Carotid imaging in cardiovascular risk assessment

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CONTENTS

CHAPTER 1

Introduction

1

CAROTID ARTERY IMAGING

Applications in cardiovascular risk assessment and pathophysiological studies on atherosclerosis

INTRODUCTION

Cardiovascular disease is one of the most important challenges in modern medicine. Atherosclerotic changes in coronary and peripheral arteries are the main contributors to clinical cardiovascular disease. These arterial changes develop due to a multitude of known and unknown risk factors as well as a patient's individual genetic susceptibility. Atherosclerotic thickening of the arteries precedes the clinical signs and symptoms of cardiovascular disease by years to decades, as illustrated in figure 1. Identifying the patients with increased cardiovascular risk at an early stage and effective modification of the specific risk factors in those patients are key components of preventing cardiovascular complications.

Measurement of carotid vessel wall thickness using high-resolution ultrasound has proven to be an important tool in cardiovascular research. In recent years magnetic resonance imaging (MRI) protocols have been developed to evaluate carotid wall geometry. The most widely used application of both techniques is in the role of intermediate end point in clinical trials. Their use in this capacity has been extensively validated and reviewed. In addition to this application, carotid thickness measurement by ultrasound and MRI has also been used in *in vivo* pathophysiological studies of the atherosclerotic process primarily through associating vessel wall thickness to many non-traditional risk factors.

Despite several decades of experience with carotid imaging in research settings it is not routinely used in clinical practice. Carotid imaging studies could potentially aide clinicians in more accurately identifying patients with high cardiovascular risk. Ultrasound as well as MRI scans can quantify the thickness of the carotid artery wall. Abnormal or premature thickening of the vessel wall is representative of smooth muscular hypertrophy and intimal hyperplasia and are indicative of early atherosclerotic changes. In addition, both imaging modalities are able to detect the presence of atherosclerotic plaques before they cause blood flow changes or stenosis. Therefore these vessel wall measurements carry a great

potential benefit for clinicians: they make it possible to identify patients with atherosclerosis at a very early stage, before they exhibit abnormalities on echo duplex examination or angiography. Diagnosing patients with atherosclerosis at such an early stage would allow for timely (lifestyle and pharmacological) interventions , thereby potentially delaying or preventing cardiovascular events in the future. Data from population-based studies as well as clinical trials offer strong arguments for the utilisation of carotid imaging in risk assessment:

- Carotid atherosclerosis can be objectified through non-invasive and highly reproducible techniques (1)
- Carotid thickness and plaques are associated with atherosclerosis in other vascular beds, including the coronaries, cerebral vasculature and other peripheral large arteries. (2), (3) (4) (5)
- Carotid atherosclerosis is independently associated with future cardiovascular events (table 1)
- Data suggests that taking carotid imaging parameters into account improves cardiovascular risk prediction (6)

Nonetheless, the role of carotid imaging in cardiovascular risk assessment has been long debated and overall general consensus is still not reached. Most current clinical guidelines (AHA (7) /ESH (8)/ESC (9)) include some form of recommendation on the use of carotid imaging but they are not uniform and implementation is far from ubiquitous in clinical practice. Several concerns need to be addressed before broad scale implementation can be advised:

- Although ample data is available associating carotid ultrasound abnormalities to elevated cardiovascular risk, imaging protocols are varied and the optimum scanning technique is subject to ongoing debate
- Much of the evidence on carotid MRI abnormalities and increased risk is extrapolated from ultrasound studies and not based on direct long term follow-up studies
- Carotid atherosclerosis has consistently been found to be independently associated with cardiovascular events but the magnitude of the additional predictive power over risk assessment models such as the Framingham risk score and the SCORE model may be marginal. (10) (11) (12) (13)
- Whether treatment decisions based partly on carotid imaging parameters would lead to better outcomes has not been studied.

In this thesis several of the challenges of carotid imaging in clinical practice will be further explored. A summary of the technical considerations on carotid ultrasound and MRI as well as a brief review of the evidence on carotid atherosclerosis and cardiovascular risk will precede the outline of the thesis.

