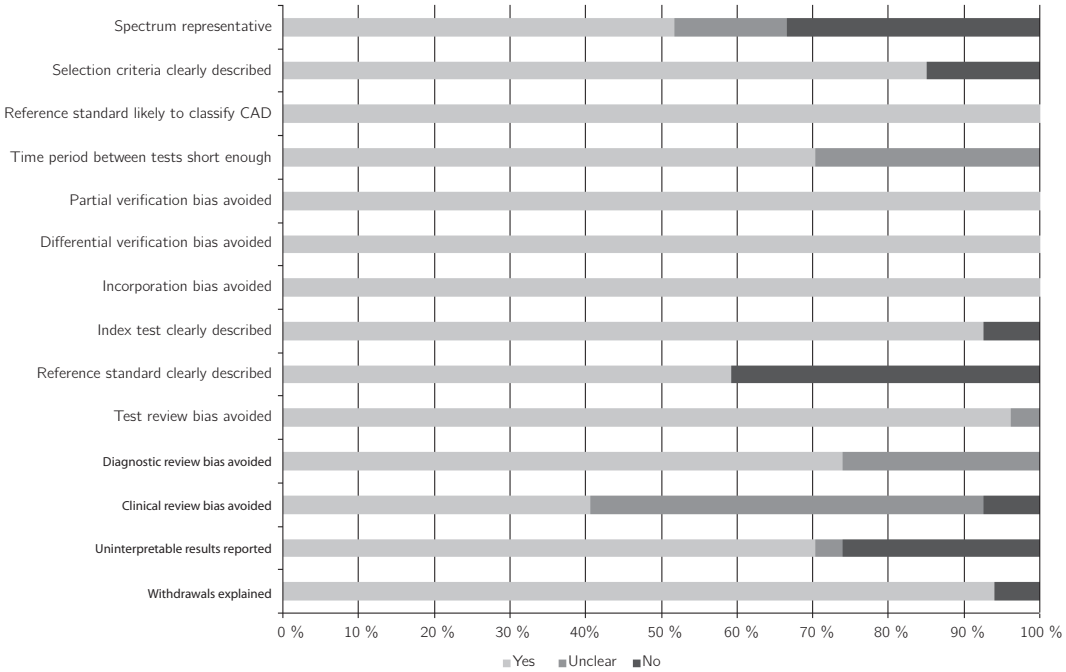


Table 2.1: Characteristics of the 27 studies included in the meta-analysis

Author	Year	Type of CT scanner	Number of patients	Mean ± SD age (years)	Male/female (*n*)	Calcium score subgroups used	Excluded segments, *n* (%)
Maffei[14]	2011	64-MDCT	1,500	58 ± 12	928/572	0, 1-10, 11-100, 101-400, 401-1,000, >1,000	0
Gang[6]	2011	320-MDCT	60	68 ± 9	38/22	0-100, >100	0
Nazeri[31]	2011	64-MDCT	168	58 ± 11	126/42	0-100, 101-418, 419-8,420	0
Zhang[5]	2010	DSCT	113	64 ± 12	82/13	0-100, 101-400, >400	11 (0.7)
Dewey[51]	2010	64-MDCT	291	59.3 ± 10	214/77	0-100, 101-300, 301-600	na
Bettencourt[32]	2009	64-MDCT	237	67 ± 10	114/123	≤10, 11-400, 401-1,000, >1,000	167 (4.1)
Meijs[24]	2009	64-MDCT	360	60 ± 6	245/115	<10, 10-99, 100-399, ≥400	na
Meng[37]	2009	DSCT	109	63 ± 9	68/41	0-100, 101-400, >400	25 (1.6)
Palumbo[39]	2009	64-MDCT	200	57 ± 13	169/31	0, 1-10, 11-100, 101-400, >400	0
Diederichsen[25]	2009	64-MDCT	109	63 ± 11	58/51	0 vs. >0, ≤50 vs. >50, ≤100 vs. >100, ≤200 vs. >200, ≤400 vs. >400	na
Marano[48]	2009	16-, 64-MDCT	350	64	265/85	0-100, 101-400, 401-1,000, >1,000	0
Stolzmann[22]	2008	DSCT	100	64.2 ± 6.5	62/38	0-315, ≥316	76 (4.8)
Budoff[33]	2008	64-MDCT	230	57 ± 10	136/94	0-400, >400	na
Ulimoen[26]	2008	64-MDCT	48	65.1	31/29	0-300, >300	177 (26.6)
Brodoefel[34]	2008	DSCT	100	62 ± 10	80/20	0-100, 101-400, >400	71 (5.5)
Alkadhi[27]	2008	64-MDCT	150	62.9 ± 12.1	103/47	0-194, >194	0

Table 1.1: continued

Author	Year	Type of CT scanner	Number of patients	Mean ± SD age (years)	Male/female (*n*)	Calcium score subgroups used	Excluded segments, *n* (%)
Brodoefel[35]	2007	64-MDCT	102	62 ± 10	82/20	0-100, 101-400, >400	26 (2.0)
Hausleiter[2]	2007	16-, 64-MDCT	243	62.0 ± 9.9	158/85	0-999, ≥1,000	0
Meijboom[50]	2007	64-MDCT	104	59	75/29	0-105, 107-375, 400-2,870	181 (10.6)
Coles[28]	2007	16-MDCT	120	61.9 ± 10.7	78/42	<100, 100-400, >400	273 (22.0)
Burgstahler[15]	2007	DSCT	41	66.2 ± 8.4	35/6	0-350, >350	0
Scheffel[23]	2006	DSCT	30	63.1 ± 11.3	24/6	<400, ≥400	46 (9.9)
Mitsutake[40]	2006	16-MDCT	92	63 ± 11	68/24	0, 1-399, ≥400	101 (11.0)
Manghat[4]	2006	16-MDCT	40	70.8 ± 10	27/13	≤400 vs. >400, ≤1,000 vs. >1,000	38 (8.4)
Ong[29]	2006	64-MDCT	134	54.5 ± 8.8	98/36	<142, ≥142	143 (9.7)
Mollet[20]	2005	64-MDCT	52	59.6 ± 12.1	34/18	0-10, 11-400, 401-1,000, >1,000	142 (16.4)
Cademartiri[18]	2005	16-MDCT	120	59 ± 11	105/15	<55, ≥55	0

CT Computed tomography, *SD* standard deviation, *MDCT* multidetector computed tomography, *DSCT* dual-source computed tomography, *na* not applicable as analyses were performed on a per-patient basis.

Meta-analysis: results

Publication bias was present in neither patient-based nor segment-based analysis.

The overall pooled sensitivity and specificity were 96.9 % (95 % CI, 96.1-97.5) and 86.4 % (95 % CI, 84.9-87.9) in patient-based analyses. On a per-segment basis the pooled results were 88.8 % (95 % CI, 88.0-89.5) and 94.9 % (95 % CI, 94.7-95.1), respectively. Sensitivity and specificity by CS categories can be found in Table 2.2. Compared to the overall pooled specificity, a CS of 0-100 scored significantly better (P <0.01). In patient-based analyses, the specificity for CS of 401-1,000 was significantly lower than overall (P <0.01). The specificity for CS over 1,000 was 84.0 % (95 % CI, 76.5-89.9), not significantly different from the overall pooled specificity. The drop in specificity for CS of 401-1,000 was not seen in segment-based analyses. In segment-based analyses, the specificity decreased significantly with increasing calcium score, from 98.4 % for CS 0-100 to 88.6 % for CS over 1,000 (P <0.01). Exclusion of low-quality studies did not alter the results.

Table 2.2: Characteristics of the 27 studies included in the meta-analysis

	Calcium score	Sensitivity (95 % CI)	Specificity (95 % CI)
Per Patient	Overall	96.9 (96.1-97.5)	86.4 (84.9-87.9)
Calcium score	0-100	95.8 (93.8-97.2)	91.2 (89.3-92.9)⋆
	100-400	95.6 (93.7-97.1)	88.2 (84.8-91.0)
	400-1,000	97.6 (95.9-98.7)	50.6 (39.5-61.7)⋆
	>1,000	99.0 (97.0-99.8)	84.0 (76.5-89.9)
Per Segment	Overall	88.8 (88.0-89.5)	94.9 (94.7-95.1)
Calcium score	0-100	93.0 (91.5-94.3)⋆	98.4 (98.2-98.6)⋆
	100-400	90.4 (88.8-91.8)	94.6 (94.2-94.9)
	400-1,000	89.8 (88.1-91.3)	90.9 (90.1-91.7)⋆
	>1,000	94.9 (93.5-96.1)⋆	88.6 (87.7-89.5)⋆

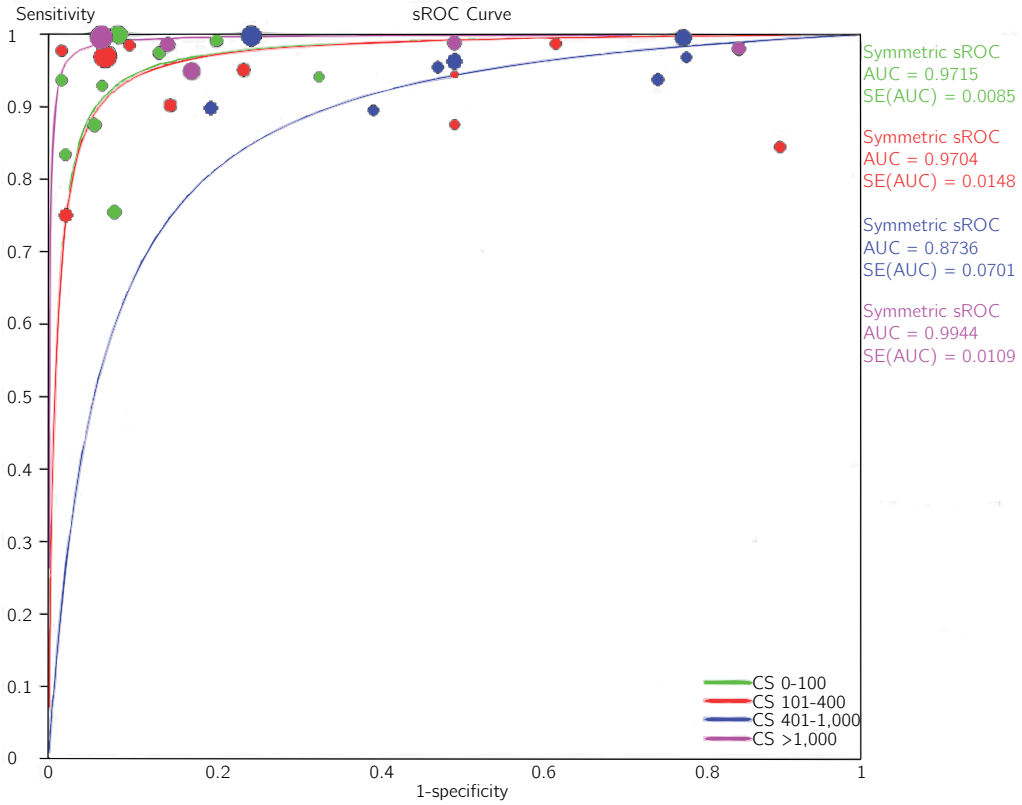
CI Confidence interval ⋆ P <0.05 in comparison to overall test characteristic

SROC curves on patient-basis are shown in Figure 2.3. Area under the curve for the ROCs was 0.97 (95 % CI, 0.96-0.99), 0.97 (95 % CI, 0.94-1.00), 0.87 (95 % CI, 0.74-1.00) and 1.00 (95 % CI, 0.97-1.00) for increasing CS categories in patient-based analysis. On a per-segment basis it was 0.99 (95 % CI, 0.97-1.00), 0.98 (95 % CI, 0.96-0.99), 0.97 (95 % CI, 0.94-0.98) and 0.98 (95 % CI, 0.96-1.00), respectively.

16-MDCT had a significantly lower specificity in patient- and segment-based analyses (P <0.01), as well as a lower sensitivity on a per-segment basis (P <0.01). 64-MDCT and newer CT systems had significantly improved sensitivity and specificity (P <0.05). Results can be found in Table 2.3.

In 16-MDCT, 3 of 6 studies reported exclusion of segments due to heavy calcification, while only 2 of 17 studies using 64-MDCT or newer CT systems reported this.

Figure 2.3: SROC curves on patient-basis, per calcium score



The median percentage of non-assessable segments was considerably higher in 16-MDCT than in 64-MDCT and newer CT systems: 6.1 (interquartile range 3.6-6.1) versus 0.0 (interquartile range 0.0-0.7), respectively (P <0.001). Even so, the mean percentage of false positive results of stenosis on cCTA was significantly higher in 16-MDCT than in 64-MDCT and newer CT systems, 9.0 (interquartile range 4.2-9.0) versus 4.3 (interquartile range 3.3-4.3) (P <0.001). The number of false positive results in case of CS >400 was minimally higher in 16-MDCT compared to 64-MDCT and newer CT systems, 3.1 (interquartile range 2.5-3.7) versus 2.5 (interquartile range 2.0-2.5) (P <0.001).

Meta-analysis: subgroup analysis

Patient- and segment-based analyses showed heterogeneity (I^2 of 86 and 96 % respectively). To assess possible sources of heterogeneity, we performed several subgroup analyses. Results for subgroups in patient-based analyses are shown in

Table 2.3: Sensitivity and specificity of coronary CT angiography by scanner generation

Subgroup	Number of patients (studies)	Sensitivity (95 % CI)	Specificity (95 % CI)
Per Patient	Overall	96.9 (96.1-97.5)	86.4 (84.9-87.9)
Scannertype			
16-MDCT	626 (3)	95.0 (92.1-97.0)	77.6 (72.2-82.3)⋆
64-MDCT	3,366 (12)	97.2 (96.3-97.9)	87.5 (85.8-89.1)
320-MDCT	60 (1)	100 (92.3-100)	92.9 (66.1-99.8)
DSCT	502 (5)	96.6 (93.9-98.4)	89.8 (84.8-93.6)
Per Segment	Overall	88.8 (88.0-89.5)	94.9 (94.7-95.1)
Scannertype			
16-MDCT	10,791 (6)	75.4 (73.1-77.6)⋆	92.4 (91.9-93.0)⋆
64-MDCT	35,074 (9)	92.9 (92.0-93.8)⋆	95.4 (95.2-95.7)⋆
320-MDCT	866 (1)	95.3 (91.0-98.0)⋆	97.6 (96.1-98.6)⋆
DSCT	9,525 (7)	92.0 (90.5-93.4)⋆	95.5 (95.0-95.9)

CI Confidence interval *MDCT* multidetector computed tomography *DSCT* dual-source computed tomography ⋆ P <0.05 in comparison to overall test characteristic

Table 2.4. Compared to overall pooled results, significantly lower sensitivity and specificity were found for multicenter trials (P <0.05). Significantly higher specificity was found for studies with consecutive inclusion of patients (P <0.05), and for studies using thinner CT slice thickness (P <0.01).

Subgroup results for segment-based analyses are shown in Table 2.5. Most results were similar to the patient-based analyses. Significantly lower specificity was found for studies with non-consecutive inclusion (P <0.01), studies with more women (P <0.01), thicker slice thickness (P <0.01), higher current (P <0.05) and higher iodine concentration (P <0.01). The lower specificity associated with high iodine contrast material and higher tube current can be explained by the number of 16-MDCT studies and the 16-MDCT study of Coles et al,[28] respectively.

Exclusion of studies that excluded over 10 % of segments did not influence diagnostic performance.

2.4 Discussion

This meta-analysis in 5,203 patients shows that specificity of cCTA for significant stenosis remained high in case of severe coronary calcification, with newer CT systems of 64-MDCT and beyond. For patients with CS over 1,000, the specificity of cCTA was 84 % (89 % in segment-based analysis), not significantly lower than overall. The test characteristics for 16-MDCT were significantly worse. The results suggest that for modern CT systems (at least 64-MDCT), a high CS should not necessarily imply cancellation of cCTA.

Table 2.4: Patient-based sensitivity and specificity of coronary CT angiography by subgroups

Subgroup	Number of patients (studies)	Sensitivity (95 % CI)	Specificity (95 % CI)
Age			
Average ≤62	3,024 (9)	97.2 (96.3-97.9)	86.5 (84.5-88.3)
Average >62	1,530 (12)	96.1 (94.4-97.4)	86.4 (83.7-88.7)
Gender			
Males ≤70 %	3,329 (13)	98.1 (97.3-98.7)	86.0 (84.2-87.7)
Males >70 %	1,225 (8)	94.3 (92.5-95.8)⋆	88.1 (84.6-91.1)
Study size			
≤120 Patients	736 (9)	97.5 (95.6-98.7)	90.5 (85.9-94.0)
>120 Patients	3,818 (12)	96.7 (95.8-97.5)	85.9 (84.3-87.5)
Study design			
Consecutive	2,479 (8)	98.1 (97.2-98.7)	90.0 (88.1-91.7)⋆
Non-consecutive	1,200 (5)	97.8 (96.1-98.9)	82.1 (77.6-86.1)
Multicenter	875 (8)	93.7 (91.6-95.5)⋆	81.4 (77.9-84.6)⋆
Slice thickness			
Slice <0.75 mm	2,489 (8)	96.7 (95.6-97.5)	91.0 (89.2-92.6)⋆
Slice ≥0.75 mm	722 (6)	96.0 (93.9-97.6)	83.1 (77.7-87.7)
Tube current			
Current <500 mAs	1,221 (8)	94.3 (92.3-95.9)⋆	87.3 (84.2-90.0)
Current ≥500 mAs	2,984 (10)	98.4 (97.6-98.9)⋆	85.9 (83.9-87.7)
Contrast			
Iodine <350 mg/ml	877 (7)	97.2 (95.2-98.5)	84.9 (81.0-88.2)
Iodine ≥350 mg/ml	2,147 (12)	95.5 (94.2-96.6)	82.6 (79.9-85.0)

CI Confidence interval ⋆ *P* <0.05 in comparison to overall test characteristic

In a recent meta-analysis on this topic that only included 64-MDCT studies, Abdulla et al.[65] concluded that cCTA was not feasible for CS over 400, with a specificity of 85 % and 66.5 % for low and high CS. However, other studies have shown the quality of DSCT has significantly improved compared to MDCT.[32,58] This was the reason for the current meta-analysis, and for including different CT systems that are deemed accurate for evaluating CS (16-MDCT and beyond). Additionally, we analyzed categories that included higher CS levels than previously, and in subgroups, to evaluate the impact of different factors on the test characteristics. Similar to the study by Abdulla,[65] we found a significant reduction in patient-based specificity for CS between 401 and 1,000. The reason for this finding is that in this specific group, the contributing studies reported few to no patients without significant stenosis on invasive coronary angiography. For a CS over 1,000, the specificity was not significantly different from the overall specificity. Interestingly, of patients with a CS over 1,000, a larger proportion did not have significant stenosis on invasive coronary angiography. We suspect selection bias of patients is a contributing factor.

In the meta-analysis the specificity of 16-MDCT was significantly lower both in

Table 2.5: Segment-based sensitivity and specificity of coronary CT angiography by subgroups

Subgroup	Number of segments (studies)	Sensitivity (95 % CI)	Specificity (95 % CI)
Age			
Average ≤62	36,885 (11)	90.8 (89.8-91.6)⋆	94.5 (94.2-94.7)
Average >62	19,371 (12)	85.0 (83.5-86.5)⋆	95.7 (95.4-96.0)⋆
Gender			
Males ≤70 %	15,029 (10)	90.1 (88.5-91.5)	93.6 (93.1-94.0)⋆
Males >70 %	41,227 (13)	88.4 (87.4-89.3)	95.4 (95.2-95.6)⋆
Study size			
≤120 Patients	13,601 (13)	90.8 (89.4-92.0)	94.7 (94.3-95.1)
>120 Patients	42,655 (10)	87.9 (86.9-88.8)	95.0 (94.7-95.2)
Study design			
Consecutive	39,661 (13)	91.8 (90.9-92.6)⋆	95.6 (95.4-95.8)⋆
Non-consecutive	11,967 (9)	88.3 (86.8-89.8)	92.1 (91.5-92.6)⋆
Multicenter	4,628 (1)	70.5 (66.7-74.1)⋆	95.8 (95.4-96.2)⋆
Slice thickness			
Slice <0.75 mm	33,362 (8)	90.1 (89.1-91.1)	95.8 (95.6-96.0)⋆
Slice≥0.75 mm	11,673 (9)	84.8 (83.0-86.4)⋆	93.7 (93.2-94.2)⋆
Tube current			
Current <500 mAs	13,881 (10)	90.6 (89.2-91.8)	96.1 (95.8-96.5)⋆
Current ≥500 mAs	37,327 (11)	90.7 (89.7-91.6)⋆	94.3 (94.1-94.6)⋆
Contrast			
Iodine <350 mg/ml	10,238 (7)	92.1 (90.7-93.4)⋆	96.1 (95.6-96.5)⋆
Iodine ≥350 mg/ml	23,737 (13)	83.8 (82.4-85.1)⋆	94.1 (93.8-94.4)⋆

CI Confidence interval ⋆ *P* <0.05 in comparison to overall test characteristic

patient-based and segment-based analysis. In patient-based analyses, there was no significant difference between newer CT systems and overall. In segment-based analyses, both sensitivity and specificity for 64-MDCT and newer CT systems were significantly better. Even in case of severe calcification there was no significant difference in sensitivity or specificity compared to overall test characteristics. The systematic review showed that there is a broad diversity in CS cut-offs proposed, although a cut-off of 400 was most commonly used. Interestingly, despite the increasing accuracy, there was a tendency for recommending lower CS cut-offs for newer CT systems. The current meta-analysis shows that accuracy of cCTA has considerably improved with 64-MDCT and newer CT systems, implicating that chosen CS cut-offs may not apply to newer CT systems. This is in discordance with the latest appropriate use criteria report, which still qualifies performing cCTA in case of a CS of 401-1,000 and over 1,000 as uncertain, under the assumption of 64-MDCT as a minimum requirement.[9] In that report, the use of cCTA was deemed appropriate in patients with a low and intermediate pre-test probability of coronary artery disease, either symptomatic or

pre-operative, similar to the patient populations included in this meta-analysis.

Some studies in the meta-analysis also included patients with high pre-test probability of CAD. A large percentage of these patients were present in the category of a calcium score between 401 and 1,000. In total 3 of 11 studies included patients with a high pre-test probability. This may partly explain the high prevalence of significant coronary artery disease in this patient group. According to the appropriate use criteria[9] there is no indication for cCTA in a high risk population. On the other hand, in the patient group with a calcium score over 1,000, more patients were at low and intermediate pre-test probability. In this group, as many as one-third of patients did not have significant stenosis. We focused on the diagnostic accuracy of cCTA for stenosis detection according to the CS. Thus, this study did not set out to answer the question whether in patients with high probability of significant CAD invasive coronary angiography rather than cCTA is indicated in case of a high CS, based on a high overall prevalence of significant stenosis. Also, according to the current guidelines, there is no indication for cCTA in asymptomatic patients with high calcium scores.