CAROTID ULTRASOUND

Technical considerations

Ultrasound-based measurement of carotid intima-media thickness was first proposed in 1986. (14) It was established that sonographic quantification of the combined thickness of the lamina intima and media of the carotid artery closely approximates microscopic measurement of these arterial structures. Using a high resolution ultrasound device longitudinal images of the carotid artery are obtained. This technique allows visualization of two echogenic lines, separated by an anechoic space. It has been demonstrated that these lines represent the blood-intima and the media-adventitia interfaces. The thickness of this intima-media complex can be measured using computer aided semi-automated edge detection software. Several studies have shown that such an imaging protocol, utilizing high resolution ultrasound and computer aided analysis can produce highly reproducible results. (1) (15) (16) The technique of offline computer aided edge detection was utilized for all the study of this thesis

Ultrasound provides a cross-sectional image of the vessel wall. Figure 2a shows an example of such an image. Because atherosclerosis is often an asymmetrical process, it has been suggested that the ultrasound scans should be performed from several angles of insonation, thereby providing an estimation of the circumferential thickness. (17) Figure 2b is a schematic representation of the different angles of insonation. The parameter of intima-media thickness used in this thesis is an average of the thickness values from four angles at the site of the common carotid artery over a length of one centimeter.

Besides intima-media thickness measurement, ultrasound can also be used to detect the presence of atherosclerotic plaque. The definition of carotid plaque varies in literature. In this thesis the following definition is used: a focal thickening of the intima-media complex of greater than 1.5mm or 1.5 times greater than the surrounding IMT. (18) The plaques can be further classified to be 1) mild if only

the aforementioned criteria are met, 2) moderate when encroaching into the arterial lumen and 3) severe if the lesion causes stenosis. Plaque detection was performed under 4 angles of insonation at the site of the common and internal carotid artery and the carotid bulb.

Carotid ultrasound and cardiovascular risk

Accurate identification of patients that are at elevated risk remains challenging for physicians. This is reflected by the observation that in up to 40% of the incident cases of myocardial infarction, the event cannot be explained by the presence of cardiovascular risk factors. (19)

Several large population-based studies have demonstrated that carotid intima-media thickness and the presence of atherosclerotic plaques are independently associated with the occurrence of future cardiovascular events (table 1). It is thought that this association is partly explained by the strong correlation between the severity of carotid atherosclerosis and coronary artery disease. (20) (21) (22) (23) (24)It has been proposed that adding carotid ultrasound parameters to individual patient evaluation strategies may improve the predictive ability of physicians (25) (26) (27) Other studies suggest that adding carotid intima-media thickness values to traditional risk calculating algorithms only modestly improves the predictive strength of the algorithm. (10) (11) (12) (13) (28) The latter findings have led to scepticism about the added benefit of carotid ultrasound parameters. However, these studies were performed on a population-based level, and did not include carotid plaque detection in the analysis. Furthermore, there is evidence that ultrasound-based carotid parameters can improve cardiovascular risk prediction in specific populations, such as hyperlipidemic (29) and hypertensive patients. (30) In summary the potential incremental value of adding vascular parameters, including carotid plaques, to a patient's work-up has not yet been conclusively elucidated, especially in the setting of a secondary referral center as opposed to the general population. Evaluation of these carotid parameters in such a specific patient population (e.g. patients referred to a vascular outpatient department), where these imaging techniques are more readily available is an essential step in facilitating broader application of the technique.

Carotid ultrasound in pathophysiological studies

Another important application of carotid ultrasound is its use as a tool in pathophysiological studies. It is an easy, cost efficient and non-invasive method to assess the association of a broad range of established and potential cardiovascular risk factors to vessel wall thickness and by extension to coronary atherosclerosis and future cardiovascular events. In the last decade numerous articles have been published relating carotid IMT to a wide variety of risk factors such as hyperhomocysteinemia (31), lipoprotein(a) (32), inflammatory cytokines (33), ethnicity (34) (35), endothelial dysfunction (36) and many more. Carotid ultrasound has played a crucial role in advancing our knowledge about human atherosclerosis by allowing *in vivo* studies on the effects of these and other parameters in relatively limited patient numbers.

CAROTID MAGNETIC RESONANCE IMAGING

Technical considerations

Magnetic resonance imaging (MRI) protocols have been developed to measure carotid vessel wall geometry. As opposed to the ultrasound methodology discussed above, MRI images provide a fully circumferential view of the arterial wall. (Figure 3) MRI therefore may have an advantage over ultrasound in measurements of asymmetrical atherosclerotic arteries. The studies reported in this thesis were performed using a 3-Tesla MRI scanner. A dual inversion recovery (black-blood), spoiled segmented k-space fast gradient echo sequence with spectral selective fat suppression was used for the acquisition of the images. This technique has been shown to be highly reproducible and operator independent. (37)

On MRI images of arterial wall thickness the constituent layers cannot be differentiated, unlike ultrasound. Therefore the entire vessel wall thickness is quantified during analysis. These quantifications have also been validated against the golden standard of light microscopic histology, although less extensively than ultrasound based measurement of intima-media thickness. (38) In recent years MRI studies on carotid atherosclerosis have focussed more on plaque burden and composition than on wall thickness and plaque presence as we have done in this thesis. MRI characterization of carotid plaque is a

potentially promising technique for clinical risk prediction (39) but has not been used in the studies in this thesis.

Carotid MRI and cardiovascular risk

To date, carotid MRI has mainly been utilized as a surrogate end point in clinical trials. (40) (41) The baseline data in these trials seems to indicate that MRI-based vessel wall parameters are associated with cardiovascular risk factors. However, there are no published data on the association of carotid MRI abnormalities with coronary atherosclerosis or the occurrence of future cardiovascular events. Therefore the role of this technique in cardiovascular risk assessment is currently uncertain. Potentially carotid MRI could prove an important tool, especially in patients with advanced atherosclerosis. These patients often have asymmetrical thickening in the carotid artery that can be overlooked by ultrasound due to the cross-sectional nature of the images. The circumferential view provided by MRI may lead to a more reliable vascular assessment in these patients.

Carotid MRI in pathophysiological studies

There are very few data on the effect of cardiovascular risk factors on MRI-based values of vessel wall thickness. So far, the most important pathophysiological studies have focussed on MRI-based visualization of carotid plaque composition (39) (42) and outward arterial remodelling *in vivo*. (43) Moreover, the inclusion of the lamina adventitia in the MRI-based thickness measurements may facilitate future studies on the role of the outer vascular layer in the atherosclerotic process. Many data from human and animal models suggest an active role for the lamina adventitia. Being able to quantify its thickness through imaging would facilitate *in vivo* studies on this topic. Thus, the main current added value of carotid MRI over ultrasound is its ability to produce fully circumferential images of the entire vessel wall and include the lamina adventitia.

OUTLINE OF THIS THESIS

The main focus of the current thesis has been to explore the role of carotid imaging in clinical cardiovascular risk assessment and pathophysiological studies on atherosclerosis.

A major obstacle for many physicians in general hospitals, preventing the routine use of carotid ultrasound measurements in risk assessment strategies is a lack of the necessary infrastructure. In some clinical centers there are no experienced vascular sonographers. More often, the specialized equipment needed for measurement and analysis of carotid ultrasound scans is absent. To address this issue a study was performed to evaluate the level of accuracy that physicians themselves can achieve when carrying out carotid ultrasound scans during normal clinical practice. Such an office-based method would greatly broaden the scope of carotid ultrasound by allowing every physician to incorporate vascular parameters in their overall cardiovascular risk assessment strategy. The results of the study are reported in **chapter 2**.

Another factor currently limiting the use of carotid ultrasound for risk assessment is the uncertainty of its additive value over traditional risk factors especially in patients with established high cardiovascular risk. This issue is addressed in **chapter 3** by exploring the prevalence of carotid atherosclerosis in the setting of a presumed high-risk population, namely patients referred to a specialized vascular unit for cardiovascular risk management. It is in this setting that carotid ultrasound is readily available, however it can be hypothesized that this specific subset of the general population is at such an increased risk that the ultrasound findings lose their additional predictive power.