Even though diagnostic accuracy of cCTA is high with modern CT technology, and a CS cut-off may no longer be necessary, there are issues to keep in mind. If the CS is based on considerable calcification limited to a small area, there is a greater probability of artefacts and false positive results of cCTA, compared to a more even distribution of coronary calcification. Considering this, cCTA can be non-diagnostic for a certain coronary segment in case of a CS of 100, while CCTA in a patient with a CS of 1,500 can have good diagnostic quality;[7,66] Coles[28] found a decrease in accuracy for proximal segments mainly due to quantity of calcium. Cademartiri[18] and Mollet[20] found that large calcium deposits led to overestimation of lesion grade, but cCTA still maintained high overall sensitivity and specificity. With modern CT systems, the percentage of non-assessable segments has significantly decreased, in this meta-analysis from 5.8 % in 16-MDCT to 1.4 % in 64-MDCT and newer CT systems, while the mean number of false positive segments has also decreased with 64-MDCT and newer CT systems. Despite the fact that more segments were analyzed, likely also including more segments with considerable calcification, the percentage of false positive segments for a CS >400 in 64-MDCT and newer CT systems showed a minimal decrease from 3.1 to 2.5 %. The studies that reported excluding some segments due to heavy calcification did not specify what the calcium score of the specific segments or of the involved patients were. However, the studies did not exclude all segments of a patient, even in case the patient had a total calcium score over 1,000.

In clinical practice, the decision whether or not to perform cCTA, if dependent on CS, is usually based on the total CS and not on the distribution of calcified lesions. In this study we could not take calcium distribution into account when investigating the performance of cCTA.

Limitations to the current study include the fact that arbitrary cut-offs were chosen

for the CS categories. However, CS cut-offs were based on values commonly reported in literature. Also, as CS categorisation for individual studies sometimes had to be fitted to the predefined categories, there could be partial overlap of CS ranges for fitted study categories with neighbouring CS categories. As this was randomly the case, we do not expect this to have caused a systematic bias, although it could have slightly attenuated differences in test characteristics between CS categories. Furthermore, we restricted the systematic review to studies that reported the CS or a CS cut-off in association with cCTA. In many articles on cCTA, the CS is not mentioned. These studies may have performed cCTA even in high CS. The results of these studies could not be included in the systematic review or the meta-analysis. Second, there are limited cCTA publications on 256- or 320-MDCT; thus we could not accurately evaluate the performance of these modern MDCT machines.

In conclusion, with 64-MDCT and newer CT systems, the sensitivity and specificity of cCTA for significant stenosis remain high in case of severe coronary calcification. Therefore a CS cut-off above which cCTA should not be performed seems no longer to be indicated.

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3

Prevalence of severe subclinical coronary artery disease on cardiac CT and MRI in patients with extra-cardiac arterial disease

M.A.M. den Dekker
J.J.A.M. van den Dungen
I.F.J. Tielliu
R.A. Tio
M.M.J.J.R. Jaspers
M. Oudkerk
R. Vliegenthart

Abstract

Objectives Patients with extra-cardiac arterial disease (ECAD) are at high risk of coronary artery disease (CAD). Prevalence of silent, significant CAD in patients with stenotic or aneurysmal ECAD was examined. Early detection and treatment may reduce CAD mortality in this high-risk group.

Methods ECAD patients without cardiac complaints underwent computed tomography (CT) for calcium scoring, coronary CT angiography (cCTA), if calcium score $\leq$1,000, and adenosine perfusion magnetic resonance imaging (APMR) if no left main stenosis. Significant CAD was defined as calcium score >1,000, cCTA-detected coronary stenosis of $\geq$50 % lumen diameter, and/or APMR-detected inducible myocardial ischemia. In case of left main stenosis (or equivalent) or myocardial ischemia, patients were referred to a cardiologist.

Results The prevalence of significant CAD was 56.8 % (95 % confidence interval 47.5-66.0 %) One-hundred-eleven patients were included. Eighty-four patients (76 %) had stenotic ECAD, and 27 (24 %) had aneurysmal disease. In patients with stenotic ECAD, significant coronary stenosis was present in 32 (38 %), inducible ischemia in 8 (12 %). Corresponding results in aneurysmal ECAD were 8 (30 %) and 2 (11 %), respectively (P for difference >0.05). Sixteen (19 %) patients with stenotic and 6 (22 %) with aneurysmal ECAD were referred to a cardiologist, with subsequent cardiac intervention in 7 (44 %) and 3 (50 %), respectively (both P >0.05).

Conclusion Patients with stenotic or aneurysmal ECAD have a high prevalence of silent, significant CAD.

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3.1 Introduction

Extra-cardiac arterial disease (ECAD) is common in the Western population, with a prevalence of 29 % in persons over 50 years.[1] ECAD can be divided in stenotic and aneurysmal disease. In ECAD patients, coronary artery disease (CAD) is the major cause of death.[2–4] Compared with individuals without ECAD, patients with ECAD have over 5 times increased mortality risk, with an annual mortality rate >3 %, mostly due to coronary heart disease.[5] In patients undergoing vascular surgery, cardiac death accounts for 45 to 67 % of the operative mortality (3.9 %).[6] In view of the high cardiac mortality in ECAD patients, early detection and evidence-based treatment of silent, severe CAD could potentially improve prognosis.

Previous studies have shown that coronary revascularization can reduce the mortality risk in case of significant CAD, even in patients without cardiac complaints. The Coronary Artery Surgery Study showed an increased survival rate from 57 to 88 % for coronary artery bypass graft surgery (CABG) compared with medical management in patients with silent severe CAD.[7] In the Asymptomatic Cardiac Ischemia Pilot study, the 2-year mortality in patients with silent significant CAD was 1.1 % for coronary revascularization compared with 6.6 % in case of conservative treatment.[8] More recently, the CONFIRM registry (Coronary CT Angiography EvaluatioN For Clinical Outcomes: An InteRnational Multicenter Registry) confirmed a 2-year mortality reduction for coronary revascularization compared with conservative treatment (5.3 to 2.3 %) in patients with silent severe CAD.[9]

In case of severe CAD with or without symptoms, invasive treatment with percutaneous coronary intervention (PCI) or CABG is recommended.[10,11] This is considered particularly appropriate in patients at high risk (annual mortality risk >3 %),[11] including ECAD patients. In ECAD, the survival benefit of coronary revascularization therapy has mainly been investigated in patients scheduled for elective vascular surgery. A study in 1,000 patients with invasive coronary angiography reported improved 5-year survival if CABG for silent severe CAD was performed prior to major vascular surgery (survival 86-92 % for patients with CABG, compared with 62-69 % in those with indication for CABG, but who did not undergo surgery).[6] In a recent randomized trial in patients undergoing carotid endarteriectomy, half of the 426 patients were screened preoperatively by invasive coronary angiography.[12] Silent severe CAD was detected and revascularized in 31 %. In the screened group, no patient (0 %) died in the 30 days postoperatively, versus 2 (4.2 %) in the non-screened group. A randomized trial with 208 patients admitted for elective major vascular surgery showed a higher 4-year coronary event-free survival in high-risk patients undergoing preoperative coronary angiography and revascularization, compared with those without (86.6 vs 69.6 %, respectively).[13]

The exact prevalence of silent coronary stenosis and myocardial ischemia in the overall population of patients with stenotic or aneurysmal ECAD is unknown. As coronary angiography is an invasive procedure with some risk of morbidity and mortality, this

procedure is generally only performed in case of strong suspicion of CAD.

With new imaging modalities, CAD can be detected non-invasively. Computed tomography (CT) without contrast agent depicts the amount of coronary calcium, expressed as a calcium score. Contrast-enhanced coronary CT angiography (cCTA) can detect coronary luminal narrowing with high diagnostic accuracy,[14] while adenosine perfusion magnetic resonance imaging (APMR) can evaluate the functional significance of coronary stenosis on the myocardium under stress. APMR is more sensitive than nuclear single-photon emission CT for detecting myocardial ischemia, with the additional advantage of lack of radiation.[15–17] Non-invasive detection and subsequent treatment of silent severe CAD could lower CAD mortality in ECAD patients in the future.

This study was performed to assess the prevalence of silent CAD in cardiac asymptomatic patients with stenotic and aneurysmal ECAD using non-invasive imaging techniques.

3.2 Materials and Methods

Patients

The GROUND2 study is a prospective multicenter study.[18] Patients were recruited from departments of vascular surgery at the University Medical Center Groningen and Deventer Hospital. Patients were eligible if aged 50 years or older and diagnosed with symptomatic ECAD (peripheral artery obstructive disease [PAOD], carotid artery stenosis or aortic aneurysm) by a vascular surgeon. PAOD was defined by an ankle-brachial index of $\leq$0.7 or previous surgical treatment. Carotid artery disease was defined as a stenosis of at least 50 % lumen diameter stenosis as diagnosed by ultrasonography (Acuson Antares, Siemens, Erlangen, Germany) or previous surgical treatment. Aortic aneurysm was defined as a maximum aortic diameter of at least 3 cm, measured in any direction, detected by ultrasonography or CT (SOMATOM Definition, Siemens, Erlangen, Germany) or previous surgical treatment. If patients had stenotic and aneurysmal ECAD, they were classified according to the most limiting disease. The most limiting disease was considered the disease one which (had) needed surgical treatment.

Exclusion criteria were history or complaints of symptomatic CAD, unable to sustain a breath-hold for 25 seconds, asthma, contra-indications to APMR examination, contra-indications to adenosine, unable to remain in supine position for at least 60 minutes, significant aortic valve stenosis, contra-indications to iodine contrast agent, renal insufficiency (serum creatinine $\geq$120 mmol/l), severe arterial hypertension (>220/120 mmHg), extreme obesity (body mass index [BMI] >40 kg/m^2), severe physical deterioration due to concomitant disease, or inability to give informed

consent. The study was approved by the local institutional review boards. All patients gave written informed consent.

In the current investigation, only patients from the University Medical Center Groningen were included, as inclusion at the latter center is still ongoing and the inclusion rate at the latter center has so far been low, which could introduce bias. A flow chart of the study is given in Figure 3.1. Of the invited patients, over 70 % agreed to participate. The primary endpoints were defined as significant CAD (calcium score >1,000, cCTA-detected coronary stenosis ≥50 %, or APMR-detected perfusion defects. Secondary endpoint was referral to a cardiologist.

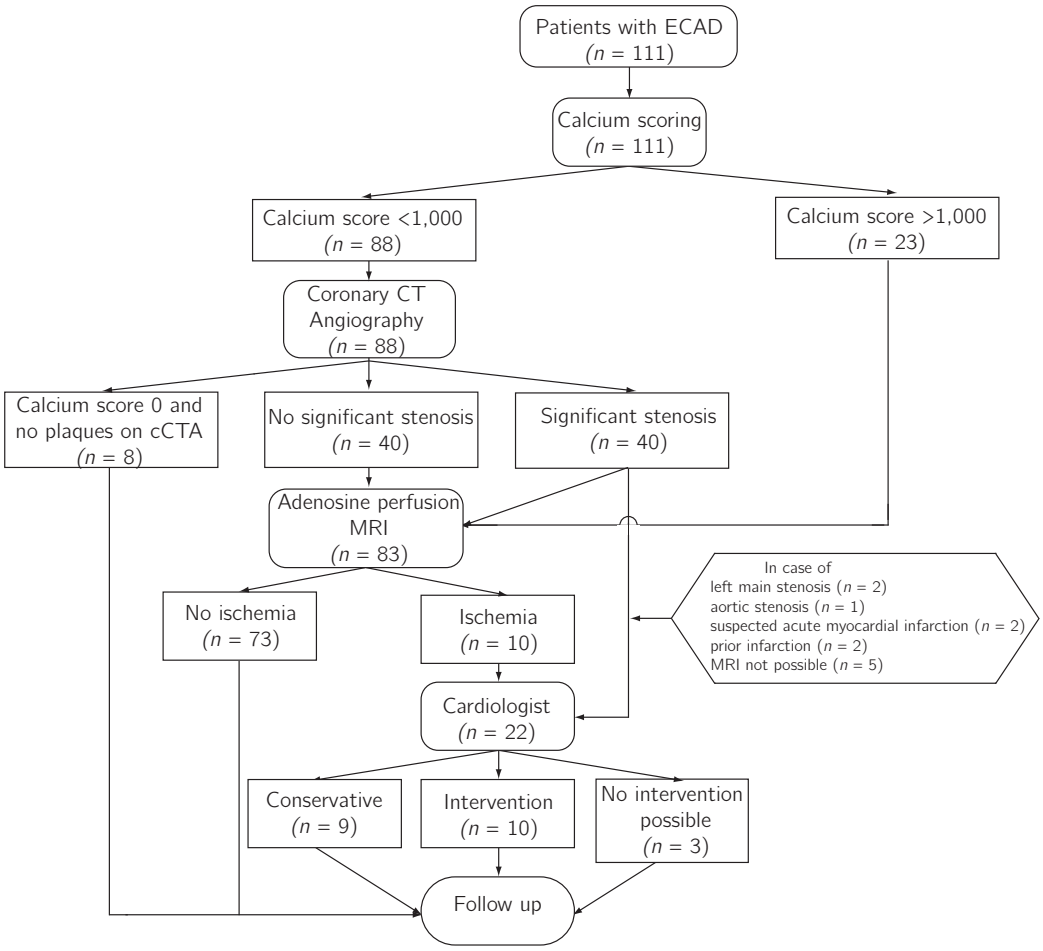
At baseline, patients completed a questionnaire on ECAD, risk factors, medication use, medical history, and family history (1st degree relatives <60 years). Height, weight and blood pressure were measured. Total cholesterol, high density lipoproteins (HDL), low density lipoproteins (LDL), triglycerides, glucose, serum creatinine and high-sensitive C-reactive protein (hsCRP) were measured at the local laboratory. Hypertension was classified as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, or medication for hypertension. Dyslipidaemia was classified as LDL-cholesterol >4.0 mmol/l, HDL-cholesterol <1.2 mmol/l (female) or <1.0 mmol/l (male), triglycerides >4.0 mmol/l, or current lipid-lowering medication. Diabetes was classified as fasting plasma glucose >7.0 mmol/l, known diabetes or medication for diabetes. Also an electrocardiogram (ECG) was performed.

CCTA imaging

A dual-source CT scanner was used (SOMATOM Definition, Siemens, Erlangen, Germany). First, a non-contrast-enhanced calcium score was performed, using prospective ECG-triggering, starting 15 mm cranial of the most superior coronary artery to 15 mm caudal of the inferior border of the heart. Calcium score calculation was performed according to Agatston.[19] Next, cCTA was performed using a standardized protocol. The following scanning parameters were used: slice acquisition 64 × 2 × 0.6 mm, gantry rotation time 330 ms, tube voltage 120 kV, BMI-adapted tube current (CARE Dose, Siemens, Erlangen, Germany). Calcium scoring was used as scout radiograph. A beta-blocker (metoprolol 5 to 20 mg, depending on heart rate) was administered intravenously to patients with a heart rate over 65 beats per minute, under blood pressure monitoring. Nitroglycerine was administered sublingually. Heart rate and ECG were continuously monitored. As contrast material iomeprol (Iomeron 400, 400 mg/ml, Bracco, Buckinghamshire, UK) was used at a flow rate adjusted for BMI.

Analysis of calcium scoring and cCTA was performed by the attending radiologist, with at least 5 years experience in cardiac imaging. According to clinical practice, a single radiologist read the cCTA; cCTA acquisition and evaluation were performed prior to APMR. In a 15-segment modified American Heart Association classification all segments, independent of image quality, were evaluated visually. Segments

Figure 3.1: Flow chart with the study design and number of patients



ECAD Extra-cardiac arterial disease *CT* computed tomography *MRI* magnetic resonance imaging.

were classified as having significant stenosis when there was $\geq$50 % lumen diameter reduction. Patients did not undergo cCTA if the calcium score exceeded 1,000.

APMR imaging

APMR was performed with a 1.5-Tesla scanner (Avanto, Siemens, Erlangen, Germany). Patients were instructed to refrain from caffeine 24 hours prior to examination. Medication interfering with adenosine was stopped 5 days prior to examination.

During the procedure, ECG was continuously monitored. Blood pressure and heart rate were recorded at baseline and every minute during adenosine infusion.

After acquisition of scout views for determination of the standard orientations of the heart, 3 parallel short-axis slices (basal, mid-papillary and apical) were planned for perfusion imaging. Hyperaemia was induced by continuous intravenous infusion of 140 μg/kg/min adenosine. After 3 minutes, a bolus of gadolinium contrast material (infusion rate and contrast material defined by local practice) was injected, followed by perfusion imaging. Minimally 5 minutes after perfusion images, MR sequences were repeated for rest imaging. Ten minutes after the last gadolinium injection, delayed contrast enhancement images were acquired to assess possible myocardial infarctions.

Analysis of the APMR was performed by the attending radiologist, with at least 5 years experience in cardiac imaging, using a 16-segment model. APMR was considered positive if a perfusion abnormality was present in at least two segments at consecutive planes during adenosine stress imaging, with normalization at rest.

Referral to Cardiologist

In the GROUND2 study, we used current guidelines to establish criteria for referral to a cardiologist for further work-up and treatment.[10,20] Patients were referred to a cardiologist if a left main coronary artery stenosis or equivalent (stenosis in proximal left anterior descending artery and circumflex artery) was detected on cCTA, or if APMR showed signs of reversible myocardial ischemia. In case of incidental non-cardiac findings, patients were referred to a dedicated physician. Invasive coronary angiography was performed according to standard procedures using a transfemoral or transradial approach. Multiple projections were obtained as deemed necessary by the angiographer. Coronary angiography images were assessed visually for diameter stenosis; no fractional flow reserve measurement was performed. Further work-up and treatment choice was left to the discretion of the cardiologist.

Statistical analysis

Patients with stenotic ECAD (PAOD, carotid stenosis) were grouped and compared with patients with aneurysmal ECAD. Descriptive statistics were calculated as mean with standard deviation or median with interquartile range and absolute numbers and percentages in case of dichotomous variables. The Student t-test was used for normally distributed, Kruskal-Wallis test for non-normally distributed and the Chi-square test for categorical variables. All statistical analyses were performed using PASW Statistics version 18.0.3 (SPSS Inc, Chicago, USA). All statistical tests are two-sided and a *P*-value of <0.05 was considered to be statistically significant. The study had 80 % power to determine a prevalence of 30 % CAD as compared with

20 % in the overall population.[6]

3.3 Results

Between December 2009 and March 2012, 111 patients were included. In total, 68 patients had PAOD, 16 carotid stenosis and 27 abdominal aortic aneurysm. Of the patients with PAOD, 3 were asymptomatic following surgery, the remainder had at least Fontaine 2. Carotid stenosis was symptomatic in 14 of 16 patients (87.5 %). Of the patients with an aortic aneurysm, 59.3 % had been interventionally treated. General characteristics are shown in Table 3.1. Ninety percent of the patients with stenotic ECAD had at least two risk factors, versus 81 % of the patients with aneurysmal ECAD (P = 0.21).

Table 3.1: Baseline characteristics of all patients and comparison of baseline characteristics in patients with stenotic versus aneurysmal extra-cardiac arterial disease

Baseline characteristics	All patients (n = 111)	Stenotic (n = 84)	Aneurysmal (n = 27)	P-value
Age (years)	65.2 ± 7.7	64.3 ± 7.6	67.9 ± 7.3	0.03
Gender (male)	84 (75.7 %)	61 (72.6 %)	23 (85.2 %)	0.08
Body mass index	25.8 [23.2-28.4]	25.2 [23.0-27.6]	26.4 [23.8-29.1]	0.24
Hypertension	92 (82.9 %)	72 (85.7 %)	20 (74.1 %)	0.16
Systolic blood pressure (mmHg)	141 ± 24	142 ± 24	134 ± 25	0.13
Diastolic blood pressure (mmHg)	78 ± 11	78 ± 10	79 ± 11	0.67
Dyslipidemia	104 (93.7 %)	78 (92.6 %)	26 (96.3 %)	0.52
Total cholesterol (mmol/l)	4.7 ± 1.9	4.7 ± 1.0	4.6 ± 1.3	0.59
Triglycerides (mmol/l)	2.06 ± 1.88	1.92 ± 1.71	2.47 ± 2.33	0.20
HDL-cholesterol (mmol/l)	1.3 ± 0.4	1.3 ± 0.4	1.2 ± 0.4	0.13
LDL-cholesterol (mmol/l)	2.7 ± 0.9	2.8 ± 0.9	2.6 ± 1.0	0.37
Diabetes mellitus	15 (13.5 %)	14 (16.7 %)	1 (3.7 %)	0.09
Glucose (mmol/l)	6.0 ± 1.9	6.1 ± 2.1	5.6 ± 1.0	0.22
Creatinin (mmol/l)	80 ± 18	79 ± 17	84 ± 21	0.24
High-sensitive CRP (mg/l)	2.1 [0.9-7.0]	1.9 [0.8-6.2]	3.8 [1.1-11.8]	0.045
Smoking				0.17
Current	37 (33.3 %)	32 (38.1 %)	5 (18.5 %)	
Past	65 (58.6 %)	46 (57.1 %)	19 (70.4 %)	
Never	9 (8.1 %)	6 (7.1 %)	3 (11.1 %)	

Values are mean ± SD, median [interquartile range] or number of subjects (percentage). *HDL* High density lipoprotein *LDL* low density lipoprotein *CRP* C-reactive protein.