Whereas ultrasound-based measurement of carotid wall thickness has been widely validated in large population based studies with clinical outcomes, the potential role of carotid MRI in cardiovascular risk stratification is still largely unknown. In **chapter 4**, the clinical relevance of carotid MRI was explored by comparing MRI measurement of arterial lumen diameter and vessel wall thickness to ultrasound-based measurement. If MRI-based values are equal to ultrasound-based measurements it may be justified to assert that clinical outcome data from ultrasound studies can be extrapolated to MRI-based studies.

It has been demonstrated that ultrasound and MRI do not measure the same part of the arterial wall. Ultrasound quantifies the thickness of the lamina intima and media whereas MRI measures the thickness of the entire vessel wall. This should be taken into account when evaluating pathophysiological studies using these different techniques. The information obtained from ultrasound and MRI may be complementary. In **chapter 5** the different associations of carotid ultrasound and MRI to cardiovascular risk factors is described. In addition, a

separate vascular parameter, indicative of adventitia-thickness is proposed using the combination of ultrasound- and MRI-based carotid thickness values.

The development of a reliable method of measuring carotid intima-media thickness has contributed significantly to our understanding of the atherosclerotic process in humans. It has facilitated pathophysiological studies on atherosclerosis *in vivo* in a way that was not possible before. In doing so it has taken a central role in cardiovascular research between the preclinical *in vitro* and animal models and the large epidemiological studies using clinical cardiovascular end-points. **Chapter 6** of this thesis illustrates this point. A literature review about the role of inflammation on atherosclerosis in the setting of diabetes mellitus is presented. The section in the review discussing carotid imaging studies bridges the sections on preclinical studies and clinical end-point studies.

Chapter 7 reports on the findings of a study comparing the effect of cardiovascular risk factors on patients of different ethnicity. While this study represents another example of how carotid ultrasound parameters can be utilized in pathophysiological studies, it also stresses the need for ongoing research with these imaging techniques, particularly in populations of different ethnic origin.

The final chapter **(chapter 8)** of the thesis reviews the literature on carotid ultrasound and risk assessment from the internist's perspective. It provides practical suggestions and recommendations on its use in day-to-day clinical practice. A brief explanation of the key technical aspects and available data from the literature will hopefully help clinicians to decide how and why carotid ultrasound may be of value and in which patients it may be relevant.

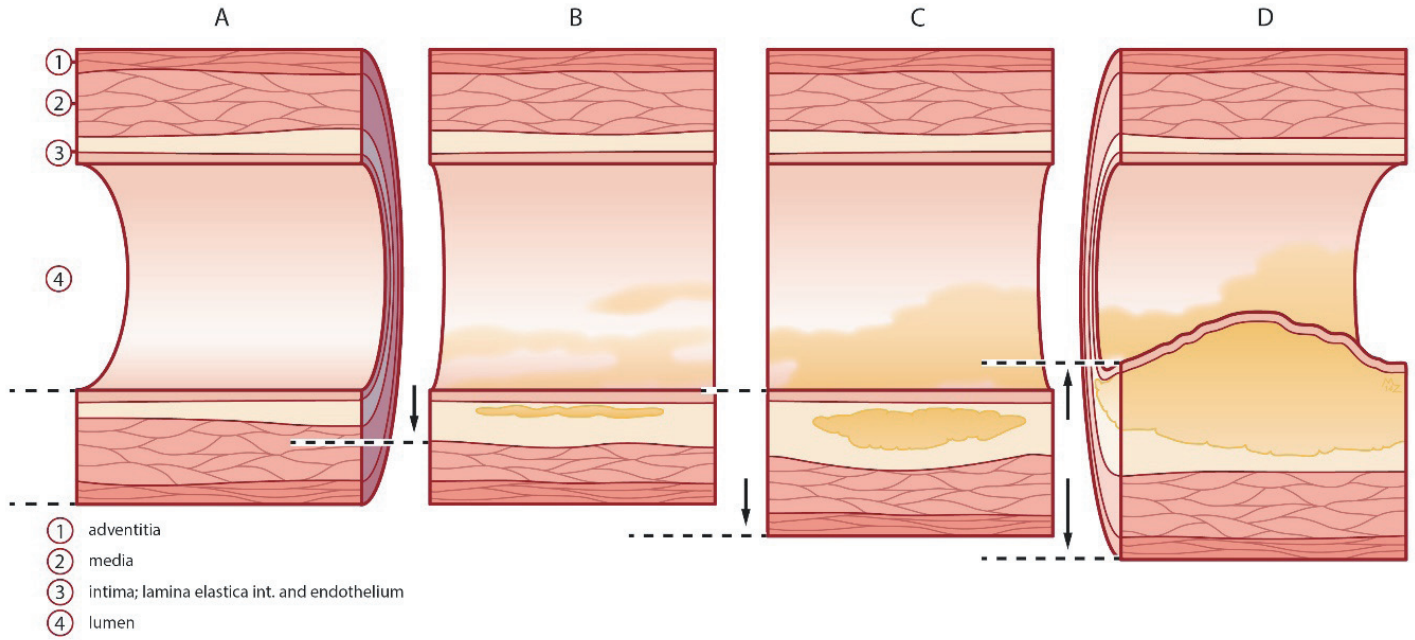

Figure 1 | Schematic representation of the progression of arterial atherosclerosis over a period of decades. Different vascular tests are relavant in the various stages:
A: flow mediated dilatation & pulse wave analyses are aimed at evaluating arterial dysfunction in early stages of disease
B/**C**: both carotid ultrasound and MRI imaging are able to visualize morphological changes inside the arterial wall, before plaques encroach the lumen
D: invasive intra-arterial contrast and pressure gradient measurements are only useful in advanced disease

FMD: flow mediated dilatation; PWV: pulse wave velocity; PWA: pulse wave analysis; IMT: intima-media thickness; CT: computed tomography; MRI: magnetic resonance imaging

Table 1

Author	n	Follow-up	Carotid parameter	Outcome parameter	Correction for traditional risk factors	HR [95% CI;p-value]]
Salonen et al 1991 (44)	1288	1m-2.5y	Bilateral CCA & BIF	Coronary artery event	none	6.71 [1.33-33.91;p<0.01] stenotic plaque 4.51 [1.51-11.47;p<0.01] minor plaque 2.17 [0.70-6.76;p=NS] increased cIMT
Belcaro et al 1996 (45)	2322	6y	Bilateral CCA, BIF, ICA	Cardiovascular event or death	none	*No formal HR; incident event distribution:* 0% events with normal ultrasound 5.5 % with increased cIMT (p<0.05) 18.4% with minor plaque (p<0.025) 42% with stenotic plaque (p<0.025)
Chambless et al 1997 (46)	14054	10.2y	Bilateral CCA, BIF, ICA	Cardiovascular events or death	Age, gender	5.07 [3.08-8.36;p<0.01] MI in women 8.54 [3.52-20.74;p<0.01] stroke in women 1.87 [1.28-2.69];p<0.01] MI in men 3.62 [1.45-9.15;p<0.01] stroke in men
Bots et al 1997 (47)	7983	2.7y	CCA	Cardiovascular events or death	Age, gender, BMI, smoking BP, lipids, diabetes, prior cardiovascular event	1.38[1.21-1.58;p<0.01] MI 2.23 [1.48-3.36;p<0.01] stroke
O'Leary et al 1999 (48)	5858	6.2y	CCA, ICA	MI, stroke	Age, gender, BP, presence of atrial fibrillation, smoking, diabetes	3.15[2.19-4.52;p<0.01] MI or stroke
Kitamura et al 2004 (49)	1289	4.5y	CCA, BIF, ICA	stroke	Age, BP, BMI	4.8 [1.9-12.0;p<0.01] stroke
Rosvall et al 2005 (50) (51)	5163	7y	CCA	MI, stroke	Age, gender, physical activity, smoking, BP, diabetes, lipids, waist circumference	1.23 [1.07-1.41;p<0.01] MI 1.21 [1.02-1.44;p<0.01] stroke
Lorentz et al 2006 (52)	5056	4.2y	CCA, BIF, ICA	MI, stroke	Age, gender, BMI, BP, lipids, smoking, diabetes	1.85 [1.09-3.15;P<0.01] combined stroke, MI or death
Polak et al 2011 (53)	2965	7.2y	CCA, BIF, ICA	Cardiovascular events or death	Age, gender, BP, lipids, smoking	1.21 [1.13-1.29;p<0.01] per 1SD increase in cIMT 1.92 [1.49-2.47;p<0.01] plaque
Polak et al 2013 (54)	6562	7.8y	CCA, ICA	Cardiovascular events or death	Age, gender, BP, lipids, smoking	1.45 [1.20-1.76;p<0.01] minor plaque 1.65 [1.34-2.03;p<0.01] stenotic plaque 1.33 [1.18-1.49;p<0.01] increased cIMT