Table 3.2 shows characteristics regarding family history. Aneurysmal ECAD was more

Table 3.2: Baseline characteristics of all patients and comparison of familial history in patients with stenotic versus aneurysmal extra-cardiac arterial disease

Familial history	All patients (n = 111)	Stenotic (n = 84)	Aneurysmal (n = 27)	P-value
High blood pressure	51 (45.9 %)	39 (46.2 %)	12 (44.4 %)	0.86
Diabetes	25 (22.5 %)	18 (21.4 %)	7 (25.9 %)	0.62
High cholesterol	35 (31.5 %)	30 (35.7 %)	5 (18.5 %)	0.09
Stroke	15 (13.5 %)	10 (11.9 %)	5 (18.5 %)	0.38
Myocardial infarction	23 (20.7 %)	19 (22.6 %)	4 (14.8 %)	0.38
Intermittent claudication	31 (27.9 %)	24 (28.6 %)	7 (25.9 %)	0.79
Dilatation of an artery	9 (8.1 %)	2 (2.4 %)	7 (25.9 %)	<0.01
Narrowing of an artery	18 (16.2 %)	16 (19.0 %)	2 (7.4 %)	0.15

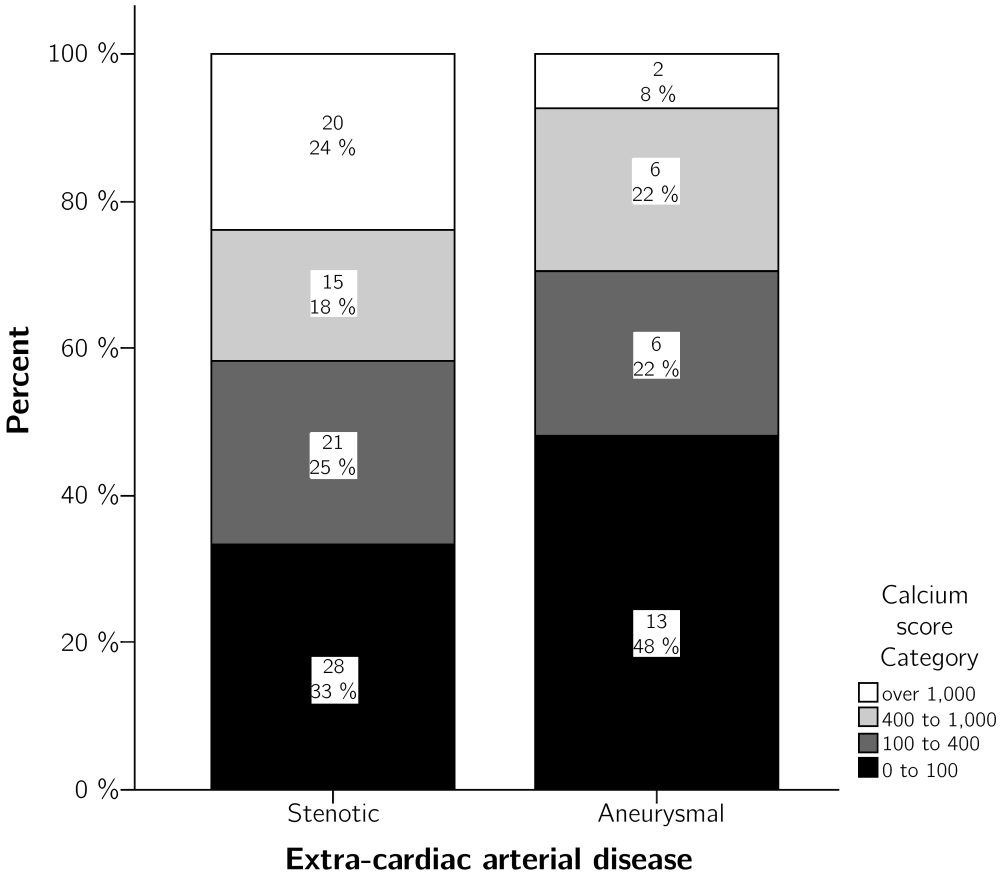
Values are number of subjects (percentage).

prevalent in the family of patients with aortic aneurysm, while there was a tendency (P = 0.15) for higher prevalence of stenotic ECAD in family of patients with stenotic ECAD.

CT was performed in all patients. Median calcium score was 239.5 for stenotic ECAD and 209.5 for aneurysmal ECAD (P = 0.21). This is demonstrated in Figure 3.2. Twenty-three patients did not undergo cCTA because their calcium scores were above 1,000. The results from cCTA were not significantly different between the two groups of patients (Table 3.3). After cCTA or calcium scoring, APMR was performed in 83 patients (74.8 %; Table 3.4). Ischemia was present in 8 patients (12.3 %) with stenotic ECAD and in 2 patients (11.1 %) with aneurysmal ECAD (P = 0.41). The prevalence of silent severe CAD was 56.8 % (95 % confidence interval 47.5-66.0 %). The waiting period between CT and APMR ranged from 1 to 6 months (70 % <2 months).

In total, 22 patients were referred to a cardiologist (Table 3.5). There was no significant difference in referral percentage between the two groups of patients. Six patients with positive cCTA and 9 patients with positive APMR underwent diagnostic coronary angiography. One patient did not have a significant stenosis on invasive coronary angiography. Of patients referred to the cardiologist, 9 received a coronary intervention (42.9 %). Two patients were advised to undergo surgery, but preferred PCI. Three more patients had a severe coronary stenosis without possibility for intervention. They were treated by maximum conservative treatment. Further details are shown in Table 3.3. Figures 3.3 and 3.4 show examples of referred patients. Of the 2 patients referred with aortic valve stenosis, it was severe in 1 patient. This patient was treated by transcatheter aortic valve implantation. In one patient with a surgically corrected aortic aneurysm a new aneurysm was found. Two patients had pulmonary lesions, comprising granulomas and previously unknown sarcoidosis. No malignancies were found.

Figure 3.2: Distribution of calcium score categories in stenotic and aneurysmal ECAD

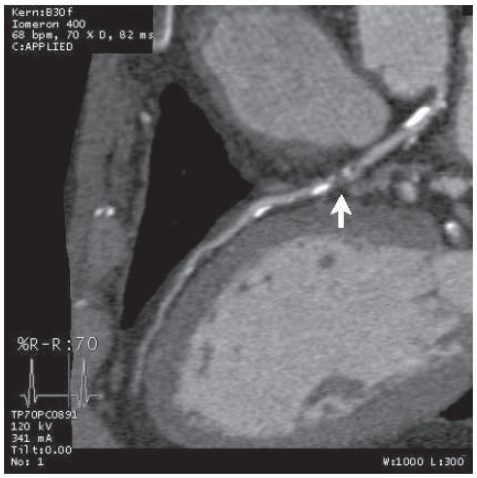


3.4 Discussion

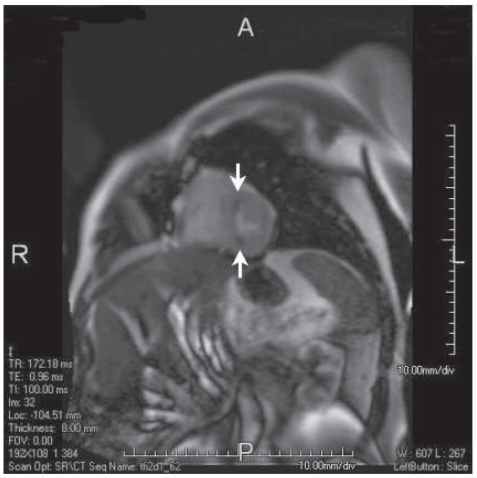
This is one of the first studies in extra-cardiac arterial disease patients assessing the presence of asymptomatic CAD using non-invasive screening. In ECAD patients without cardiac symptoms or history, we found a high percentage of silent, severe coronary artery disease on non-invasive imaging. Referral to a cardiologist because of severe CAD was deemed necessary in nearly one fifth of all patients, resulting in coronary intervention in 9.0 % of all patients. CT showed significant coronary stenosis in a third, while an additional 21 % of patients had a very high calcium score (>1,000). This does not imply CAD is present in all patients with ECAD. There was a small, but distinct group of patients without any coronary calcification or stenosis (7.2 %).

ECAD patients have a high risk of coronary events. Leng et al. found that 7 %

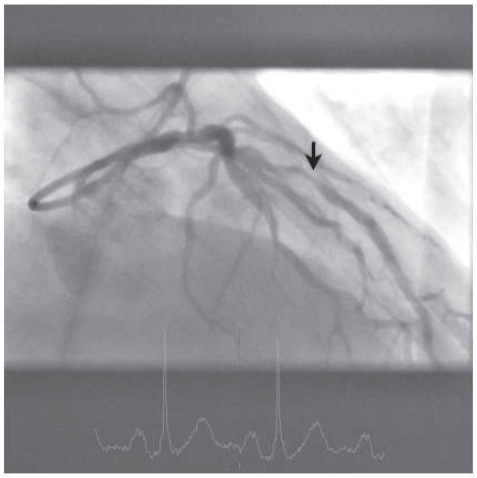
Figure 3.3: Example of a 76-year-old patient with PAOD. (a) Coronary CT angiography image. White arrow points to a mixed plaque in the proximal left anterior descending artery, with stenosis of the lumen. Calcium score was 553. (b) Short axis image of the adenosine perfusion MRI examination. White arrows point to a subendocardial perfusion defect in the septal wall, during stress. The perfusion defect was not present in the rest series, thus indicating inducible ischemia. (c) Invasive coronary angiography image of the left coronary arteries. Black arrow points to a significant stenosis, corresponding with the location seen on CT. (d) Invasive coronary angiography image after intervention. Good result after percutaneous coronary intervention with stent placement.



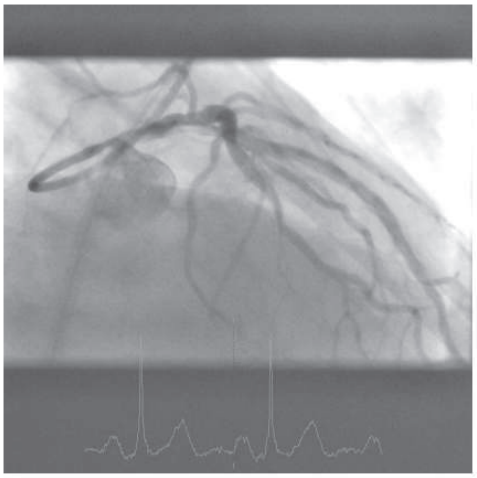
(a) Coronary CT angiography



(b) Adenosine Perfusion MRI



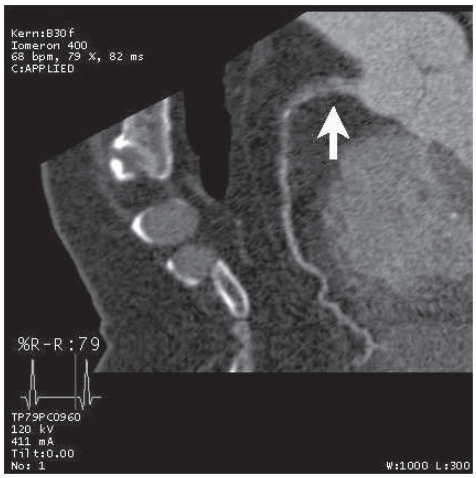
(c) Invasive Coronary Angiography



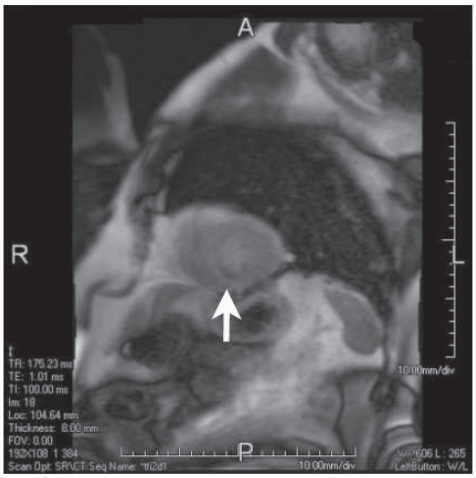
(d) After Percutaneous Coronary Intervention

of patients with ECAD suffered a myocardial infarction within 5 years in the Edinburgh Artery Study, a cohort study of 1,592 subjects.[21] In a large study, 1,000 patients with either stenotic or aneurysmal arterial disease, considered for elective

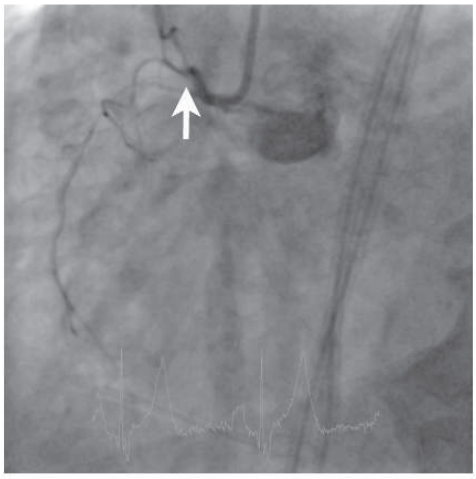
Figure 3.4: Example of a 68-year-old patient with PAOD. (a) Coronary CT angiography. White arrow points to a soft plaque in the proximal right coronary artery, narrowing the lumen more than 50 %. (b) Short axis image of the adenosine perfusion MRI examination. White arrow points to a perfusion defect of the inferior wall during stress. This perfusion was not present in rest, indicating reversible ischemia. (c) Invasive coronary angiography image of the right coronary artery. The right coronary artery is proximally occluded (white arrow). No percutaneous intervention was possible at this stage. Due to the asymptomatic status and good collateral circulation (visible as contrast filling distal to the occlusion), no surgery was performed.



(a) Coronary CT angiography



(b) Adenosine Perfusion MRI



(c) Invasive Coronary Angiography

vascular reconstruction, underwent invasive coronary angiography. Approximately 30 of the patients had significant CAD, warranting coronary revascularization. The authors concluded that non-invasive cardiac screening could be important to assess the

Table 3.3: Results from non-invasive imaging and cardiologist

	All patients (n = 111)	Stenotic (n = 84)	Aneurysmal (n = 27)	P-value
Computed tomography	111 (100 %)	84 (100 %)	27 (100 %)	
Calcium score	223.4 [33.4-850.2]	239.5 [48.4-991.8]	209.5 [26.8-457.0]	0.21
Coronary CT angiography				0.88
No calcium or stenosis	7 8 (7.2 %)	6 (7.1 %)	2 (7.4 %)	
No significant stenosis	40 (36.0 %)	29 (34.5 %)	11 (40.7 %)	
Significant Stenosis	40 (36.0 %)	32 (38.1 %)	8 (29.6 %)	
Calcium score >1,000 AU	23 (20.7 %)	17 (20.2 %)	6 (22.2 %)	
Magnetic resonance imaging	83 (74.8 %)	65 (77.4 %)	18 (66.7 %)	0.41
No ischemia	73 (88.0 %)	57 (87.7 %)	16 (88.9 %)	
Ischemia	10 (12.0 %)	8 (12.3 %)	2 (11.1 %)	
Cardiologist	22 (19.8 %)	16 (19.0 %)	6 (22.2 %)	0.66
Invasive angiography	16 (72.7 %)	13 (81.3 %)	3 (50.0 %)	
Conservative treatment	9 (40.9 %)	6 (37.5 %)	3 (50.0 %)	
Intervention	10 (45.5 %)	7 (43.7 %)	3 (50.0 %)	
PCI	7 (70.0 %)	7 (100 %)	0 (0.0 %)	
CABG	2 (20.0 %)	0 (0.0 %)	2 (66.7 %)	
Other	1 (10.0 %)	0 (0.0 %)	1 (33.3 %)	
No treatment possible	3 (14.2 %)	3 (18.8 %)	0 (0.0 %)	

Values are median [interquartile range] or number of subjects (percentage).
CT Computed tomography *AU* Agatston units *PCI* percutaneous coronary intervention *CABG* coronary artery bypass graft.

necessity for invasive coronary angiography.[6,22]

Little is known about the prevalence of severe, silent CAD. This is mainly because previously, invasive coronary angiography was the only method to detect coronary stenosis. This procedure is not without complications and therefore not usually performed in patients without clinical signs of CAD. Only recently, CT and MRI have emerged as non-invasive imaging methods for imaging of the coronary arteries and myocardium. A previous study (n = 3,263) on the association between ECAD en CAD, examined with intravascular ultrasound, revealed that patients with ECAD had more extensive and calcified CAD, impaired arterial remodelling and greater disease progression, compared with patients without ECAD.[23] This suggests that patients with ECAD could have a more aggressive form of systemic atherosclerosis. ECAD may actually directly promote the development and progression of CAD. Two mechanisms can be suggested. First, ECAD limits mobility, not only in patients with PAOD, but also in other forms of ECAD. This may lead to a lower frequency of exercise-induced cardiac symptoms in ECAD patients with CAD. Second, ECAD could have a direct relation with myocardial ischemia through impaired peripheral

endothelial function with reduced vasodilatation and vasoconstriction in response to stress.[24,25] A beneficial effect from risk factor reduction on atherosclerosis progression was demonstrated by Hussein et al.[23] Risk factor modification with lifestyle changes such as smoking cessation and optimal medical treatment with statins, beta-blockade and anti-platelet therapy is recommended, especially in patients with several cardiac risk factors undergoing high-risk vascular surgery, although only perioperatively for aneurysm surgery.[26,27] Unfortunately, adherence to the guidelines regarding risk factor reduction is often performed less reliably in patients with ECAD than in patients with proven CAD.[24,25]

The Coronary Artery Revascularization Prophylaxis study showed no overall survival benefit from pre-operative coronary revascularization before elective vascular surgery in patients with single- or multi-vessel CAD. However, pre-operative coronary revascularization did positively impact survival in patients with unprotected left main coronary artery stenosis, which was present in 4.6 % of patients.[28] Another study stated that in patients undergoing elective vascular surgery, risk stratification screening for CAD is indicated, possibly with non-invasive imaging.[26] Patients who have clinical atherosclerotic disease, such as ECAD, are also considered to be at high risk of CAD.[29] Drawback of the available risk stratification models for cardiovascular risk is the relative inaccuracy, for example to predict peri-operative death after elective open abdominal aortic aneurysm repair.[30] Thus, non-invasive imaging of CAD can be an important addition to risk stratification in appropriately selected ECAD patients.

The value of coronary assessment prior to carotid endarteriectomy has previously been studied. Illuminati et al.[12] found a reduction of postoperative cardiac ischemic events when invasive coronary angiography, if necessary combined with percutaneous coronary intervention, was performed preoperatively. This was also beneficial in cardiac asymptomatic patients, and supports cardiac screening prior to major vascular surgery.[22] Coronary stenosis was found in about a third of patients, in line with our results. While in this study only preoperative patients were included, we evaluated a broader range of ECAD patients. It is unknown if screening for CAD in the entire population of ECAD patients is recommendable. Current guidelines do not advocate routine CAD-imaging in all ECAD patients. While our study shows silent, severe CAD in a considerable part of ECAD patients, a beneficial effect of CAD screening by non-invasive imaging on occurrence of coronary events in the following years still needs to be proven. For this, large trials should be performed.

There are limitations to our study that must be mentioned. First of all, the study population was relatively small. Inclusion of vascular patients for our cardiac imaging study was complicated by the fact that many of these patients already had symptomatic CAD or comorbidity. Probably, this also at least partly explains the lack of studies on non-invasive cardiac imaging in this patient group, and adds to the value of the current study. We estimate that approximately 10 % of patients were considered suitable for inclusion. It is probable that the setting of the tertiary, university hospital has contributed to low inclusion rate, as the patients who are treated in this center

Table 3.4: Reasons for not performing adenosine perfusion magnetic resonance imaging (APMR)

APMR not performed	28
Not possible (i.e. aortic valvular stenosis, aortic stent)	11
Zero calcium score and no coronary plaque	8
Primary referral to cardiologist after CT	4
Refusal to undergo APMR	3
Endpoint reached (death or myocardial infarction)	2

CT Computed tomography.

are more often complicated cases. Thus, it is likely that the inclusion rate would be substantially higher in a general hospital. Secondly, although the two vascular patient groups are not significantly different in risk factors, they are different in size. The group with aneurysmal vascular disease was smaller. This can be explained by the fact that stenotic atherosclerosis is much more prevalent in the vascular surgery outpatient clinic, compared with aortic aneurysms. Lastly, at this stage follow-up of the patient population is not known.

Table 3.5: Reasons for referral to cardiologist

Referral Cardiologist	22
Extreme high calcium score	1
Left main coronary artery stenosis	1
Significant stenosis cCTA, without possibility APMR	4
Aortic valvular stenosis	2
Perfusion defect APMR	10
Atrial myxoma	1
Cardiac complaints	3

Values are absolute numbers.
cCTA Coronary computed tomography angiography *APMR* adenosine perfusion magnetic resonance imaging.

This study is one of the first to demonstrate a high prevalence of silent severe coronary artery disease by non-invasive imaging in patients with extra-cardiac arterial disease. The rate of CAD was not significantly different in patients with stenotic compared with aneurysmal ECAD. Further studies may show screening in these high risk patients to be beneficiary.

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4

Hemodynamic significance of coronary stenosis by vessel attenuation measurement on CT compared with adenosine perfusion MRI

M.A.M. den Dekker
G.J. Pelgrim
G. Pundziute
E.R. van den Heuvel
M. Oudkerk
R. Vliegenthart

Abstract

Objectives We assessed the association between corrected contrast opacification (CCO) based on coronary computed tomography angiography (cCTA) and inducible ischemia by adenosine perfusion magnetic resonance imaging (APMR).

Methods Sixty cardiac asymptomatic patients with extra-cardiac arterial disease (mean age 64.4 $\pm$ 7.7 years; 78 % male) underwent cCTA and APMR. Luminal CT attenuation values (Hounsfield Units) were measured in coronary arteries from proximal to distal, with additional measurements across sites with >50 % lumen stenosis. CCO was calculated by dividing coronary CT attenuation by descending aorta CT attenuation. A reversible perfusion defect on APMR was considered as myocardial ischemia.

Results In total, 169 coronary stenoses were found. Seven patients had 8 perfusion defects on APMR, with 11 stenoses in corresponding vessels. CCO decrease across stenoses with hemodynamic significance was 0.144 $\pm$ 0.112 compared to 0.047 $\pm$ 0.104 across stenoses without hemodynamic significance (P = 0.003). CCO decrease in lesions with and without anatomical stenosis was similar (0.054 $\pm$ 0.116 versus 0.052 $\pm$ 0.101; P = 0.89). Using 0.20 as preliminary CCO decrease cut-off, hemodynamic significance would be excluded in 82.9 % of anatomical stenoses.

Conclusion CCO decrease across coronary stenosis is associated with myocardial ischemia on APMR. CCO based on common cCTA data is a novel method to assess hemodynamic significance of anatomical stenosis.

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4.1 Introduction

Computed tomography (CT) has high diagnostic accuracy for the detection and exclusion of coronary artery disease (CAD), compared with invasive coronary angiography.[1,2] Coronary stenosis detected on coronary CT angiography (cCTA), however, shows poor correlation with lesion-specific and global myocardial ischemia.[1,2] In the cath lab, fractional flow reserve (FFR) is used to assess the functional significance of coronary stenosis.[3] This lesion-specific index reflects the effect of stenosis on myocardial perfusion. However, FFR measurement is an invasive procedure with associated costs, and is only performed in symptomatic patients undergoing invasive coronary angiography. Recent imaging techniques can assess the hemodynamic significance of CAD without the risk associated with an invasive procedure. Adenosine perfusion magnetic resonance imaging (APMR) and positron emission tomography are safe and preferable non-invasive alternatives for vessel-specific ischemia detection with similar high diagnostic accuracy.[4,5] APMR has an excellent sensitivity and specificity of 91 and 94 % respectively, as compared with FFR.[6]

In view of the increased use of cCTA to assess CAD, it would be extremely valuable if this same non-invasive test could determine the hemodynamic significance of the anatomical stenoses that are readily detected. Recently, an estimate of coronary flow was obtained using common cCTA data. In calculations based on cCTA, reduced flow across an intermediate-grade coronary stenosis points to the presence of hemodynamically significant CAD. Two multicenter studies have demonstrated the preliminary clinical value of this approach, using computational fluid dynamics.[7–9]This method involves a lengthy and complicated computation, potentially hindering its introduction in clinical practice. A simpler calculation named the corrected contrast opacification (CCO)[10–12] can also estimate coronary artery flow. So far, clinical studies have compared CT-derived flow measurements with FFR measurements. Due to the nature of FFR evaluation, these studies involved a selected population of symptomatic patients. This is the first study in which CT-derived CCO is compared with non-invasive imaging of functionally significant CAD. The aim of this study, in a high-risk, cardiac asymptomatic population, is to investigate the association between CCO as estimate of coronary flow and ischemia detection by APMR.

4.2 Materials and Methods

Patients

This is a substudy of the prospective GROUND2 study, in which cardiac asymptomatic patients with extra-cardiac arterial disease (ECAD) underwent non-invasive cardiac imaging.[13] This substudy included sixty patients who had undergone cCTA

and APMR at our institution. The study protocol was approved by the institutional review board of the University Medical Center Groningen; all patients gave written informed consent.

Computed tomography

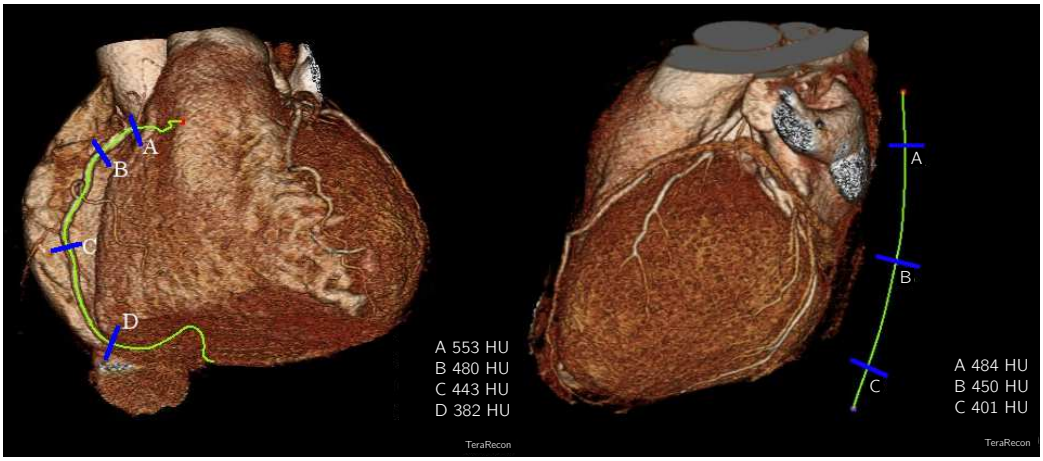
CT scanning was performed using a dual-source CT scanner (SOMATOM Definition, Siemens, Erlangen, Germany). CCTA was performed in spiral mode, with retrospective electrocardiographic (ECG)-gating. Scanning parameters were: 32 × 0.6 mm collimation, image acquisition 64 × 2 × 0.6 mm by z-flying spot, increment 0.4 mm, 330 ms rotation time, tube voltage and current according to weight, tube current modulation on (CARE Dose, Siemens, Erlangen, Germany), pitch depending on heart rate, field-of-view 205 mm. Images were reconstructed as consecutive 0.6 mm slices. Coronary evaluation was performed by the attending radiologist, with at least 5 years of experience in cardiac imaging. According to clinical practice, a single radiologist read the cCTA; cCTA acquisition and evaluation were performed prior to APMR. Axial slices as well as curved multi-planar reconstruction images were analysed, using Syngo software (Siemens, Erlangen, Germany). Using the 15-segment modified AHA classification,[14] coronary segments were evaluated visually, independent of image quality. Segments were classified as having anatomically significant stenosis when there was >50 % lumen diameter reduction, compared to the proximal lumen diameter. In case of significant stenosis, distinction was made between 50-70 % and >70 % stenosis.

CT contrast opacification

Coronary luminal attenuation values (Hounsfield Units [HU]) were assessed semi-automatically on curved multi-planar reconstruction images, using Aquarius iNtuition software version 4.4.11Beta (TeraRecon, San Mateo, USA). Segments were excluded from analysis when diameter was <1.5 mm. Vessels were excluded when calcification at the origin prohibited semi-automated measurements. No vessels were excluded based on stenosis severity. In remaining coronary arteries, HU-measurements were performed at the origin, and in the proximal, mid and distal segment. In case of >50 % stenosis, four additional HU-values were measured, two HU-measurements proximal and two distal to the stenosis (within 2 cm of the lesion). Examples of levels of coronary HU-measurements are shown in Figure 4.1 and 4.2. Length, plaque composition and segmental location of stenosis were recorded.

Normal coronary arteries show a gradient in opacification along their course.[11,15] Therefore, we measured the descending aorta attenuation at the same height as the proximal, mid and distal segments of the coronary arteries, to approach the CCO (Figure 4.1).[12] We calculated the CCO by dividing coronary attenuation by descen-

Figure 4.1: Volume-rendered computed tomography (CT) image of the heart, showing the locations of attenuation measurements at the origin (A), proximal (B), mid (C) and distal (D) segment in the right coronary artery (left frame). The right frame demonstrates the anatomical location of attenuation measurements in the descending aorta, relative to the coronary arteries

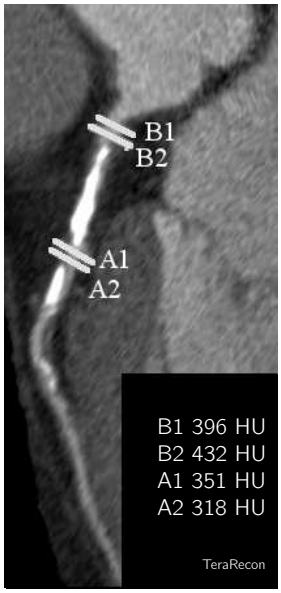


ding aorta attenuation. We used the lower of the two measurements surrounding stenosis to calculate the CCO decrease across stenosis. When a vessel ran perpendicular to the aorta, measurements were corrected at the same level, following Chow et al.[12]

Magnetic resonance imaging

APMR was performed with a 1.5-Tesla scanner (Avanto, Siemens, Erlangen, Germany). Patients were instructed to refrain from caffeine for at least 24 hours prior to the examination. Medication interfering with adenosine was stopped five days prior to the examination. During the procedure, ECG was continuously monitored. Blood pressure and heart rate were recorded at baseline and every minute during adenosine infusion. APMR analysis was performed by the attending radiologist, with at least 5 years of experience in cardiac imaging, using the 16 segment model. APMR was considered positive if a perfusion defect was present in at least two segments at consecutive planes during adenosine perfusion imaging, with normalization in rest.[16] The most likely culprit artery for the perfusion defect was assessed. If more than one stenosis exists in the culprit artery, the specific stenosis causing ischemia is not always identifiable. Therefore, in a patient with perfusion defect, we labelled all anatomically significant stenoses in the culprit artery as hemodynamically significant.

Figure 4.2: Curved multiplanar reconstruction of the left anterior descending artery, with a stenosis in the proximal segment. Measurements before (B1 and B2) and after (A1 and A2) stenosis are shown, all within 2 cm of the lesion



Statistical analysis

For numerical values, the mean ± standard deviation (SD) was calculated; median [25^{th}, 75^{th} percentile] when the distribution was observed non-normal. For categorical variables, frequency with percentage was provided. To test differences between patients with positive and negative APMR, t-test was applied for continuous, normally distributed variables; Mann-Whitney U test for non-normally distributed numerical variables, and Chi-square test for categorical variables. The Mann-Whitney U test was used to compare differences in CCO gradients between normal and stenotic arteries. Spearman correlation coefficients were calculated to examine the relationship between CCO and stenosis characteristics. Linear regression was applied with CCO decrease as outcome to test the effect of perfusion defect, corrected for length, composition and location of stenosis. Analyses were performed by vessel and by stenosis. To assess inter-reader agreement for CCO measurement, 20 randomly selected cCTA scans were evaluated by a second reader, and the intraclass correlation coefficient (ICC) was calculated. Consistency of agreement across coronary arteries was assessed by the likelihood ratio test. In Bland-Altman plot analysis, we assessed difference in CCO between the readers. Post-hoc power analysis was performed on the estimated effect size. Finally, receiver-operator characteristics (ROC) analyses were created to determine a potential cut-off value for determining hemodynamic significance of coronary stenosis. Statistical analyses were performed using IBM SPSS Statistics version 20.0.0.1 (SPSS Inc, Chicago, USA) or SAS version 9.3 (SAS In-

stitute Inc, Cary, USA). Statistical tests were two-sided. A P-value of <0.05 was considered statistically significant.

4.3 Results

Overview of cohort in terms of patients and vessels

Sixty patients (mean age 64.4 ± 7.7 years; 78 % male) were included. Of these, 10 had no coronary plaques on cCTA, 21 had plaques causing 0-50 % stenosis and 29 had >50 % stenosis. Seven patients had a positive APMR test, with eight perfusion defects. Characteristics did not differ significantly between patients with and without ischemia on APMR. Only the percentage of diabetics was higher among patients with perfusion defect (Table 4.1).
Figure 4.3 gives an overview of the coronary arteries. Twenty-eight of the 180 coronary arteries could not be included in CCO analysis, due to inability to measure attenuation at the origin or distally. Anatomical stenoses were present in 87 of the 152 remaining vessels. Eight stenotic arteries showed a perfusion defect in the corresponding vascular territory.

Anatomically and hemodynamically significant stenoses

In total, 169 anatomical stenoses were found. Seventy-seven were located in the left anterior descending artery (LAD), 32 in the circumflex artery (LCX) and 60 in the right coronary artery (RCA). One stenosis was caused by soft plaque, 59 by partially calcified plaque, and 109 by calcified plaque. Of the anatomical stenoses, 68 narrowed the lumen diameter >50 %, and 14 of these >70 %. Stenoses ≤50 % showed no hemodynamic significance. Eleven >50 % stenoses were present in the vessel territories with ischemia on APMR. Information about the stenoses in culprit vessels is provided in Table 4.2. Culprit arteries contained 7 partially calcified and 4 calcified plaques causing >50 % stenosis, compared to 1 soft plaque, 52 partially calcified, and 105 calcified in non-culprit arteries (P = 0.12). Overall stenosis length and number of stenoses were not significantly different for hemodynamically versus hemodynamically non-significant stenoses (length: 11.9 mm [25^{th}, 75^{th} percentile: 7.41, 30.90] versus 9.14 mm [6.01, 14.88], P = 0.17; number: 1.4 ± 0.7 versus 1.7 ± 0.9; P = 0.32).

Contrast attenuation in aorta and coronary arteries

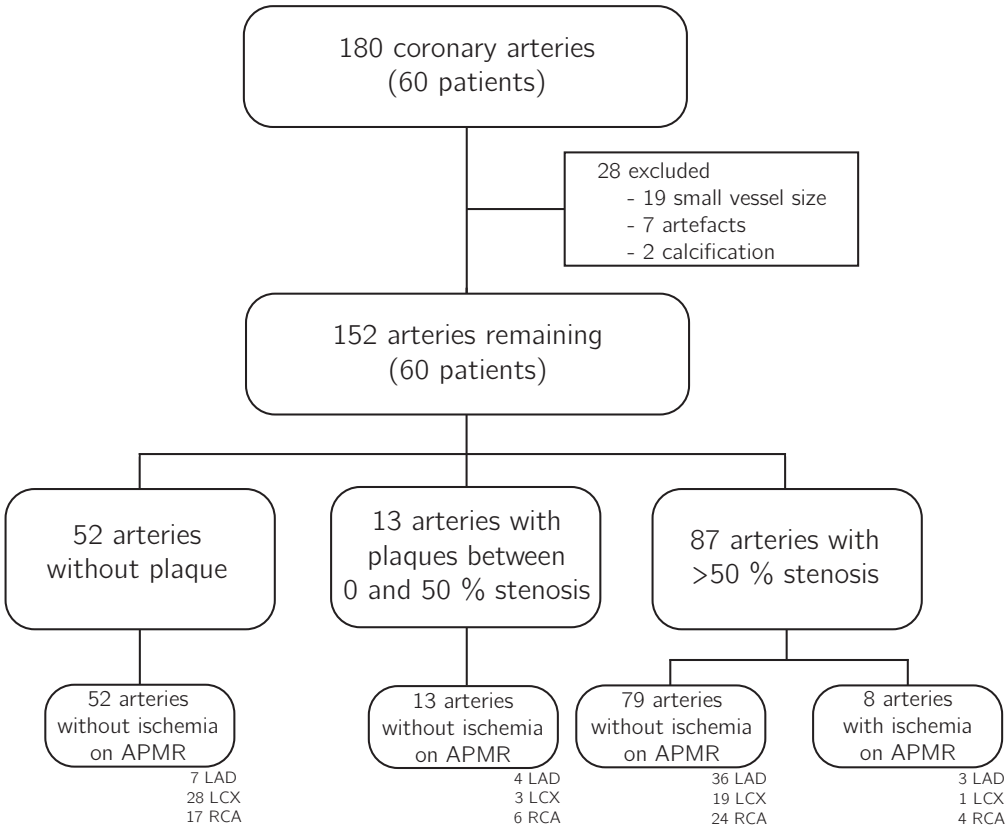
Median attenuation values in the descending aorta were 351.5 [300.8, 400.3] HU, 332.0 [288.3, 386.5] HU and 316.5 [266.8, 392.8] HU at the level of proximal, mid

Table 4.1: Characteristics of all patients and comparison of characteristics between patients with hemodynamically significant versus hemodynamically non-significant coronary artery disease, as assessed by adenosine perfusion magnetic resonance imaging

	All patients (n = 60)	APMR negative (n = 53)	APMR positive (n = 7)	P-value
Age (years)	64.4 ± 7.7	63.9 ± 7.5	68.7 ± 8.5	0.12
Gender (male)	47 (78.3 %)	41 (77.4 %)	6 (85.7 %)	0.61
Body mass index	26.3 ± 3.6	26.5 ± 3.7	25.9 ± 3.8	0.68
Hypertension	49 (81.7 %)	42 (79.2 %)	7 (100 %)	0.18
Systolic BP (mmHg)	140 ± 23	140 ± 24	134 ± 13	0.52
Diastolic BP (mmHg)	80 ± 10	80 ± 10	75 ± 9	0.19
Antihypertensive treatment	42 (70.0 %)	37 (69.8 %)	5 (71.4 %)	0.93
Dyslipidemia	54 (90.0 %)	47 (88.7 %)	7 (100 %)	0.34
Total cholesterol (mmol/l)	4.5 [3.9-5.7]	4.3 [3.8-5.6]	5.2 [4.3-6.0]	0.22
Triglycerides (mmol/l)	1.53 [1.02-2.23]	1.49 [1.04-2.16]	1.94 [0.91-2.28]	0.63
HDL-cholesterol (mmol/l)	1.2 [0.9-1.4]	1.2 [0.9-1.5]	1.0 [0.8-1.2]	0.32
LDL-cholesterol (mmol/l)	2.8 [2.2-3.6]	2.5 [2.2-3.6]	3.2 [2.9-4.1]	0.13
Cholesterol lowering treatment	46 (76.7 %)	42 (79.4 %)	4 (57.1 %)	0.19
Diabetes mellitus	5 (8.3 %)	3 (5.7 %)	2 (28.6 %)	0.04
Glucose (mmol/l)	5.4 [5.0-6.0]	5.3 [5.0-6.0]	5.5 [4.8-7.0]	0.80
Metabolic syndrome	7 (11.7 %)	6 (11.3 %)	1 (14.3 %)	0.82
Creatinin (mmol/l)	79.5 [69.0-93.0]	79.0 [68.5-92.5]	90.0 [74.0-115.0]	0.27
High-sensitive CRP (mg/l)	4.1 [1.3-7.7]	3.7 [1.2-7.9]	4.8 [1.6-4.8]	1.00
Calcium score	266.2 [79.4-895.9]	357.1 [109.7-902.6]	68.3 [0.0-294.9]	0.07
Number of lesions	9.9 ± 6.4	9.7 ± 6.5	12.5 ± 5.8	0.41
Smoking				0.21
Current	25 (41.7 %)	23 (43.4 %)	2 (28.6 %)	
Past	29 (48.3 %)	26 (49.1 %)	3 (42.9 %)	
Never	6 (10.0 %)	4 (7.5 %)	2 (28.6 %)	
ECAD				0.11
PAOD	35 (58.3 %)	31 (58.5 %)	4 (57.1 %)	
Carotid stenosis	11 (18.3 %)	8 (15.1 %)	3 (42.9 %)	
AAA	14 (23.3 %)	14 (26.4 %)	0 (0.0 %)	

Values are mean ± SD, median [interquartile range] or number of subjects (percentage). *APMR* Adenosine perfusion magnetic resonance imaging *BP* blood pressure *HDL* high density lipoprotein *LDL* low density lipoprotein *CRP* C-reactive protein *ECAD* extra-cardiac arterial disease *PAOD* peripheral arterial occlusive disease *AAA* abdominal aortic aneurysm

Figure 4.3: Overview of the coronary arteries included and excluded from the analyses, and of the presence of stenoses and perfusion defects in these arteries



and distal coronary segments, respectively. Median coronary contrast attenuation in the proximal segments was 377.0 [332.0, 408.5] HU, and in the distal segments 316.0 [269.0, 371.0] HU. Decrease in contrast attenuation from origin to distal coronary segment was 128.5 [99.3, 151.5] HU in vessels with hemodynamically significant stenosis and 60.5 [17.3, 115.8] HU in those without (P = 0.02). Decrease in vessels with and without anatomical stenosis was 61.0 [18.0, 122.0] HU and 69.0 [18.0, 106.5] HU, respectively (P = 0.72).

CCO in vessel-based analysis

Mean CCO_{origin} was 1.022 ± 0.080 in coronary arteries with hemodynamically significant stenosis, and 1.095 ± 0.177 in those without (P = 0.25). Mean CCO_{distal} was 0.733 ± 0.131 and 1.028 ± 0.240, respectively (P <0.001). For anatomically significant stenosis, mean CCO_{origin} was 1.098 ± 0.175, and 1.084 ± 0.173 in ves-

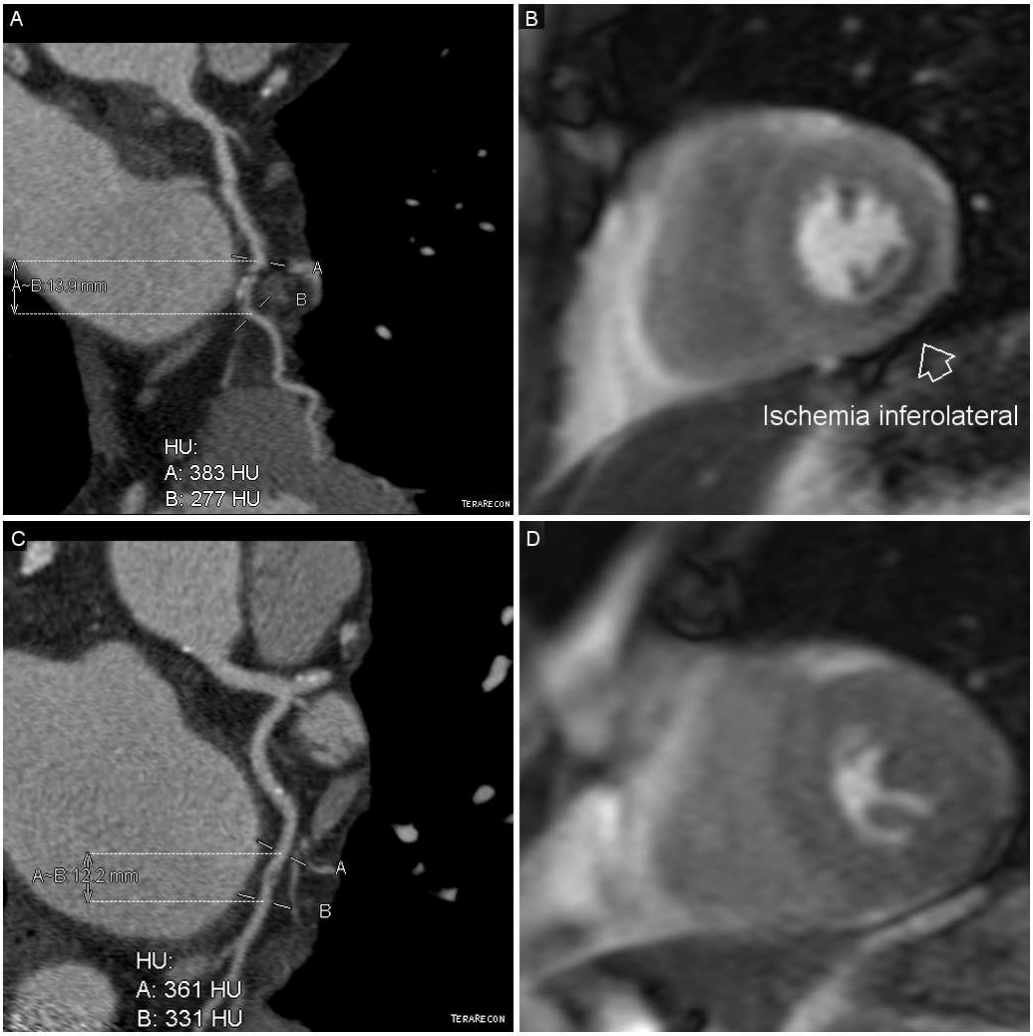
Table 4.2: Overview of cases with perfusion defect on adenosin perfusion magnetic resonance imaging

Patient	Age (years)	Gender	Location perfusion defect	Expected culprit artery	Anatomically significan stenosis on CT	Type of plaque
1	76	Male	Anterior	LAD	LAD prox (>50 %)	Mixed
1	76	Male	Inferior subendocardial (basal, midventr. and apical	RCA	RCA all (>50 %)	Mixed
2	65	Male	Inferolateral (basal and midventr.)	LCX	LCX dist (>50 %)	Mixed
3	58	Male	Septal subendocardial (basal)	LAD	LAD prox and mid (>70 %)	Calcified
4	69	Male	Inferior subendocardial (basal)	RCA	RCA prox (100 %)	Mixed
5	83	Male	Anteroseptal subendocardial (basal, midventr. and apical)	LAD	LAD prox (>50 %)	Mixed
6	62	Female	Inferolateral (apical)	RCA	RCA prox (>50 %)	Mixed
7	68	Male	Inferior (midventr. and apical)	RCA	RCA prox (>70 %)	Mixed

Shown are number of anatomic significant stenoses on coronary computed tomography angiography, location of perfusion defect on APMR and the cornoary artery expected for causing the perfusion defect.
RCA Right coronary artery *LCX* circumflex coronary artery *LAD* left anterior descending coronary artery *prox* proximal part of coronary artery *mid* middle part *dist* distal part *midventr* midventricular

sels without (P = 0.60). Mean CCO_{distal} was 1.005 $\pm$ 0.220 and 1.030 $\pm$ 0.272, respectively (P = 0.47). CCO decrease across vessels with hemodynamically significant stenoses was larger than in vessels without associated ischemia: 0.289 $\pm$ 0.132 versus 0.068 $\pm$ 0.258 (P = 0.02). In contrast, CCO decrease for vessels with and without anatomical stenoses was not different (0.097 $\pm$ 0.251 versus 0.055 $\pm$ 0.265; P = 0.32). When limited to >70 % stenoses, the difference in CCO decrease for

Figure 4.4: Example of decrease in corrected contrast opacification (CCO) for 2 patients with similar anatomical severity of stenosis, but with opposite result in adenosine perfusion magnetic resonance imaging (APMR). A and B: 65-year old male patient with a 50-70 % stenosis in the circumflex artery. Contrast attenuation measurements above and below the stenosis are shown; CCO decrease was 0.33 (A). the APMR revealed ischemia in the inferolateral wall of the left ventricle, matching the circumflex artery (B). C and D: a 75-year old male, also with a 50-70 % stenosis in the circumflex artery. CCO decrease was 0.09 (C), no perfusion defect was present in APMR (D)



vessels with and without stenoses remained not significant (0.114 $\pm$ 0.230 versus 0.070 $\pm$ 0.259; P = 0.18).