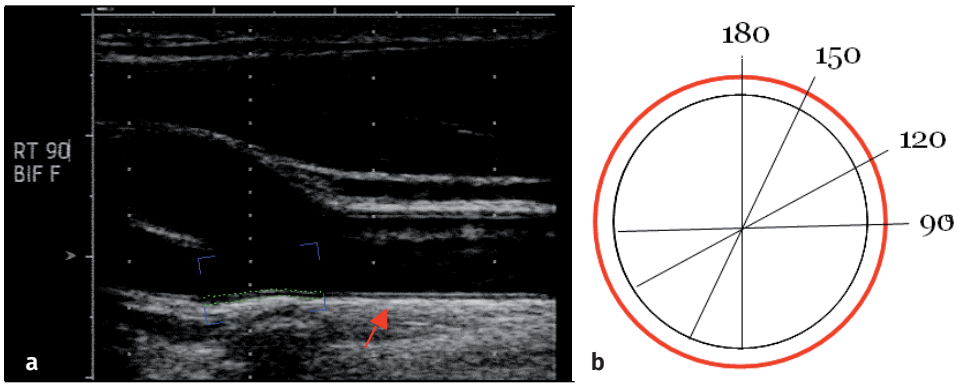

Figure 2a, 2b | Representative example of a longitudinal, cross-sectional carotid ultrasound image of a 62-year old male patient (**a**). Red arrow indicates the intima-media complex in the common carotid artery. The blue box shows an example of a region of interest for computer aided edge detection (green dotted lines). (**b**) Is a schematic representation of a transverse cross-section through the right common carotid artery, illustrating the four angles of insonation at which the carotid artery is examined.

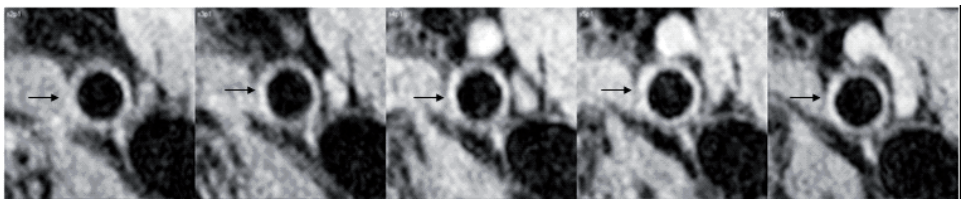

Figure 3 | Representative example of 3-Tesla MRI transverse cross-sectional image of the carotid artery of a 62-year old male subject. Black arrows indicate the carotid artery. Five contiguous transverse slices of 2mm, covering the most distal 1cm of the common carotid artery.

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CHAPTER 2

Accuracy of Carotid Plaque Detection and Intima-Media Thickness Measurement with Ultrasonography in Routine Clinical Practice

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ABSTRACT

Background

Current guidelines in cardiovascular disease prevention advocate the use of carotid ultrasound measurements for risk stratification. Carotid abnormalities (plaques or increased intima-media thickness (IMT)) are associated with high risk of coronary and peripheral artery disease. An office-based measurement by clinicians would considerably broaden the clinical applicability of carotid ultrasound. In the present study we have assessed the accuracy of ultrasound detection of carotid plaques and intima-media thickness by trained internists in a routine outpatient setting.

Methods & Results

Carotid ultrasound was performed in 112 vascular outpatients by internists, after a six week training period. The internists' results were independently compared to the reference standard, consisting of carotid ultrasound performed in a specialized vascular laboratory. Sensitivity and specificity were calculated for plaque detection and IMT determination. The mean time required to perform the scans on the outpatient department was 7.3 minutes (range 4.5 to 16.7 minutes). A high level of accuracy for detecting plaques (sensitivity 78.5%; specificity 93.6%) was achieved. Identifying abnormal IMT had lower sensitivity but adequate specificity of 46.7% and 87.6%, respectively.

Conclusions

In conclusion, our findings demonstrate that clinicians can be trained well enough in six weeks to accurately and efficiently detect carotid plaques in an outpatient setting. As IMT abnormalities were less accurately detected, this may require a follow-up IMT, for instance in a specialized vascular laboratory. Incorporating vascular imaging results in treatment decisions may improve clinicians' ability to provide individualized patient care.

INTRODUCTION

Cardiovascular disease (CVD) is a major cause of morbidity and mortality. Identifying patients at high risk for future cardiovascular events and peripheral artery disease is an important challenge for clinicians, as the presence of traditional risk factors predicts only 60-65% of incident events [1]. It has repeatedly been established in longitudinal studies that increased intima-media thickness (IMT), as well as the presence of atherosclerotic plaques in the carotid artery, is associated with increased risk of future myocardial infarction and stroke [2-6]. Reported increases in relative risk for cardiovascular events vary between 1.2 and 3.6, in subjects with abnormal compared with normal IMT [3,6,7]. Furthermore it has been shown that IMT is independently associated with a host of cardiovascular risk factors [8,9], and with atherosclerotic changes in other vascular beds, including the coronary and femoral arteries [10-14]. The presence of plaque in the carotid artery increases cardiovascular risk by approximately three- to six-fold compared to plaque-free controls, depending on plaque severity [2,6,8,13]. Therefore carotid IMT measurement and plaque detection has been proposed as a means to improve risk stratification by identifying patients that may require more aggressive preventive interventions [2,7,15,16]. The European Society of Cardiology advises the use of carotid plaque detection and IMT measurement in risk assessment in hypertensive patients [17] and the American Society of Echocardiography as well as the American Heart Association concluded that carefully performed IMT measurement and plaque detection may add incremental information over traditional risk factors in selected patients [18,19]. However the ultrasound protocols of a vascular laboratory are time consuming, generally taking around 40-45 minutes. Furthermore, most general hospitals lack the specialized equipment needed for quantitative analysis of the scans. Recently, it has been demonstrated that IMT measured in normal clinical practice by trained sonographers was sufficiently reproducible with intra- and interobserver variability of 4.2% and 7.3% respectively [20]. Importantly, in this study the measurements were not compared with a reference standard which is a critical step in the evaluation of a diagnostic test [21].

Whether carotid ultrasound can be adequately performed by internists and incorporated in a normal clinical setting has not been studied extensively. Office-based measurement by internists would considerably broaden the clinical

applicability of carotid IMT and plaque detection. Results of a multicenter study showed that non-sonographer clinicians (doctors, nurses, etc) can accurately measure carotid IMT and plaques using a handheld ultrasound device [22]. Moreover, ultrasound screening for carotid plaque in an office setting has been shown to potentially alter treatment plans [23,24]. Therefore, the aim of the current study was to assess the level of accuracy that can be achieved by trained internists in identifying atherosclerotic plaque and increased IMT in a routine vascular outpatient setting.

MATERIALS & METHODS

Patients

Patients were recruited from the vascular outpatient department (OPD) at the Leiden University Medical Center. Oral and written informed consent was obtained from all patients; the study protocol was approved by the Institutional Review Board. To ensure the inclusion of a broad clinical spectrum, both patients with a history of CVD (prior myocardial infarction or stroke, coronary revascularization or known peripheral vascular disease) as well as subjects free of clinically manifest CVD were recruited. There were no predefined exclusion criteria. The presence of hypertension was defined as an average of 3 measurements over 140/90 mmHg or the use of anti-hypertensive medication. Subjects were designated as having a positive family history if at least one first degree relative had a cardiovascular event or revascularization intervention before the age of 60 years. Smoking habits were recorded, and patients who had not smoked in the last 12 months were designated as ex-smokers.