CCO in stenosis-based analysis

Figure 4.4 shows an example of CCO results in patients with and without ischemia on APMR, with similar anatomical stenosis severity. CCO decrease across stenoses with hemodynamic significance was larger than across those without (0.144 ± 0.112 and 0.047 ± 0.104; P = 0.003). In contrast, CCO decrease between lesions with and without anatomically significant stenosis was similar (0.054 ± 0.116 and 0.052 ± 0.101, respectively; P = 0.89; Figure 4.5). Also stenosis composition did not differ significantly (P = 0.47). However, plaques causing anatomically significant stenosis were relatively longer than non-obstructive plaques (11.9 [7.1, 24.7] mm versus 9.0 [5.6, 12.9] mm; P = 0.008).
Mean CCO decrease was 0.025 ± 0.106 across proximal stenoses, 0.077 ± 0.096 across stenoses in mid segments and 0.053 ± 0.122 across distal stenoses (P = 0.02). When corrected for length, composition and location of stenosis, CCO decrease remained significantly different for stenoses with versus those without hemodynamic significance (P = 0.003). For anatomically significant stenoses, the CCO decrease remained comparable in multivariable analysis (P = 0.90). Post-hoc power analysis revealed a power of 84.9 % (using effect size of 0.09645, standard error of 0.03203, and degrees of freedom of 162).

Reproducibility and inter-reader variability

The overall ICC was 0.917, indicating excellent agreement. The ICC was 0.941, 0.872 and 0.964 for the LAD, LCX and RCA, respectively (P = 0.122). In Bland-Altman analysis, the mean difference in CCO between readers was -0.0283 (95 % CI: -0.222, 0.166).

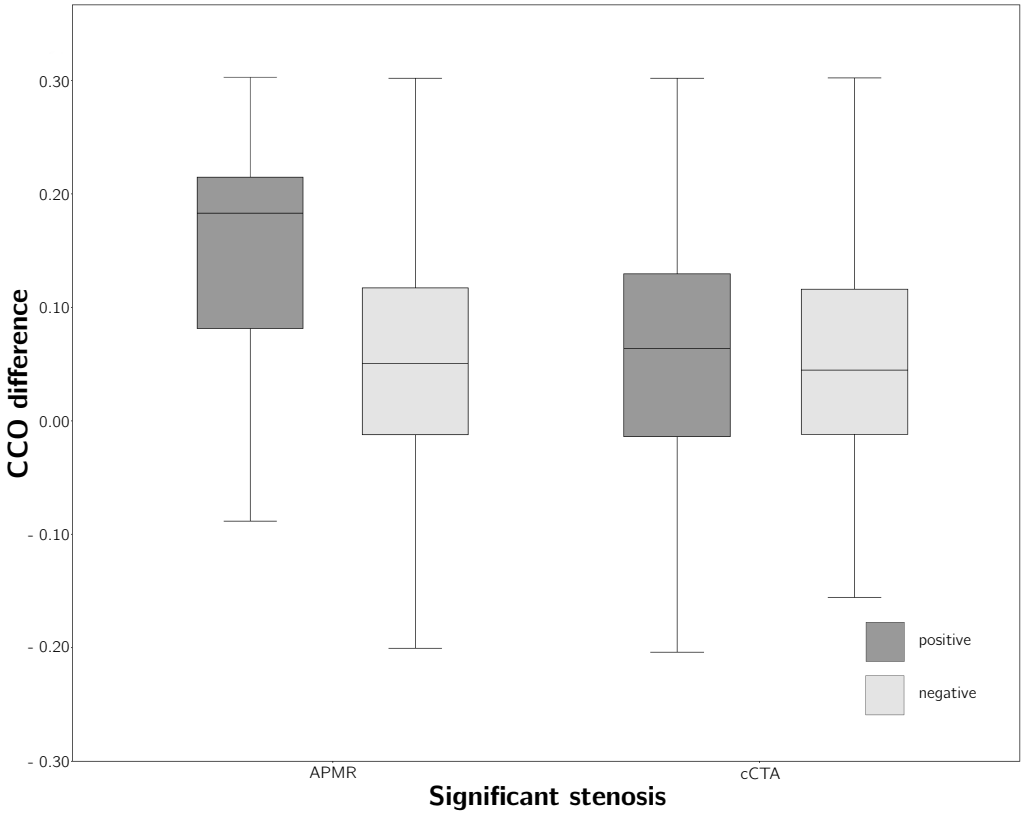
ROC analysis

The sample size was insufficient to derive a reliable cut-off point for CCO decrease to detect ischemia. For a CCO decrease cut-off value between 0.20 and 0.25, the sensitivity and specificity were relatively high (Figure 4.6). For instance, for a CCO decrease of 0.20, the sensitivity was 87.5 % and the specificity was 71.5 %, while for a CCO decrease of 0.25 the sensitivity was 75.0 % and the specificity was 79.2 %. Based on CCO assessment, with a decrease in CCO of 0.20 as cut-off value, hemodynamic significance would be excluded in 82.9 % of anatomical stenoses.

4.4 Discussion

In this study, CCO, derived from common cCTA data, showed a strong association with hemodynamically significant CAD, as determined by APMR. For mere anatomical stenoses, no such relationship was present. These results suggest that additional information from standard cCTA data may assist in differentiation between a coronary stenosis with and without hemodynamic significance.

Figure 4.5: Boxplot of corrected contrast opacification (CCO) decrease across stenoses with and without anatomical and hemodynamic significance. CCO decrease was significant for stenoses with versus those without hemodynamic significance (P = 0.003), but not for stenosis with versus those without anatomical significance (P = 0.89)



Many coronary stenoses do not cause a relevant reduction in coronary flow. It is difficult to predict which anatomical stenosis causes ischemia.[17,18] FFR, the reference standard, is used during invasive angiography to assess hemodynamic significance and guide therapy.[1,3] The correlation between anatomical stenosis detected by invasive coronary angiography or cCTA, and FFR is poor.[1,2] Thus, testing of functional significance of CAD is usually necessary to evaluate whether invasive treatment is

needed, even in case of a positive cCTA. The ultimate goal is one non-invasive, comprehensive imaging test for CAD.

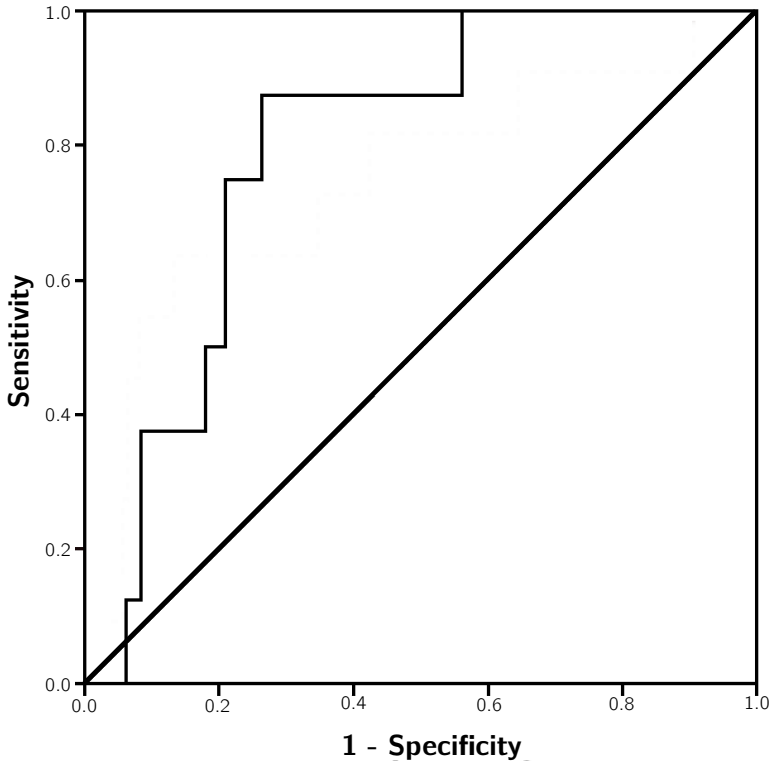
There is increasing interest in coronary CT imaging, especially in symptomatic patients at intermediate CAD risk.[19] Previously, cCTA only resulted in anatomical information on CAD. New technologies can yield an estimate of coronary flow by measuring changes in contrast attenuation.[10] Chow et al. designed the CCO method, as was also used in this study.[12] In a proof-of-concept study, CCO differences were useful to identify abnormal resting coronary flow as assessed in invasive coronary angiography.[12] A second method based on manual HU measurements, the transluminal attenuation gradient (TAG) was described by Choi et al.[20] TAG measurement was found to improve the accuracy of severity classification of calcified stenoses by cCTA, compared to invasive coronary angiography.[20] However, a validation study of both TAG and CCO showed only moderate associations between TAG/both measures and hemodynamic stenoses as assessed with FFR.[21] With the use of 320-MDCT, the diagnostic accuracy of TAG was improved.[22] However, without automated image processing, TAG remains a time-consuming method.[23] Two recent multicenter trials used computational fluid dynamics to estimate lesion-specific FFR,[7,9,24] by calculating the ratio of coronary pressure and mean aortic pressure. It was demonstrated that non-invasive FFR measurement was superior to cCTA for diagnosing ischemic lesions.[25] Drawbacks of this method are the extra patient information required, and the lengthy calculation time.[9]

Previous studies on CT assessment of coronary flow were performed in symptomatic patients waiting for invasive coronary angiography, and comparison was made with FFR. This population has a high pre-test probability for functionally significant CAD, which may have led to overoptimistic results. In contrast, our study included cardiac asymptomatic patients at increased risk of CAD. We compared CCO to non-invasive assessment of functionally significant CAD by APMR, as invasive FFR measurement in this asymptomatic population is unethical. The prevalence of silent ischemia by APMR was considerable (12 %). Silent ischemia is a very relevant finding: asymptomatic patients with positive ischemia test have 3.6 to 6.9-fold increased risk for coronary events in the next 5 years.[26,27] The most widely used non-invasive ischemia test is single photon emitting CT (SPECT). Compared to SPECT, there are important advantages of APMR: superior diagnostic accuracy, lack of radiation, and higher spatial resolution, enabling detection of subendocardial perfusion defects.[5,28] Compared to FFR, APMR has an excellent diagnostic accuracy.[6,29]

Distinguishing the culprit stenosis leading to ischemia in patients with serial stenoses in the culprit coronary artery can be difficult. When two stenoses are present, both affect coronary flow, even if individual stenoses are only moderate in severity. This may still warrant an intervention in one or both stenoses.[30] This was the reason that we measured CCO decrease across the stenosis as well as across the entire vessel, and that we assigned all >50 % stenoses in the culprit artery causing ischemia as hemodynamically significant.

Our study has limitations. Firstly compared to the CCO method in literature,[12] we propose a slightly altered approach, based on semi-automated HU measurements using curved multiplanar reconstructions. We found high reproducibility and excellent inter-reader agreement. Using our method, CCO assessment requires less skill and could be faster, and thus, can be more easily applicable in clinical practice. Secondly, a low number of patients had inducible ischemia on APMR. The calculated power shows that the sample size was large enough to find significant associations. Although the outcome rate was low, significant relationships were already found between CCO decrease and myocardial ischemia. Finally, only cardiac asymptomatic subjects were included in our study. Studies in symptomatic populations should confirm our findings.

Figure 4.6: Receiver-Operator-Characteristic (ROC) curve. Solid line represents vessel-based analysis. AUC was 0.79 (0.68-0.91)



In conclusion, decrease in CCO across coronary stenosis is associated with myocardial ischemia on APMR. CCO based on common cCTA data is a novel method to assess functional significance of anatomical stenosis. Mere anatomical stenosis did not cause a difference in CCO decrease. The CCO may offer a rather simple and

straightforward measurement to evaluate the hemodynamic significance of stenosis on cCTA without additional radiation dose. CCO can potentially exclude the majority of hemodynamically insignificant coronary stenoses from further workup. Studies in larger patient populations should assess whether CCO calculation may obviate the need for ischemia testing, and derive CCO cut-off points to distinguish normal versus reduced coronary flow.

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5

Relationship between epicardial adipose tissue and subclinical coronary artery disease in patients with extra-cardiac arterial disease

M.A.M. den Dekker
R. Takashima
E.R. van den Heuvel
J.J.A.M. van den Dungen
R.A. Tio
M. Oudkerk
R. Vliegenthart

Abstract

Objectives Epicardial adipose tissue (EAT) and mediastinal adipose tissue (MAT) are linked to coronary artery disease (CAD). The association between EAT, MAT and severity of CAD in known extra-cardiac arterial disease was investigated.

Methods Sixty-five cardiac asymptomatic patients (mean age 65 $\pm$ 8 years, 69 % male) with peripheral arterial disease, carotid stenosis, or aortic aneurysm underwent coronary computed tomography angiography. Patients were divided into non-significant (<50 % stenosis, n = 35), single vessel (n = 15) and multi-vessel CAD (n = 15). EAT and MAT were quantified on computed tomography images using volumetric software.

Results Subgroups did not significantly differ by age, gender, or cardiovascular risk factors. Median EAT was 99.5, 98.0, and 112.0 cm^3 (P = 0.38) and median MAT was 66.0, 90.0, and 81.0 cm^3 (P = 0.53) for non-significant, single vessel, and multi-vessel CAD, respectively. In age- and gender-adjusted analysis, only EAT was significantly associated with CAD (odds ratio [OR] 1.12 [95 % confidence interval, 1.01-1.25] per 10 cm^3 increase in EAT; P = 0.04). This remained in multivariate-adjusted analysis (OR 1.21 [1.04-1.39]; P = 0.01).

Conclusion In patients with known extra-cardiac arterial disease, CAD is correlated with EAT, but not with MAT. These results suggest that EAT has a local effect on coronary atherosclerosis, apart from the endocrine effect of visceral fat.

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5.1 Introduction

Visceral adipose tissue deposits have been found to exert various effects.[1] Mesenteric and thoracic adipose tissue, one of the largest deposits of visceral adipose tissue, has been associated with atherosclerosis markers.[2] Epicardial adipose tissue (EAT), like visceral adipose tissue, is metabolically active and produces hormones, although its exact function is still unclear. EAT covers about 80 % of the heart, and surrounds the epicardial coronary arteries and is assumed to play a role in the pathogenesis of coronary artery disease (CAD).[3–5] Possibly there is an additional, direct effect of EAT on the development of coronary atherosclerosis, on top of the endocrine effect of different visceral fat deposits. However, this has yet to be confirmed. Mediastinal adipose tissue (MAT) is separated from the heart by the pericardium. It is more closely related to cardiovascular risk factors than EAT. However, EAT demonstrates a stronger association with vascular calcifications, possibly due to the paracrine effects.[6]

First attempts to visualize and quantify EAT thickness were performed by echocardiography, however, echocardiography does not yield an adequate window of all cardiac segments.[7] Furthermore, it depends on acoustic windows, which can make it inadequate to assess obese patients.[8] With more recent developments in computed tomography (CT) and magnetic resonance imaging (MRI), accurate volumetric measurements of EAT have become possible.[9] CT has a high temporal and spatial resolution with submillimeter collimation, and provides a three-dimensional view of the heart and epicardial surface.[10] MRI, on the other hand, is somewhat limited in spatial resolution.

Several studies have demonstrated an association between EAT volume and the presence of obstructive CAD.[11–16] These studies were performed in low to intermediate risk patients without known atherosclerotic disease. Whether the effect of adipose tissue on the coronary arteries is due to local or endocrine effects cannot be teased out as there is a correlation between epicardial adipose tissue and other visceral adipose tissue deposits. In a homogenous group of patients with known vascular disease, it is assumed that the endocrine effect of visceral adipose tissue on the vessel wall is relatively similar and accounted for. This offers the possibility to examine the potential, additional local effect of EAT on CAD.

The aim of this study is to examine the relationship between EAT, MAT, and the prevalence of CAD in patients with extra-cardiac arterial disease.

5.2 Materials and Methods

Patients

The current investigation was performed as a sub study of the GROUND2 study.[17] In this study, cardiac asymptomatic patients with a history of extra-cardiac arterial disease (intermittent claudication, carotid stenosis or abdominal aortic aneurysm) underwent non-invasive cardiac imaging by CT and MRI. The study protocol was approved by the internal institutional review board of the University Medical Center Groningen. In the current study sixty-five patients, who had undergone coronary CT angiography (cCTA) performed at our institution, were included.

The following risk factors for cardiovascular disease were assessed and measured: smoking, overweight (defined as a body mass index >25 kg/m^2), diabetes mellitus, hypertension (defined as as systolic blood pressure $\geq$140 mmHg and/or diastolic blood pressure $\geq$90 mmHg or current anti-hypertensive medication) and dyslipidaemia (defined as low density lipoprotein of more than 4.0 mmol/l, high density lipoprotein of less than 1.2 mmol/l for women or 1.0 mmol/l for men, or triglycerides of over 4.0 mmol/l or current cholesterol-lowering therapy).

Computed tomography

All CT scans were performed on a dual-source CT scanner (SOMATOM Definition, Siemens, Erlangen, Germany) using a standard scanning protocol.

CCTA was performed in spiral mode, using retrospective electrocardiographic gating. Scanning parameters were as follows: 32 $\times$ 0.6 mm collimation, image acquisition 64 $\times$ 2 $\times$ 0.6 mm with flying z-spot, increment 0.4 mm, 330 ms rotation time, tube voltage and tube current according to weight, pitch depending on heart rate of the patient, field-of-view 205 mm. Images were reconstructed as consecutive 0.6 mm slices.

Assessment of coronary artery disease

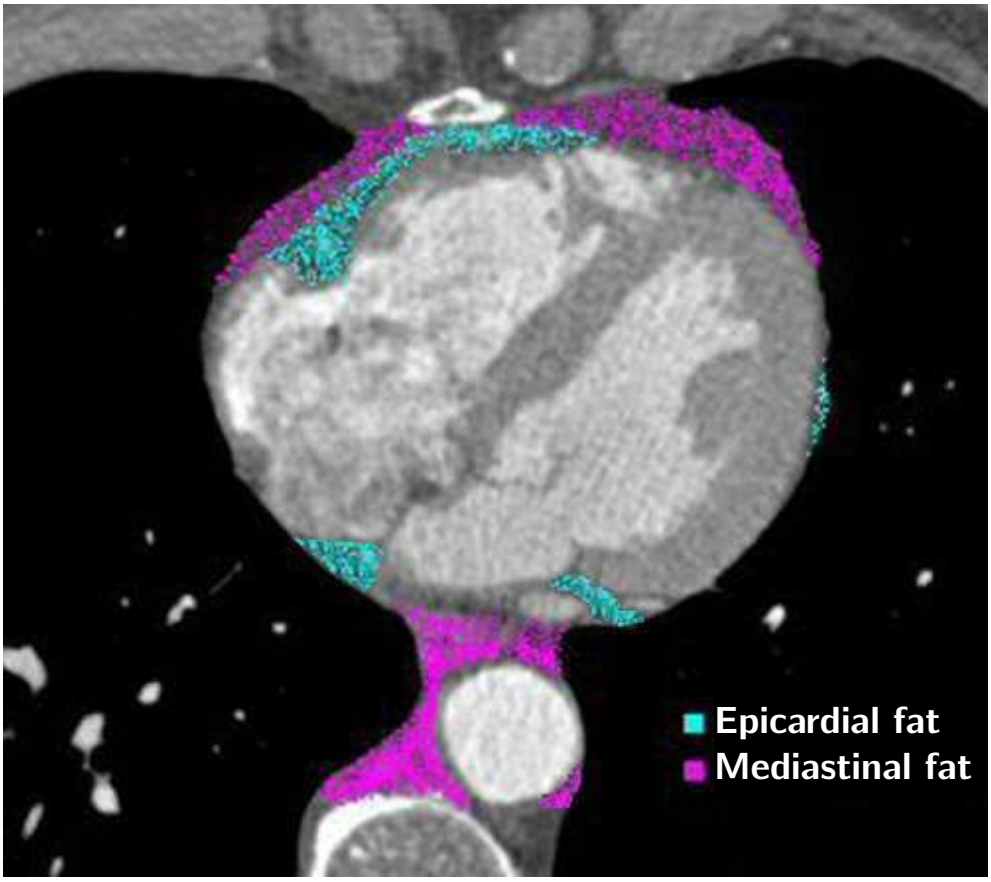
Analysis of the cCTA was performed by the attending radiologist, with experience in cardiac CT ranging from 5 to over 10 years. Syngo (Siemens, Erlangen, Germany) was used for coronary evaluation. In the 15-segment modified AHA classification,[18] all segments were evaluated independently of image quality. Segments were classified as having significant stenosis when there was $\geq$50 % lumen diameter reduction by visual assessment.

Patients were classified as having no significant CAD, significant CAD in a single vessel, or significant CAD in multiple vessels.

Measurement of EAT and MAT

EAT and MAT were quantified as total volume (cm^3) using dedicated software (Aquarius iNtuition, TeraRecon, San Mateo, USA) based on the cCTA reconstructions. The superior border for EAT and MAT was defined as the center of the right pulmonary artery, the inferior border was the caudal border of the pericardial sac. EAT, defined as adipose tissue within the visceral layer of the pericardium, was measured by manually tracing the pericardium. The anterior border for MAT was defined as the sternum, the posterior border as the front of the vertebral column. Hounsfield units from -195 to -45 were used to isolate adipose tissue within the total selected volume. Adipose tissue was then automatically quantified by the software. To calculate the MAT, EAT was subtracted from the total selected adipose tissue. Figure 5.1 shows an example of EAT and MAT. In additional analysis, EAT and MAT measurements were divided by body surface area to correct for subcutaneous adipose tissue.

Figure 5.1: Example of measurements of thoracic adipose tissue, showing epicardial adipose tissue in light blue, and mediastinal adipose tissue in pink



Statistical analysis

Continuous values are presented as mean with standard deviation for normally distributed variables and median with $25^{th}/75^{th}$ percentile for non-normally distributed variables. For dichotomous variables, percentages are given. To investigate differences between the groups, a t-test was applied for normally distributed, continuous variables, a Kruskal-Wallis test for continuous variables with non-normal distribution, and Chi-square test for categorical variables. The Jonckheere-Terpstra test was used to investigate the trend between extent of coronary atherosclerosis in the three groups and the amount of fat.

Spearman correlation analyses were performed to examine the relationship between cardiovascular risk factors and EAT as well as MAT, and between different adipose tissue measurements. Then, multinomial logistic regression analyses were performed to assess the association between EAT volume, MAT volume and levels of CAD independent of confounding factors. For this, two models were created. The first model corrected for age and gender.

The second model corrected for all cardiovascular risk factors, including body mass index. The odds ratio with the 95 % confidence interval (CI) of EAT and MAT volume was calculated for each 10 cm^3 increase in volume. The statistical analyses for EAT and MAT adjusted for body surface area were repeated. In the logistic regression analyses, body mass index was removed from the second model.

All statistical analyses were performed using PASW Statistics version 19.0.0.1 (SPSS Inc, Chicago, USA). All statistical tests were two-sided, and a P-value of <0.05 was considered to be statistically significant.

5.3 Results

Patient characteristics are shown in Table 5.1. CCTA was successfully performed, and of good diagnostic quality for coronary evaluation in all 65 patients. The majority of the patients were male (69 %). Mean age was 65 years, 53 % of patients were overweight. Non-significant, single vessel and multi-vessel disease on cCTA was present in 54, 23 and 23 %, respectively. EAT ranged from 47.5 to 288.0 cm^3, with a median of 100.0 (25^{th}, 75^{th} percentile 82.3, 133.0). Amount of MAT ranged from 17.4 to 453.0 cm^3; median was 77.0 (51.1, 109.5). Median EAT was 99.5, 98.0 and 112.0 cm^3 ($P = 0.38$) and median MAT was 66.0, 90.0 and 81.0 cm^3 ($P = 0.53$) for the 3 groups, respectively. Most cardiovascular risk factors were correlated with the amount of EAT and MAT (see Table 5.2), except for male gender in case of EAT, and except for blood pressure and hypertension in case of MAT.

Figure 5.2 and 5.3 show box plots of the distribution of EAT and MAT, respectively, by level of CAD. There was a tendency towards a higher median EAT in patients with multivessel CAD compared to non-significant and single vessel CAD, although

Table 5.1: Clinical characteristics of the study population (n = 65)

	Non-significant (n = 35)	Single vessel CAD (n = 15)	Multi-vessel CAD (n = 15)	P-value
Age (years)	64 ± 8	66 ± 7	67 ± 8	0.57
Male gender (%)	62.9	73.3	80.0	0.45
Body mass index (kg/m^2)	26.0 ± 4.6	25.8 ± 2.4	26.7 ± 4.0	0.82
Systolic BP (mm Hg)	145 ± 23	138 ± 19	137 ± 22	0.46
Diastolic BP (mm Hg)	79 ± 9	81 ± 11	78 ± 8	0.70
Hypertension (%)	80.0	73.3	100	0.12
Cholesterol (mmol/l)	4.8 ± 1.4	5.2 ± 0.9	4.8 ± 0.9	0.45
Triglycerides (mmol/l)	1.45 (0.79, 2.66)	2.10 (1.12, 2.91)	1.68 (1.05, 2.37)	0.39
HDL (mmol/l)	1.1 (0.8, 1.6)	1.1 (0.9, 1.4)	1.3 (1.0, 1.5)	0.77
LDL (mmol/l)	2.7 (2.1, 3.5)	3.0 (2.2, 3.9)	2.5 (2.2, 3.3)	0.70
Dyslipidemia (%)	97.1	93.3	80.0	0.11
Glucose (mmol/l)	5.7 (5.9, 6.0)	5.2 (5.1, 5.9)	5.7 (5.2, 6.4)	0.35
Diabetes mellitus (%)	22.9	20.0	40.0	0.37
Smoking (%)	37.1	33.3	20.0	0.49
Extra-cardiac arterial disease				0.05
Intermittent claudication (%)	48.6	60.0	80.0	
Carotid stenosis (%)	25.7	0.0	13.3	
AAA (%)	25.7	40.0	6.7	

Continuous variables are expressed as mean ± SD or median (25^{th}, 75^{th} percentile), dichotomous variables are expressed as percentages. *CAD* Coronary artery disease *BP* blood pressure *HDL* high density lipoprotein *LDL* low density lipoprotein *AAA* Abdominal Aortic Aneurysm

this tendency did not reach statistical significance. In patients with single vessel CAD, median EAT was not higher compared to patients with non-significant CAD. For median MAT, no tendency of increasing fat measures was visible with increasing severity of CAD.

Table 5.3 outlines the odds ratios with 95 % confidence intervals in the different logistic regression models. In age- and gender-adjusted analysis, EAT was significantly associated with CAD (Odds ratio per 10 cm^3 increase in EAT = 1.12, 95 % CI = 1.01-1.25, P = 0.04). When we corrected for all cardiovascular risk factors, this relationship remained significant (OR = 1.21, 95 % CI = 1.04-1.39, P = 0.01). There was no association between increase in MAT and probability of increasing level of CAD, not even when corrected for risk factors.

EAT adjusted for body surface area ranged from 27.4 to 141.9 cm^3/m^2, with a median of 54.3 (43.9, 70.2). MAT adjusted for body surface area ranged from 9.7 to 202.6, with a median of 39.4 (27.3, 53.6). EAT adjusted for body surface area was strongly correlated with EAT (r = 0.944, P <0.001). MAT adjusted for body surface

Table 5.2: Univariate correlation analysis between cardiovascular risk factors and thoracic fat measurements

	EAT		MAT	
	Spearman *r*	*P*-value	Spearman *r*	*P*-value
Male gender	0.05	0.69	0.27	0.03
Systolic BP	-0.27	0.04	-0.16	0.22
Diastolic BP	-0.29	0.03	-0.06	0.65
HDL-cholesterol	-0.45	<0.001	-0.45	<0.001
Tryglycerides	0.51	<0.001	0.39	0.002
BMI	0.44	<0.001	0.55	<0.001
Overweight	0.48	<0.001	0.50	<0.001
Hypertension	-0.25	<0.001	0.07	0.57

EAT Epicardial adipose tissue *MAT* mediastinal adipose tissue *BP* blood pressure *HDL* high density lipoprotein *BMI* body mass index

area was also highly correlated with MAT (r = 0.975, P <0.001). The association between EAT adjusted for body surface area and CAD was significant only when we corrected for all cardiovascular risk factors (OR per 10 cm^3/m^2 increase in adjusted EAT = 1.41, 95 % CI = 1.08-1.84, P = 0.01). MAT adjusted for body surface area was not significantly correlated with CAD, not even when corrected for risk factors.

5.4 Discussion

In patients with extra-cardiac arterial disease, a significant, although moderate, association between EAT and CAD was demonstrated. This association persisted after correction for traditional risk factors, and was still present when EAT was adjusted for body surface area. No such association was present for MAT or MAT adjusted for body surface area, as measures of purely endocrine-acting visceral fat. The fact that an association was found for EAT but not for MAT supports our hypothesis of a local, direct interaction of EAT with the coronary arteries through paracrine or vasocrine pathways, in contrast to the more endocrine effect of MAT that is likely already accounted for due to the specific study population, with symptomatic atherosclerosis elsewhere.

The strength of the relationship between EAT and CAD in our study was only moderate compared to the strength reported in other studies. A possible explanation for this could be that patients with extra-cardiac arterial disease already have higher values of EAT and MAT compared to patients with suspected CAD but without known atherosclerotic disease. In other studies, the volume of EAT in patients without coronary artery disease was indeed lower compared to our study population.[19–23] Only patients with a high clinical suspicion of CAD or demonstrated CAD had a higher

Table 5.3: Odds ratios for increasing levels of coronary artery disease by 10 cm^3 increase in fat measurements

	Model 1 Odds ratio (95 % CI)	*P*-value	Model 2 Odds ratio (95 % CI)	*P*-value
EAT (per 10 cm^3)	1.12 (1.01-1.25)	0.04	1.21 (1.04-1.39)	0.01
MAT (per 10 cm^3)	0.93 (0.85-1.01)	0.09	0.88 (0.77-1.01)	0.07
Adj. EAT (per 10 cm^3/m^2)	1.25 (1.00-1.56)	0.05	1.41 (1.08-1.84)	0.01
Adj. MAT (per 10 cm^3/m^2)	0.87 (0.72-1.06)	0.17	0.81 (0.63-1.02)	0.07

CI Confidence interval *EAT* epicardial adipose tissue *MAT* mediastinal adipose tissue *BP* blood pressure *HDL* high density lipoprotein *BMI* body mass index Model 1 was corrected for age and gender. Model 2 was corrected for all cardiovascular risk factors (age, gender, body mass index, hypertension, dyslipidemia, diabetes mellitus and smoking). Adjusted EAT is defined as EAT divided by body surface area. Adjusted MAT as MAT divided by body surface area

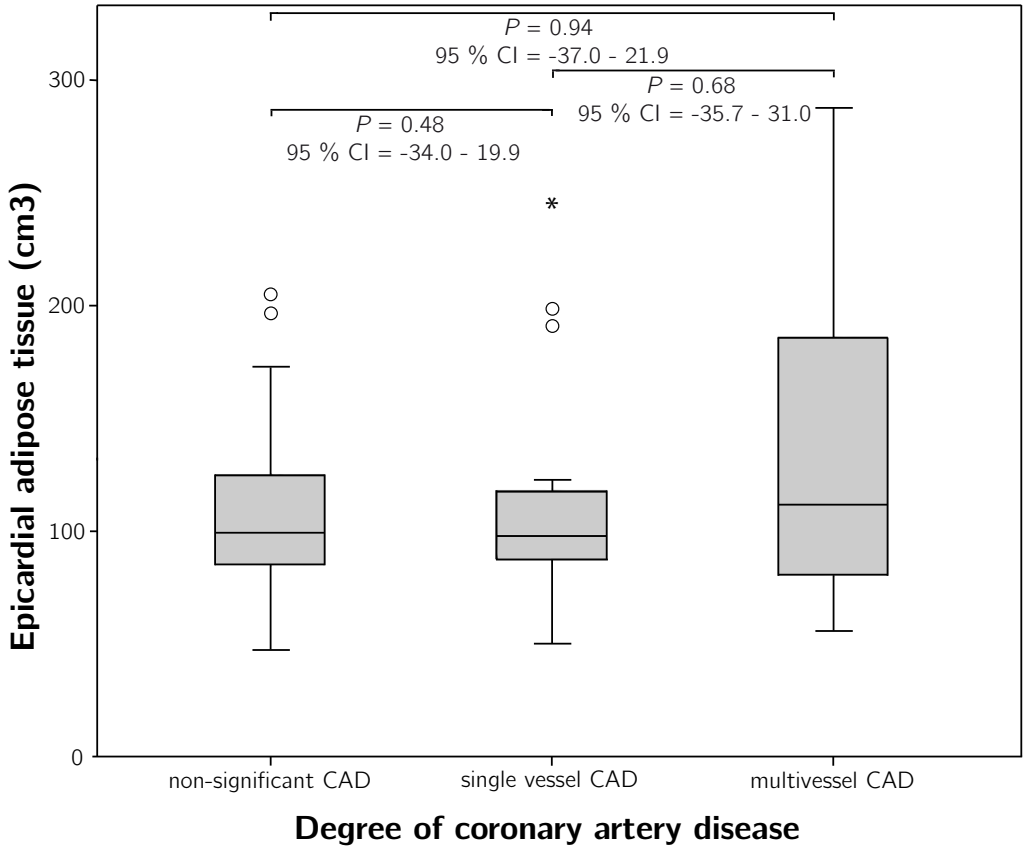
epicardial adipose tissue volume.[12,13,24] On a positive note, since extra-cardiac arterial disease, as expression of a generalized, endocrine effect of visceral adipose tissue, was present in all study subjects, this allowed us to tease out the local, additional effect of EAT on coronary atherosclerosis. In contrast to EAT, there is no agreed definition on the measurement of MAT. Therefore, it is not possible to compare the MAT volume of our population to values in the literature.

Other studies have shown a correlation with EAT and CAD in different study populations. Ueno et al.[14] demonstrated a significant correlation with adjusted EAT and the severity of CAD. Furthermore, they concluded that adjusted EAT was higher in patients with a history of acute coronary syndrome and coronary occlusions. Iwasaki et al.[13] reported a relationship between EAT and significant coronary stenosis on invasive coronary angiography. A study among 112 patients suspected for CAD, but without known history of CAD showed a relationship between adjusted EAT and ischemia on positron-emission tomography (PET)/CT.[25] Several studies have demonstrated that EAT is independently associated with the coronary calcium score,[20,21,26,27] although other studies did not show any relationship between EAT and calcium score[27] unless patients had a low body mass index.[24] Studies have also shown a correlation between MAT and cardiovascular risk factors.[6,28]

EAT can be measured as total volume or as thickness, usually on the right ventricular free wall. Gorter et al.[29] found a good correlation between both fat area and thickness. They concluded that thickness is an easier and faster way to measure EAT, but volume measurement is a more reproducible method. Other studies have confirmed that volumetric EAT measurement is highly reproducible, even with the use of different CT scanner generations.[12,30] In this study, the contrast-enhanced cCTA was used for the measurement of adipose tissue. It is also possible to measure EAT on the non-enhanced scan used for coronary calcium scoring, which is advanta-

geous in way of less radiation and no need for iodine contrast injection. This would be more acceptable for assessment of adipose tissue as a risk factor and has already been used in literature.[11,30] However, coronary stenosis as sign of significant CAD can not be determined on a non-enhanced CT scan. It is not known how the results of fat measurements based on contrast- and non-contrast-enhanced scans correlate. Future research on this issue is needed.

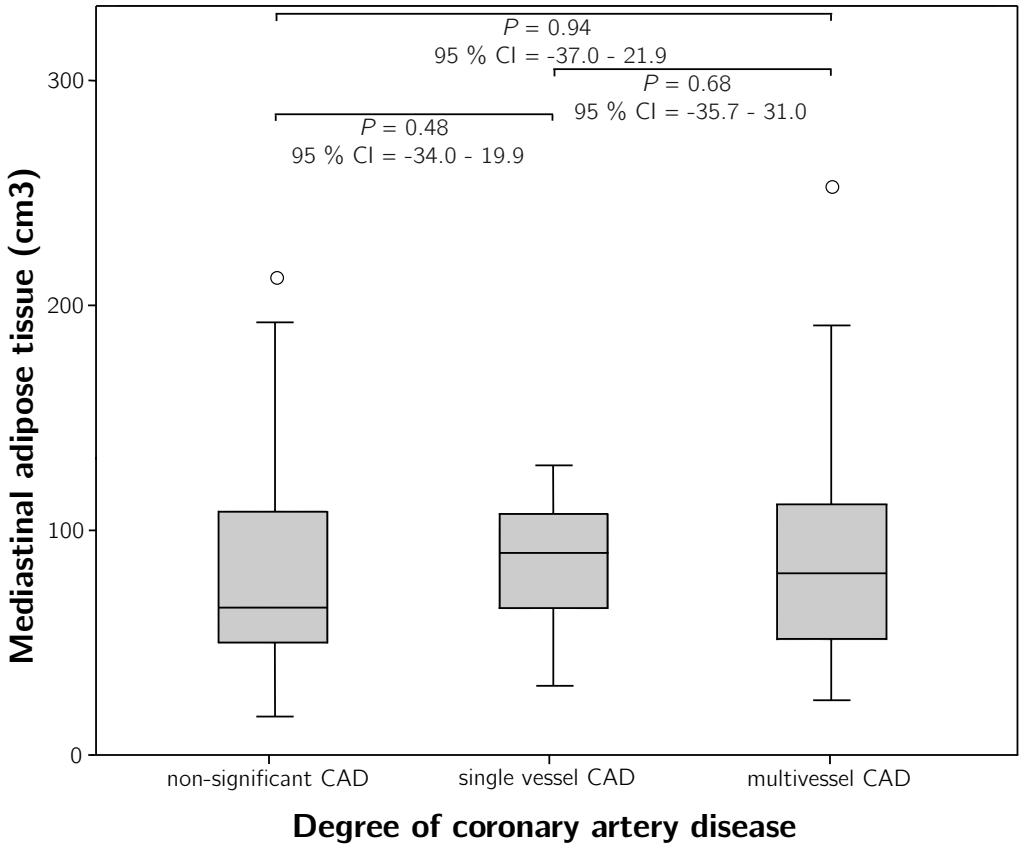
Figure 5.2: Boxplot of median epicardial adipose tissue at different levels of coronary artery disease (CAD). Epicardial adipose tissue volume is higher, although not significantly, in patients with multivessel CAD



There are several effects thought to be associated with EAT. EAT produces pro-inflammatory and pro-atherogenic cytokines, interleukins, chemokines and tumor necrosis factors, which can locally induce atherosclerosis.[10,31] On top of these paracrine effects, there are endocrine effects of visceral adipose tissue, leading to hypertension and insulin resistance.[32] Moreover, adipose tissue can cause compression on the ventricles, leading to diastolic dysfunction.[33] Possible local protective effects are absorption of free fatty acids, protecting the myocardium, or buffering the coro-

nary arteries from torsion induced by the arterial pulse wave.[3] Another role might be a temporary extra source of energy for the myocardium or protection against hypothermia.[31] Furthermore, EAT has paracrine effects, such as production of anti-inflammatory and anti-atherogenic adipokines.[10,31] The balance between protective and damaging effects is still unknown.[34,35] The exact biomechanical effects of MAT are unknown, however, it is clear that there can be no direct influence on the coronary arteries such as the one that could be possible with EAT due to its proximity to the coronary arteries.[4]

Figure 5.3: Boxplot of median mediastinal adipose tissue at different levels of coronary artery disease (CAD). No increase in mediastinal adipose tissue volume in patients with increasing severity of CAD was present



This study has strengths and limitations. As a major advantage, a very specific population of patients with vascular disease was included. In these patients, it is assumed that the endocrine effect of visceral fat on the vessel wall is relatively similar and already taken into account. This enables to investigate a potential additional, direct effect of EAT on the severity of CAD. It is not exactly known if and how this

can be translated to a more general population. Intra-abdominal visceral adipose tissue or waist circumference was not measured for comparison with EAT and MAT. Although this visceral fat depot is described to have similar effects as EAT, it was outside of the CT scan range. We used MAT as measure of endocrine-acting visceral fat as this could be calculated on the same scan. As all patients included in the study have symptomatic peripheral vascular disease, the prevalence of cardiovascular risk factors is high. The degree of subclinical CAD was not known before the cCTA, which was performed as part of this study. We did not find statistically significant differences in the prevalence of cardiovascular risk factors, as assessed at baseline, by CAD group. We assume that the percentages varied among the three CAD groups is mostly due to chance. As the current study reports the baseline risk factor levels and cCTA results, prior to any potential change in therapy based on cCTA results, we consider the possibility of confounding by indication unlikely. To limit the possibility of bias in our study, the radiologists who read the cCTA scans had no knowledge of the risk factors or vascular treatment (invasive or pharmacological) of the scanned participants. The study participant was referred to a cardiologist in case of a left main stenosis or equivalent on cCTA. Potential additional work-up or treatment due to the severity of subclinical CAD, as evident from the cCTA scans, was left at the discretion of the cardiologist. The current study was likely underpowered due to the relatively small cohort of patients. This is mainly caused by the fact that many patients with extra-cardiac arterial disease already have symptomatic CAD for which they are treated. In these patients, there is currently no accepted role for cCTA in the assessment of the extent of CAD.[36] This exclusion criterion of the GROUND2 study considerably restricted the pool of eligible patients. Thus, the findings in the current study should be considered preliminary findings. Nevertheless, we found a clear, statistically significant association between the amount of EAT and the severity of subclinical CAD. This relationship merits further study in larger patient populations.

In conclusion, there was a positive but moderate association between epicardial adipose tissue and the presence of CAD in patients with known extra-cardiac atherosclerosis. This association persisted when EAT was adjusted for body surface area. No such relationship was present for mediastinal adipose tissue. The results suggest that there could be a local, direct effect of EAT on coronary atherosclerosis, apart from the endocrine effect of visceral adipose tissue. Although our study was likely underpowered, we found a significant association between EAT and degree of subclinical CAD. These preliminary results merit further study in larger patient populations.

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6

Skin autofluorescence, a non-invasive marker for AGE accumulation, is associated with the degree of atherosclerosis

M.A.M. den Dekker
M. Zwiers
E.R. van den Heuvel
L.C. de Vos
A.J. Smit
C.J. Zeebregts
M. Oudkerk
R. Vliegenthart
J.D. Lefrandt
D.J. Mulder

Abstract

Objectives Advanced glycation endproducts (AGEs) may be involved in the development of atherosclerosis, beyond diabetes and renal disease. Skin autofluorescence (AF) is a non-invasive marker for AGEs. We examined whether skin AF is increased in (subclinical) atherosclerosis and associated with the degree of atherosclerosis independent of diabetes and renal function.

Methods A cross-sectional study of 223 patients referred for primary (n = 163) or secondary (n = 60) prevention between 2006 and 2012 was performed. Skin AF was measured using the AGE-Reader. Ultrasonography was used to assess plaques in carotid and femoral arteries and computed tomography for the calculation of the coronary artery calcium score (in primary prevention only). Primary prevention patients were divided into a group with subclinical atherosclerosis defined as >1 plaque or calcium score >100 (n = 67; age 53 years [interquartile range 48-56]; 49 % male) and without (controls; n = 96; 43 [38-51]; 55 %). Secondary prevention were patients with extra-cardiac arterial disease (n = 60; 64 [58-70]; 73 %).

Results Skin AF was higher in subclinical and clinical atherosclerosis compared with controls (skin AF 2.11 [interquartile range 1.83-2.46] and 2.71 [2.15-3.27] vs. 1.87 [1.68-2.12] respectively; P = 0.0005 and <0.001). In a multivariate analysis, the association of skin AF with the atherosclerosis categories was independent of age, sex, diabetes, presence of the metabolic syndrome, Framingham Risk Score, and renal function. Skin AF correlated with most cardiovascular risk factors, Framingham risk score, and IMT and calcium score.

Conclusion Skin AF is increased in documented subclinical and clinical atherosclerosis, independent of known risk factors such as diabetes and renal disease. These data suggest that AGEs may be associated with the burden of atherosclerosis and warrant a prospective study to investigate its clinical usability as a risk assessment tool for primary prevention.

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6.1 Introduction

Atherosclerosis is characterized by chronic low grade inflammation and oxidative stress leading to plaque formation and ultimately calcification.[1] While formerly only implicated in diabetes and renal disease, evidence for an important role of advanced glycation endproducts (AGEs) in cardiovascular disease (CVD) beyond these conditions is growing.[2] AGEs are formed by non-enzymatic glycation and oxidative reactions leading to stable structures accumulating on long-lived proteins. They promote cellular stress responses by engagement of the receptor for AGEs (RAGE). AGE epitopes have been detected in human plaques.[3] Lowering AGEs or blocking RAGE in murine models has been found to attenuate plaque formation, supporting the involvement of AGEs in atherosclerosis.[3,4]

Measurement of tissue AGEs may be preferable over plasma measurement, since long-lived proteins accumulate in the tissues in which chronic complications develop.[5] Thus, blood and urine AGEs do not necessarily reflect their tissue levels.[6] We developed and validated a non-invasive technique to quantify tissue AGEs by measuring skin autofluorescence (AF).[7,8] It has been validated with skin biopsies in patients with diabetes or renal disease and healthy controls[7–9] and was shown to correlate strongly with plasma circulating AGEs and with corneal and lens fluorescence in type 1 diabetes.[10] Skin AF is elevated in diabetes mellitus and end-stage renal disease and is associated with cardiovascular mortality, independent of known CVD risk factors.[8,11] Skin AF is also elevated in coronary artery disease,[12,13] correlates with carotid intima media thickness (IMT),[14] and is elevated in patients with carotid artery stenosis and extra-cardiac artery disease (ECAD),[15,16] irrespective of diabetes or renal disease.

Atherosclerosis is a generalized disease that develops years before clinical events occur. Previous studies have only focused on symptomatic disease in a single vascular bed (coronary, carotid, or femoral). It is yet unclear whether skin AF is already increased in subjects with subclinical atherosclerosis. We hypothesized that skin AF is increased in patients with subclinical atherosclerosis, independent of diabetes and renal function, and that skin AF is positively associated with the degree of atherosclerosis. Therefore, we compared skin AF in subjects without and with subclinical atherosclerosis as ascertained by non-invasive imaging measures, and in patients with clinically overt and established atherosclerosis.

6.2 Materials and Methods

Patients

We performed a cross-sectional study of 223 patients, at least 18 years of age, visiting the outpatient vascular clinic of our hospital for primary (n = 163) or secondary

(n = 60) cardiovascular prevention between 2006 and 2012. The study was approved by the local institutional review board at the University Medical Center Groningen and all participants gave written informed consent. Eligible patients of the primary prevention group were referred for counselling because of an increased CVD risk based on conventional cardiovascular risk factors and did not have a history of CVD or symptoms of coronary artery disease, cerebrovascular disease or ECAD. The primary prevention group was divided in patients with and without evidence of subclinical atherosclerosis, the latter forming the control group. Subclinical atherosclerosis was defined as the presence of one or more plaques in carotid and femoral arteries using high resolution ultrasonography or a coronary artery calcium score >100 on computed tomography (CT). These cut-offs were chosen on the basis of previous reports showing that subjects meeting these criteria are at substantially increased CVD risk.[17,18] The secondary prevention group consisted of patients with proven ECAD, which was ascertained by a resting ankle-brachial index $\leq$0.90 or a toe-brachial index $\leq$0.70 if possible in case of non-compressible calf arteries, or a history of radiological or surgical intervention for ECAD. ECAD was confirmed by CT angiography, magnetic resonance angiography or catheter angiography. For both primary and secondary prevention, exclusion criteria consisted of an estimated glomerular filtration rate of <60 ml/min/1.73 m^2, a history of renal transplantation, a recent acute coronary syndrome or cerebrovascular attack, or sepsis (all within the past 3 months), cognitive impairment, or current cancer or autoimmune disease because these conditions have previously been shown to increase skin AF. Furthermore, those with a brown or black skin (Fitzpatrick type V skin) were excluded because skin AF could not be reliably measured with the device used in the current study due to excessive light absorption in this skin type. In the primary and secondary prevention group, skin AF and calcium score measurements were performed. Since the secondary prevention group of ECAD patients by definition had clinically established and proven atherosclerotic disease, no ultrasonographic plaque scoring was performed in this group.

Risk factor assessment

The following risk factors were assessed: smoking status, dyslipidaemia (fasting low density lipoprotein (LDL) cholesterol >4.0 mmol/l, high density lipoprotein (HDL) cholesterol <1.2 mmol/l (female) or HDL-cholesterol <1.0 mmol/l (male), triglycerides >4.0 mmol/l, or current lipid lowering treatment), hypertension (blood pressure $\geq$140/90 mmHg or drug treatment for hypertension), obesity (body mass index (BMI) $\geq$30 kg/m^2), family history of premature CVD (in first degree relatives, male <55 years and female <65 years), and diabetes mellitus (known diabetes, fasting plasma glucose >7.0 mmol/l or random plasma glucose >11.1 mmol/l). Medication use was documented and additional measurements of serum creatinine and high-sensitive C-reactive protein (hsCRP) were performed. Metabolic syndrome was defined according to the International Diabetes Federation, in which BMI substituted

waist circumference, because the latter was unavailable.[19] Framingham risk score was defined as the 10 year risk of coronary heart disease (i.e. myocardial infarction or death from coronary heart disease).[20] Kidney function was estimated using the MDRD formula.[21]

Measurement of skin autofluorescence

Skin AF was assessed using the AGE Reader (DiagnOptics Technologies BV, Groningen, the Netherlands). This is a non-invasive desk-top device that uses the characteristic fluorescent properties of certain AGEs to estimate the level of AGEs accumulation in the skin. The method has been extensively validated and strongly correlates with individual AGE compounds measured in skin biopsy dermal tissue homogenates taken from the same site as skin AF measurement.[22] Technical details concerning the optical technique have been described elsewhere.[7] In short, the right forearm was positioned on top of the device which is the standard and most practical measuring site for skin AF. The AGE Reader illuminates a skin surface of 4 cm^2 with an excitation light source with a peak excitation of 370 nm. Emission light (fluorescence in the wavelength of 420-600 nm) and reflected excitation light (with a wavelength of 300-420 nm) from the skin is measured with a spectrometer. Skin AF is calculated as the ratio between the emission light and reflected excitation light, multiplied by 100 and expressed in arbitrary units. A series of three consecutive measurements was carried out, taking less than a minute of time. Mean skin AF was calculated from these three consecutive measurements and used in the analyses. The method is observer independent and has an intra-patient coefficient of variation of 5 %.[7]

Plaque assessment by intima media thickness

Plaque assessment was performed by measurement of carotid and femoral IMT, as described previously.[23] High resolution B-mode ultrasonography (Acuson 128XP10, Acuson Corporation, Mountainview, USA) with a 7 MHz linear array transducer was used with the subject in a supine position. For both carotid arteries, a mean value over 10 mm length of the far wall segments of the common and internal carotid artery and the carotid bulb were imaged from a fixed lateral transducer position. The same procedure was used for the femoral arteries, with measurement of segments of the common femoral artery and the superficial femoral artery. Mean and maximum IMT of each of the 10 segments was calculated. Presence of a plaque was based on the Mannheim consensus statement. A plaque is defined as a focal structure that encroaches onto the arterial lumen at least 0.5 mm or 50 % of the surrounding IMT value or demonstrates a thickness of $\geq$1.5 mm as measured from the media-adventitia interface to the intima-lumen interface.[24] A total plaque score was calculated summing all segments with presence of at least one plaque, consequently ranging from 0 to 10. The sonographers were unaware of the risk factors

of the studied persons. The measurements were analyzed offline by an independent image analyst who also was unaware of the clinical status of the patient.

Measurement of coronary calcium score

Calcium score was measured either with electron beam CT (C-150 Imatron, San Francisco, USA) or dual-source CT (SOMATOM Definition, Siemens, Erlangen, Germany). A standard scanning protocol was applied. The scan range was from the carina to 1.5 cm below the base of the heart. Images were acquired at 80 % of the cardiac cycle for electron beam CT or 70 % for dual-source CT, with electrocardiographic triggering, during a single breath-hold. For electron beam CT 38 contiguous, 3 mm thick slices were obtained, with a scan time of 100 ms per slice. Tube voltage was 120 kV, with a tube current of 64 mA and a field-of-view of 260 mm. For dual-source CT a sequential scanning protocol was used, with 6×3 mm collimation, 330 ms rotation time, tube voltage 120 kV, tube current dependent on the weight of the patient and a field-of-view of 250 mm. Quantification of calcium score was performed with the use of dedicated software (AccuImage Diagnostics Corporation, South San Francisco, USA for electron beam CT, and Siemens Syngo, Erlangen, Germany for dual-source CT) by trained readers, according to the method described by Agatston.[25] The calcium score of the dual-source CT was corrected to correlate better with the values of the reference standard, electron beam CT.[26]

Laboratory analyses

Venous blood samples were collected into EDTA-containing tubes (1.5 mg/ml). Plasma cholesterol and triglycerides were assayed by routine enzymatic methods (Roche Diagnostics GmbH, Mannheim, Germany). HDL-cholesterol was measured with a homogeneous enzymatic colorimetric test (Roche/Hitachi). HsCRP was determined by nephelometry with a lower limit of 0.175 mg/l (BNII N; Dade Behring, Marburg, Germany). Glucose was measured with an APEC glucose analyzer (APEC Inc., Danvers, USA).

Statistical analysis

Based on previous studies in asymptomatic subjects, we expected a standard deviation of 0.3 for skin AF and considered a mean difference of 0.2 arbitrary units (AU) between patients with subclinical and those without subclinical atherosclerosis as clinically relevant. With a power of 80 % and an alpha of 0.05 and under the assumption of normality, at least 36 subjects were needed to reject the null hypothesis of no difference in a 2-sided independent t-test.

Normally distributed parameters are shown as mean with standard deviation, non-normally distributed values are given as median (interquartile range) and categorical variables are reported as number (percentage). To investigate differences between the three risk groups, the Kruskal-Wallis test for continuous variables and the Chi-square test for categorical variables was used. In case of a significant difference between the three groups, the Mann-Whitney U test was used to compare the subclinical atherosclerosis group with the control group. Several ordinal logistic regression models were developed to examine the relationship between the atherosclerosis groups and skin AF in addition to other risk factors. In the first model, age, sex, diabetes mellitus and renal function were entered as covariates and in the second model, cardiovascular risk factors defined in the Framingham risk score were added to the first model. In the third model, age, sex, renal function and metabolic syndrome were entered. The odds ratio of increasing level of atherosclerosis (control, subclinical, clinical atherosclerosis) was calculated for each AU increase in skin AF.

The Spearman correlation coefficient was used to examine the univariate association between skin AF and other factors. Those with a P <0.10 were included in the linear multiple regression model using stepwise selection of variables. All statistical analyses were performed using PASW Statistics version 18.0.3 (SPSS Inc, Chicago, USA). All statistical tests are two-sided. A P-value of <0.05 was considered statistically significant.

6.3 Results

Patients

A total of 223 patients participated. Characteristics are shown in Table 6.1, 6.2 and 6.3. In the primary prevention group, 67 subjects had subclinical atherosclerosis, while 96 subjects did not meet our criteria for subclinical atherosclerosis and were assigned to the control group. Age, sex, BMI, systolic blood pressure, fasting glucose, hsCRP, lipids, kidney function, the presence of diabetes mellitus, smoking status, hypertension, and cardiovascular drug use differed significantly between groups. Subjects of the subclinical atherosclerosis group were older, had a higher BMI, systolic blood pressure, and fasting glucose levels as compared with the control group. As expected from their need for secondary prevention, ECAD patients used statins more frequently, and consequently had lower lipid levels.

Skin autofluorescence

Skin AF was significantly higher in patients with subclinical atherosclerosis and ECAD compared with controls, and skin AF was higher in the ECAD group compared with

Table 6.1: Clinical characteristics of the study groups

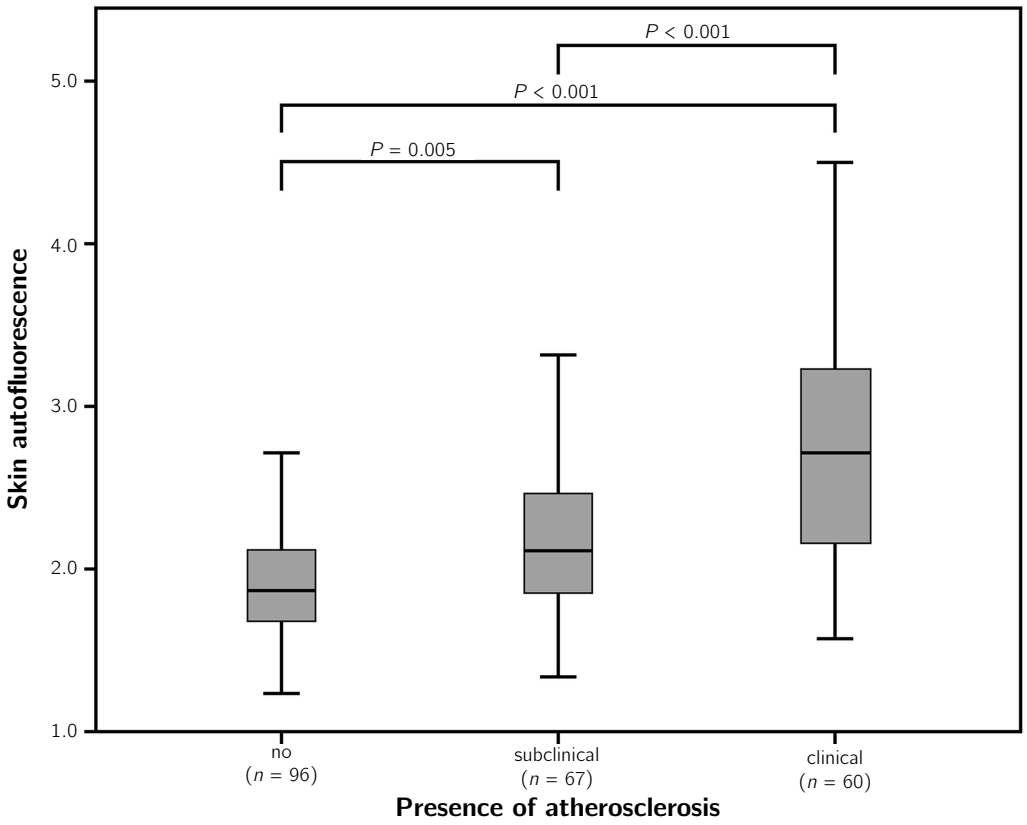
	Controls (n = 96)	Subclinical atherosclerosis (n = 67)	Clinical atherosclerosis (n = 60)	P-value
Age (years)	43.8 ± 9.5	51.8 ± 7.8	63.5 ± 7.6	<0.001⋆
Sex (Male/Female)	53/43	32/35	44/16	0.01
Skin autofluorescence (AU)	1.87 (1.68-2.12)	2.11 (1.83-2.46)	2.71 (2.15-3.27)	<0.001⋆
Body mass index (kg/m^2)	25.0 (23.1-27.7)	26.6 (23.8-29.8)	26.3 (24.2-29.5)	0.03⋆
Systolic BP (mmHg)	130 (120-140)	136 (128-146)	139 (127-164)	0.005⋆
Diastolic BP (mmHg)	80 (73-90)	82 (75-90)	80 (75-85)	0.57
Fasting glucose (mmol/l)	5.2 (4.9-5.8)	5.3 (5.1-5.8)	5.5 (5.0-6.4)	0.09
High sensitive CRP (mg/l)	1.20 (0.60-3.00)	1.50 (0.58-3.33)	3.80 (1.35-7.10)	<0.001
Cholesterol (mmol/l)	5.7 (4.7-6.7)	6.0 (4.9-6.7)	4.3 (3.9-5.4)	<0.001
LDL-cholesterol(mmol/l)	3.8 (2.9-4.7)	4.0 (3.3-4.5)	2.5 (2.1-3.2)	<0.001
HDL-cholesterol (mmol/l)	1.3 (1.1-1.7)	1.3 (1.0-1.6)	1.3 (1.1-1.4)	0.35
Triglycerides (mmol/l)	1.36 (0.91-2.10)	1.67 (1.09-2.74)	1.69 (1.12-2.11)	0.12
Serum creatinine (μmol/l)	73.5 (68.0-84.0)	70.0 (64.0-76.0)	79.0 (69.0-94.0)	<0.001⋆
GFR	93.7 (83.3-103.8)	96.0 (85.3-105.9)	83.1 (68.3-97.2)	<0.001
Diabetes mellitus	3 (3.1)	2 (3.0)	13 (21.7)	<0.001
Smoking				0.009
Yes	29 (30.2)	27 (40.3)	22 (36.7)	
No	31 (32.3)	17 (25.4)	5 (8.3)	
Past	36 (37.5)	23 (38.8)	33 (55.0)	
Dyslipidaemia	77 (80.2)	59 (88.1)	51 (85.0)	0.39
Hypertension	43 (44.8)	41 (61.2)	52 (86.7)	<0.001⋆
Metabolic syndrome	24 (25.0)	25 (37.3)	21 (35.0)	0.19
Framingham Risk Score	2.0 (0.4-4.1)	5.1 (2.5-11.7)	9.5 (3.2-18.3)	<0.001⋆

⋆ P <0.05 between subclinical atherosclerosis and controls.
BP Blood pressure *GFR* glomerular filtration rate using MDRD formula

the group with subclinical atherosclerosis (Figure 6.1). Within the control group, 40 subjects had no detectable plaques and a calcium score of 0 while 56 subjects had only a single plaque and/or calcium score of 0-100; skin AF did not differ between these two groups (skin AF 1.93 [1.72-2.16] vs. 1.83 [1.67-2.05], P = 0.30). Within the subclinical atherosclerosis group, 17 subjects had plaques only, 10 had an increased calcium score only, and 40 had more than one plaque and increased calcium score. Between these three groups, skin AF also did not differ (2.14 [1.74-2.45] vs. 2.03

[1.80-2.23] vs. 2.16 [1.85-2.55], P = 0.47). Finally, in the ECAD group, skin AF was not significantly different between those with calcium score below and those with calcium score above 100 (2.65 [2.10-3.35] vs. 2.72 [2.26-3.20], P = 0.92).

Figure 6.1: Boxplot of skin autofluorescence between patients with increasing degree of atherosclerosis



The three models for ordinal regression analysis are outlined in Table 6.4. The difference in skin AF between the 3 groups was independent of age, sex, diabetes, and renal function (P = 0.009). Addition of Framingham risk score or replacement of diabetes with metabolic syndrome in this model did not change the results (P = 0.017 and 0.009). In all models, the odds of having a higher degree of atherosclerosis were 2-fold increased per unit of skin AF. To investigate factors associated with skin AF, univariate correlations were calculated in the entire group. Skin AF correlated with age (r = 0.55; P <0.001), Framingham risk score (r = 0.35; P <0.001), diabetes (r = 0.23; P <0.001), hypertension (r = 0.19; P=0.005), body mass index (r = 0.15; P = 0.02), calcium score (r = 0.34; P <0.001) and systolic blood pressure (r = 0.14; P = 0.04), but not with diastolic blood pressure. Skin AF also correlated with plasma glucose (r = 0.20; P = 0.003), triglycerides (r = 0.17; P = 0.01), hsCRP (r = 0.31;

P <0.001) and inversely with LDL (r = -0.16; P = 0.02). No correlations between medication and skin AF were observed. In the linear multiple regression model, skin AF was independently associated with age and plasma glucose (model: r^2 = 0.29, P <0.001; age: standardized beta 0.46, P <0.001; glucose: r = 0.19, P = 0.005). Because of the low prevalence of diabetes, analyses were repeated after removal of the patients with diabetes. This did not alter the results. In the primary prevention group, skin AF also correlated with plaque score (r = 0.21; P = 0.008), mean IMT of carotid and femoral arteries (r = 0.22; P = 0.004 and r = 0.13; P = 0.11) and max IMT (r = 0.26; P = 0.001 and r = 0.21; P = 0.007), respectively. The correlation with skin AF and calcium score did not reach significance (r = 0.13; P = 0.09).

6.4 Discussion

In the current study we demonstrate that skin AF – a non-invasive marker for tissue AGE accumulation – is elevated in subjects with evidence of subclinical atherosclerosis and subjects with clinical atherosclerosis. Furthermore, skin AF increases with the degree of atherosclerosis, independently of factors known to be associated with accumulation of AGEs, including age, sex, diabetes or metabolic syndrome, kidney function and Framingham risk score. To the best of our knowledge, this is the first study to demonstrate an association of skin AF with varying degrees of atherosclerosis. This was ascertained not only by measuring IMT, but also by plaque assessment and calcium score, all of which are validated methods for measuring subclinical atherosclerosis. Earlier, we reported that skin AF is increased and a strong predictor of mortality in diabetes mellitus and end stage renal disease compared with healthy controls.[8,11] Other groups have confirmed the association between skin AGEs and coronary artery disease in patients with type 1 diabetes, using a different setup, referred to as skin intrinsic fluorescence.[27] The current study is in line with these observations and presents the new finding of a higher accumulation of AGEs in patients with subclinical atherosclerosis, independent of diabetes and renal function, with the simultaneous use of more than one technique.[2]

In line with the hypothesis of a role of AGEs beyond diabetes and renal disease, we demonstrated earlier that skin AF is elevated in ECAD,[15,16] coronary artery disease[12] and myocardial infarction.[13] Skin AF was shown to correlate with markers of subclinical atherosclerosis, i.e. IMT[14,28] and small artery elasticity,[10] the soluble RAGE and with markers of inflammation, endothelial activation, and oxidative stress in other studies.[12,13] These previous studies were all performed in populations with known cardiovascular disease, diabetes, renal disease, or autoimmune diseases. This may have confounded the relation between skin AF and atherosclerosis. The diseases per se, especially autoimmune diseases, could cause increases in skin AF.[29] The study by Lutgers[14] demonstrated an association between skin AF and IMT in healthy subjects without diabetes. However, in this particular study plaques were not documented

Table 6.2: Medication use of study groups

	Controls (n = 96)	Subclinical atherosclerosis (n = 67)	Clinical atherosclerosis (n = 60)	P-value
Statin use	38 (39.6)	31 (46.3)	46 (76.7)	<0.001
Other lipid lowering medication	8 (8.3)	7 (10.4)	3 (5.0)	0.53
ACEi / ARB use	8 (8.3)	11 (16.4)	37 (61.7)	<0.001
Other antihypertensive medication	14 (14.6)	15 (22.4)	34 (56.7)	<0.001
Aspirin use	4 (4.2)	8 (11.9)	48 (80.0)	<0.001
Coumarin use	0 (0.0)	1 (1.5)	8 (13.3)	<0.001

ACEi Angiotensin-converting enzyme inhibitor *ARB* angiotensin receptor blocker

and no coronary calcium score was measured. Also, a preliminary version of the AGE-Reader was used, which has not been developed further and was not validated to skin biopsy.

Table 6.3: Classification variables for patient group definition

	Controls (n = 96)	Subclinical atherosclerosis (n = 67)	Clinical atherosclerosis (n = 60)
Calcium score (AU)	0.0 (0.0-6.9)	130.0 (0.0-248.0)	389.8 (107.6-984.0)
Mean carotid IMT	0.65 (0.57-0.74)	0.83 (0.67-0.98)	NA
Max carotid IMT	0.71 (0.60-0.82)	0.97 (0.80-1.11)	NA
Mean femoral IMT	0.55 (0.48-0.67)	0.70 (0.53-1.05)	NA
Max femoral IMT	0.60 (0.50-0.75)	0.80 (0.63-1.25)	NA

AU Arbitrary units *IMT* intima media thickness *NA* not applicable

Correlations between (non-invasive) assessments for different vascular beds i.e. IMT, carotid and coronary plaque scores, and calcium score) is moderate, even in post-mortem studies.[30] No single method seems to adequately reflect overall atherosclerosis burden. In line, we found only a weak concordance between the presence of plaques and calcium score, with most subjects presenting with plaques or coronary calcifications only. This supports our choice to study multiple vascular techniques and vascular beds. The IMT value is strongly age dependent and a relatively weak predictor of CVD. Therefore, we preferred to define atherosclerosis as the presence of plaques, which is considered a more accurate surrogate for subclinical atherosclerosis[31] and a better predictor of future CVD.[32] Although we used the Mannheim consensus definition, some plaques may have been missed, since we only quantified plaques that were present at the far wall and did not scan outside the designated segments.

Calcium score is a stronger predictor of future coronary events[33] and to a lesser extent with CVD in general. Calcium score correlates strongly with histopathologic coronary disease and that absence of calcification is highly suggestive for the absence of CAD.[17] We chose a cutoff of >100 AU for subclinical atherosclerosis, since this is considered optimal to separate asymptomatic subjects at high from those at low risk. Since calcium score only detects the presence of a calcified plaque, so-called "soft" plaques are missed.[34] Furthermore, with the techniques we used it is not possible to discriminate "active" or vulnerable from stable plaques. This would necessitate more sophisticated and currently experimental techniques.

Table 6.4: Odds ratios for increasing degree of atherosclerosis (control, subclinical atherosclerosis, symptomatic peripheral arterial disease) by 1 unit increase of skin autofluorescence (Skin AF).

	Skin AF Odds ratio (95 % CI)	*P*-value
Model 1	2.11 (1.21, 3.68)	0.009
Model 2	1.99 (1.13, 3.50)	0.017
Model 3	2.13 (1.21, 3.74)	0.009

Model 1 was only corrected for age, sex, diabete mellitus and renal function. In model 2, we corrected additionally for cardiovascular risk factors as Framingham risk score. Model 3 was corrected for age, sex, renal function and metabolic syndrome.

Skin AF is an indirect marker for AGEs in the skin, and is influenced by other skin fluorophores. Most AGEs are not fluorescent. Nonetheless, in validation studies, skin AF strongly and consistently correlated with fluorescent as well non-fluorescent AGEs, including the major and most extensively studied AGE Nε-(carboxymethyl)lysine.[35] We did not collect extra plasma samples and could therefore not assess plasma AGE levels. The disadvantage of plasma AGEs is that they can be influenced and fluctuate due to several factors (i.e. smoking, nutrition, renal function) whereas skin AF remains stable. Furthermore, plasma AGE measurement has an impaired reproducibility due to a lack of uniformity in assays, and are not independently associated with CVD.[36] A restriction to measuring skin AF is that it could not be reliably measured in persons with a dark skin with the AGE-Reader used in this study. However, with the new set-up of the AGE-Reader it is possible to perform accurate measurements in darker skin types, even in Asian or African populations.[37]

The use of cardiovascular drugs may have influenced AGE levels. Statins and aspirin have been shown to reduce plasma levels of the soluble receptor for AGEs.[38,39] Although no such evidence exists for the effects on AGE levels in serum or tissue, this cannot be excluded. If statin use would have caused mitigation of the association between skin AF and extent of atherosclerosis (bias toward zero), then we expect the actual relationship between skin AF and atherosclerosis to be stronger. The majority of patients referred for secondary prevention were treated with lipid and

blood pressure lowering drugs according to the latest guidelines, which explains the relatively low levels of cholesterol and blood pressure in these patients.

Since this is a cross-sectional study, a direct etiological role of skin AF or AGEs in atherosclerosis cannot be proven. Furthermore, the relationship between skin AF and severity of atherosclerosis is confounded by the conventional cardiovascular risk factors. Statistical correction inevitably results in interaction and overadjustment, since these factors are strongly interrelated and age dependent. Because of the small study population, we did not correct for all risk factors separately and chose to cluster them in the Framingham risk score and metabolic syndrome. Skin AF should not be considered a diagnostic marker to detect (subclinical) atherosclerosis, but as an additional non-invasive marker for cardiovascular risk.

In conclusion, in this cross-sectional study we confirm the hypothesis that skin AF, a non-invasive marker for AGE accumulation, is elevated in subclinical atherosclerosis and subjects with clinical atherosclerosis. These data add further evidence that accumulation of AGEs is linked to atherosclerosis, even at a subclinical level, independent of age and sex and, importantly, diabetes and kidney disease. However, before applying this method in clinical practice for primary prevention, these results need conformation in a prospective cohort study.

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7

Summary

Summary

Extra-cardiac arterial disease (ECAD) is common in the Western population, with a prevalence of 29 % in persons over 50 years. ECAD can be divided in stenotic and aneurysmal disease. ECAD is associated with an elevated risk of coronary artery disease (CAD). Patients with aneurysmal or stenotic ECAD may have similar risk of coronary events. Screening for CAD before symptoms occur, has become a realistic possibility with recent developments in computed tomography (CT) and magnetic resonance (MR) imaging. Coronary CT angiography (cCTA) can detect coronary stenosis, i.e. anatomical disease. Adenosine perfusion MR evaluates the effect of stenosis on perfusion of the myocardium, thus whether myocardial ischemia occurs in stress. Non-invasive cardiac imaging of these patients, combined with a dedicated treatment algorithm, may beneficially affect the prognosis in ECAD patients. In this thesis, novel biomarkers of CAD based on non-invasive CT and MR imaging in ECAD patients were studied. **Chapter 1** is a general introduction on the background of atherosclerosis and the detection of CAD.

With newer CT scanner generations, the accuracy of cCTA for detection of obstructive coronary stenosis compared to invasive coronary angiography has improved. cCTA has a very high sensitivity for coronary stenosis. The specificity of cCTA may be affected by coronary calcification, since severe calcification limits lumen assessment due to blooming artifacts. The amount of coronary calcification is quantified in a calcium score, which is a marker of overall coronary atherosclerosis. In case of a high calcium score (CS), cCTA can yield false positive results. In a meta-analysis in over 5,000 patients (**Chapter 2**), it was found that the specificity of cCTA for significant stenosis remained high in case of severe coronary calcification with newer CT systems of 64-MDCT and beyond. For patients with CS over 1,000, the specificity of cCTA was 84 % (89 % in segment-based analysis), not significantly lower than overall specificity. The test characteristics for 16-MDCT scanners were significantly worse. The results suggest that for modern CT systems (at least 64-MDCT), a CS cut-off above which cCTA should not be performed seems no longer indicated.

The exact prevalence of silent CAD is in the population of ECAD patients is unknown. As coronary angiography, the reference standard, is an invasive procedure with some risk of morbidity and mortality, this procedure is generally only performed in case of strong suspicion of CAD. **Chapter 3** shows the results of one of the first studies in ECAD patients assessing the presence of asymptomatic CAD using non-invasive screening. In 111 ECAD patients without cardiac symptoms or history, A high percentage of silent, severe coronary artery disease based on cCTA and adenosine perfusion MR was found. Referral to a cardiologist because of severe CAD was deemed necessary in nearly one fifth of all patients, resulting in coronary intervention in 9.0 % of all patients. The rate of CAD was not significantly different in patients with stenotic compared with aneurysmal ECAD. Further studies may show screening in these high risk patients to be beneficiary.

Many anatomical coronary stenoses do not cause myocardial ischemia in stress and thus, are not functionally relevant. Usually, multiple tests are needed to derive a final diagnosis of hemodynamically significant CAD, with disadvantages in terms of patient discomfort and costs. In view of the increased use of cCTA to assess CAD, it would be extremely valuable if this same non-invasive test could determine the hemodynamic significance of the anatomical stenoses that are readily detected. In **Chapter 4**, a novel method to assess the hemodynamic significance of coronary stenoses was evaluated, based on regular cCTA examinations, obtained in the group of ECAD patients. Using semi-automatic software, the corrected contrast opacification (CCO) was calculated, estimating the effect of stenosis on coronary flow. Decrease in CCO across coronary stenosis was associated with myocardial ischemia on adenosine perfusion MR. Mere anatomical stenosis did not cause a difference in CCO decrease. The CCO may offer a rather simple and straightforward measurement to evaluate the hemodynamic significance of stenosis on cCTA without additional radiation dose. CCO can potentially exclude the majority of hemodynamically insignificant coronary stenoses from further workup. Studies in larger patient populations should assess whether CCO calculation may obviate the need for ischemia testing, and derive CCO cut-off points to distinguish normal versus reduced coronary flow.

Quantitative risk markers may improve cardiovascular risk prediction. In **Chapters 5** and **6** two of these new biomarkers have been investigated. Cardiac CT without contrast for calcium scoring can yield additional information such as the amount of fat surrounding the heart, called epicardial adipose tissue (EAT) and mediastinal adipose tissue (MAT). EAT and MAT can be quantified with dedicated software. Several studies have demonstrated an association between EAT and significant CAD, but it is unknown whether the effect of fat tissue on the coronary arteries is due to local or to endocrine effects. In a homogenous group of patients with known vascular disease, the endocrine effect of visceral fat tissue on the vessel wall can be assumed to be relatively similar and accounted for. In the ECAD patients there was a positive but moderate association between EAT and the presence of CAD (**Chapter 5**). This association persisted when EAT was adjusted for body surface area. No such relationship was present for MAT. These results suggest that EAT has a local effect on coronary atherosclerosis, apart from the endocrine effect of visceral fat. These preliminary results merit further study in larger patient populations.

Finally, in **Chapter 6** a novel biomarker to measure advanced glycation endproducts with skin autofluorescence (AF) was studied. Skin AF has been associated with atherosclerosis in patients with diabetes or renal disease. It is yet unclear whether skin AF is already increased in subjects with subclinical atherosclerosis on non-invasive imaging. In this study in 223 individuals, the level of skin AF in individuals without subclinical atherosclerosis was compared to levels in patients with subclinical and clinically overt atherosclerosis. Skin AF was already higher in individuals with subclinical atherosclerosis, independent of risk factors such as diabetes and renal disease. Also, skin AF increased with the degree of atherosclerosis. This suggests that advanced

glycation endproducts may be associated with the burden of atherosclerosis. Before applying this method in clinical practice for primary prevention, the results need conformation in a prospective cohort study.

In this thesis, several new biomarkers for the detection of coronary atherosclerosis were investigated. The results can guid further clinical studies and randomized controlled trials. It is expected that cCTA and additional non-invasive biomarkers can serve as a one-stop shop in the detection and screening for coronary artery disease, in particular in patients with extra-cardiac arterial disease.

8

Samenvatting

Samenvatting

Perifeer vaatlijden (ECAD) is een veelvoorkomende ziekte in de Westerse wereld, en komt voor in 29 % van de mensen ouder dan 50 jaar. Perifeer vaatlijden kan worden onderverdeeld in vernauwend (stenotisch) en verwijdend (aneurysmatisch) vaatlijden. Perifeer vaatlijden geeft een verhoogde kans op coronairlijden. Zowel perifeer vaatlijden als coronairlijden hebben soortgelijke risicofactoren. Screenen op coronairlijden voordat klachten optreden, is een reële mogelijkheid met de nieuwe ontwikkelingen in computer tomografie (CT) en magnetic resonance (MR) imaging. Met coronaire CT angiografie (cCTA) kunnen coronaire vernauwingen (stenosen) worden aangetoond, als anatomische ziekte. Adenosine perfusie MR kan het effect van de stenose op de doorbloeding van de hartspier bepalen, en dus of er zuurstoftekort (ischaemie) optreedt bij inspanning. Niet-invasieve cardiale beeldvorming in patiënten met perifeer vaatlijden, in combinatie met een goed toegepast speciaal behandelprogramma, kan hun risico gunstig beïnvloeden. In dit proefschrift zijn nieuwe biomarkers voor coronairlijden onderzocht, met behulp van niet-invasieve CT en MR onderzoeken bij patiënten met perifeer vaatlijden. **Hoofdstuk 1** is een algemene introductie, gebaseerd op de achtergrond van atherosclerose en het opsporen van coronairlijden.

Nieuwere CT scanners hebben een verbeterde betrouwbaarheid van cCTA voor het opsporen van coronairlijden ten opzichte van invasieve coronairangiografie. cCTA heeft een hoge sensitiviteit voor coronaire stenosen. De specificiteit van cCTA kan worden beïnvloed door coronaire verkalkingen, aangezien ernstige verkalkingen in de vaatwand van de coronair de beoordeling van het bloedvat kan belemmeren door 'blooming artefacten', waarbij de stenose overschat wordt. De hoeveelheid kalk in een coronair kan worden gemeten met de calcium score, die een maat is voor totale coronaire atherosclerose. In het geval van een hoge calcium score (CS) kan cCTA fout-positieve resultaten geven. In een meta-analyse van meer dan 5,000 patiënten (**Hoofdstuk 2**) is aangetoond dat de specificiteit van cCTA voor significante stenosen hoog bleef in het geval van ernstig coronair kalk bij gebruik van 64-detectorrijen of meer. Bij patiënten met een CS van meer dan 1,000 was de specificiteit van cCTA 84 % (89 % op segment-basis) niet significant lager dan de totale specificiteit. De testresultaten voor 16-detectorrijen waren wel significant slechter. Deze resultaten suggereren dat er voor de moderne CT scanners (ten minste 64-detectorrijen) geen afkapwaarde voor de CS meer nodig is.

De exacte prevalentie van stil coronairlijden (patiënten hebben geen bijbehorende klachten) bij patiënten met perifeer vaatlijden is nog niet bekend. Coronairangiografie, de gouden standaard, is een invasieve procedure met een klein risico op morbiditeit en sterfte. Deze procedure is over het algemeen alleen toegepast bij patiënten met een sterke verdenking op coronairlijden. In **Hoofdstuk 3** worden de resultaten beschreven van een van de eerste studies waarin de aanwezigheid van stil coronairlijden onderzocht werd bij patiënten met perifeer vaatlijden, met behulp van niet-invasieve screening. Bij 111 patiënten met perifeer vaatlijden, zonder cardiale

klachten, is een hoge prevalentie van stil, ernstig coronairlijden gevonden met behulp van cCTA en adenosine perfusie MR. Ongeveer een vijfde deel van alle patiënten werd naar de cardioloog verwezen voor ernstig coronairlijden, met als resultaat dat bij 9 % van alle patiënten een interventie geïndiceerd was. Er was geen verschil in aanwezigheid van coronairlijden voor patiënten met stenotisch of aneurysmatisch vaatlijden. Vervolgstudies zijn nodig om de mogelijke voordelen van screening in deze hoog-risico patiëntengroep te bevestigen.

Veel anatomische stenosen veroorzaken geen zuurstoftekort in de hartspier en zijn dus niet functioneel relevant. Gewoonlijk zijn er meerdere onderzoeken nodig om een definitieve diagnose van functioneel relevante of haemodynamisch relevante coronairlijden vast te stellen, met de nadelen van extra patiëntbelasting en de kosten. Met het toenemende gebruik van cCTA voor het opsporen van coronairlijden zou het zeer waardevol zijn als dit niet-invasief onderzoek ook de functionele relevantie van eerder aangetoonde anatomische stenosen in beeld zou kunnen brengen. In **Hoofdstuk 4** is een nieuwe methode onderzocht om de haemodynamische significantie van coronaire stenosen te beoordelen, via de normale cCTA onderzoeken, bij patiënten met perifeer vaatlijden. Met semi-automatische software werd de gecorrigeerde contrastopacificatie (CCO) berekend, als voorspelling van het effect van een stenose op de bloedstroom in de coronair. Contrastopacificatie is een maat voor de aankleuring van bloedvat door de hoeveelheid contrastmiddel. Een hogere contrastopacificatie betekent meer contrastmiddel dat door het bloedvat stroomt. Een afname in de CCO over een coronaire stenose was geassocieerd met zuurstoftekort bij adenosine perfusie MR. Simpele anatomische stenosen zonder functionele relevantie gaven geen CCO afname. CCO kan een eenvoudige meting zijn om de functionele relevantie van een stenose op cCTA te beoordelen, zonder aanvullende straling. CCO kan mogelijk het grootste deel van de haemodynamisch niet-significante stenosen uit te sluiten van verder vervolgonderzoek. Wel zijn er nog studies nodig met grotere groepen patiënten om een afkapwaarde van CCO te bepalen. In de toekomst is het mogelijk om met een enkel onderzoek onderscheid te maken tussen normale en verminderde coronaire doorbloeding.

Nieuwe risico-markers kunnen de voorspelling van het cardiovasculaire risico verbeteren. Daarom zijn in **Hoofdstuk 5** en **6** twee van de deze nieuwe biomarkers onderzocht. Een blanco CT coronairen kan worden gebruikt voor de meting van de calcium score, maar aanvullende informatie kan uit deze scan worden verkregen, zoals over het epicardiaal vetweefsel (EAT) en mediastinaal vetweefsel (MAT). EAT en MAT kunnen worden gemeten met behulp van speciale softwareprogramma's. Meerdere studies hebben een verband aangetoond tussen EAT en ernstig coronairlijden, maar het is nog niet duidelijk of dit ten gevolge is van lokale of endocriene effecten. In een gelijkwaardige groep patiënten met bekend perifeer vaatlijden, is het te verwachten dat het endocriene effect van vetweefsel vergelijkbaar is. In deze patiëntengroep was een milde, maar positieve milde relatie tussen EAT en de aanwezigheid van coronairlijden, die ook na correctie voor het lichaamsoppervlak aanwezig bleef

(**Hoofdstuk 5**). Er is geen relatie gevonden tussen MAT en de aanwezigheid van coronairlijden. Deze resultaten suggereren dat EAT een lokaal effect heeft op coronaire atherosclerose, naast het endocriene effect van visceraal vet. Deze voorlopige resultaten geven aanleiding voor verder onderzoek.

Tenslotte werd in **Hoofdstuk 6** een nieuwe niet-invasieve biomarker voor advanced glycation endproducts met behulp van autofluorescentie via de huid (skin AF) bestudeerd. Er bestaat een verband tussen skin AF en atherosclerose bij patiënten met diabetes of nieraandoeningen. Er is echter nog nooit met behulp van niet-invasieve beeldvorming onderzocht of skin AF ook al verhoogd is bij patiënten met subklinische atherosclerose. In deze studie werden 223 personen onderzocht, waarbij de hoogte van skin AF bij personen zonder subklinische atherosclerose werd vergeleken met waardes in patiënten met atherosclerose, zonder en met klachten. Skin AF bleek reeds verhoogd bij subklinische atherosclerose, onafhankelijk van bekende risicofactoren zoals diabetes en nierziekte. Verder was skin AF verhoogd in relatie tot de mate van atherosclerose. Dit suggereert dat advanced glycation endproducts geassocieerd kunnen zijn met de atherosclerose last. Voordat deze techniek in de praktijk kan worden toegepast voor primaire preventie, moeten de resultaten worden bevestigd in een grote bevolkingsstudie.

In dit proefschrift zijn meerdere nieuwe biomarkers bestudeerd voor de detectie van coronaire atherosclerosis. Deze resultaten kunnen richting geven aan verdere klinische studies en gerandomiseerde trials. Het is te verwachten dat cCTA, eventueel met aanvullende niet-invasieve biomarkers dienen als een one-stop shop voor de detectie en screenen naar coronairlijden, met name bij patiënten met perifeer vaatlijden.

9

Dankwoord

Dankwoord

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Curriculum Vitae

Curriculum Vitae

Martijn den Dekker was born on the 9th of November, 1978 in Oss. He grew up in Uden and attended high school at the Kruisheren Kollege, where he graduated in 1997. The same year, he started to study Medicine at the Katholieke Universiteit Nijmegen, St. Radboud. Part of his internships took place in the University Medical Center Nijmegen, and his final internship was at the department of Internal Medicine. He graduated as Medical Doctor in August 2004. After this, he worked at the department of Cardiology at the Canisius Wilhelmina Ziekenhuis in Nijmegen and the Medisch Spectrum Twente in Enschede. In December 2009, he started researching at the department of Radiology, which led to this thesis. He presented his work at the annual meeting of the European Society of Cardiac Radiology in 2010, 2011, 2012 and 2013, at the European Congress of Radiology in 2012, 2013 and 2014, at the CardioVasculaire Conferentie in 2011 and at the Radiologendagen in 2013. At the annual meeting of the European Society of Cardiac Radiology, he received a Certificate of Merit for his scientific exhibit on âĂIJPrevalence of coronary artery disease in patients with extra-cardiac arterial disease as detected with cardiac CT and MRI: Results from the GROUND2 studyâĂİ. Furthermore, his work was presented at the annual meeting of the Radiological Society of North America in 2012 and 2013. He started his residency in Radiology in November 2013 at the University Medical Center Groningen.