Study design

Two trained internists performed the carotid ultrasound scans on the OPD (see below for ultrasound protocol). After the scans on the OPD, carotid ultrasound examination was performed in a specialized vascular laboratory within two weeks, by a single blinded sonographer. The ultrasound on the vascular OPD always preceded the scans in the vascular laboratory. Relevant medical history was recorded and physical examination and blood sampling were performed only after the IMT scans to minimize pre-scan bias on the part of the

sonographers. Quantitative analysis of IMT was performed on the scans made in the vascular laboratory as described below. As per design, the OPD scans were not quantitatively analyzed but rather evaluated in real-time as described below. However in a look-back at the false positive and false negative OPD scans these were analyzed in a quantitative manner. This allowed for a secondary analysis of the reasons for false adjudication on the OPD.

Ultrasound training

Two internists specialized in vascular medicine were trained over a 6 week period of by a certified sonographer with specific experience in vascular ultrasonography. On average, approximately 4 hours per week were spent learning and practicing the ultrasound protocol, leading to a total of 24 hours of training time in patients. In addition the internists were given a set of 25 frozen images of previously performed scans to become visually skilled in distinguishing normal IMT thickness from increased thickness, as well as plaque recognition. Initially the scans were performed on young healthy volunteers to get accustomed to adhering to the protocol. When the instructor was satisfied about the ability of the physicians to adequately visualize and recognize the vascular wall and anatomical structures such as the carotid bifurcation, older patients were included in the practice sample. Vascular abnormalities such as plaques and increased IMT were more prevalent in the older patients. The internists performed all the practice scans under supervision of the instructor. The ultrasound protocol was practiced 10 times in healthy volunteers and 20 times on older patients.

Ultrasound protocol on the outpatient department

On the OPD all scans were performed using an Aloka SSD-1400 high-resolution ultrasound device with a 7.5MHz linear transducer. Subjects were examined in prone position with the head tilted to the side counter lateral to the site of examination. The left and right carotids were both scanned. First, a transverse scan was performed for orientation, starting at the clavicle and moving cranially up to the mandible, hereby locating the height of the carotid bifurcation. Subsequently, longitudinal images were obtained. This technique allows visualization of two echogenic lines, separated by an anechoic space. It has previously been established that these lines indicate the blood-intima and the media-adventitia interfaces, and that the distance between the lines represents

a histologically reliable measure for the thickness of the lamina intima and media [25]. The caudal tip of the flow divider (i.e. the wall structures between the internal and external carotid arteries) was used as the anatomical landmark to localize the most distal 1cm of the common carotid artery. The artery was visualized at four angles (anterior, two antero-lateral projections and lateral). The internists estimated the IMT of the common carotid artery in real time by placing electronic callipers around the intima-media complex. Subsequently, the presence of plaques was assessed both in the common carotid artery and the bifurcation, defined as focal thickening of >1.5mm or >1.5 times the surrounding IMT. The presence, at any angle, of either a maximum IMT of >1mm or atherosclerotic plaque resulted in a categorization of the scan as abnormal. If no plaques were detected and the maximum IMT was <1mm at all angles, the scan was considered normal. Designating a scan 'normal' or 'abnormal' was done by the internists in writing, immediately after performing the scan. On the scan result-forms the internists could illustrate their findings on a schematic diagram of the carotid artery (pre-printed on the form) and add comments. It has been recently shown that a similar ultrasound protocol, limited to plaque detection and IMT measurement in the common carotid artery, produces the same results as more comprehensive protocols that include quantification of IMT in the carotid bifurcation and internal carotid artery [26]. All scans were recorded on sVHS videotapes for independent analysis of the outpatient scans at a later point in time. In addition, in order to identify the factors leading to discrepancies between OPD and vascular laboratory findings, all false positive and false negative scans were retrospectively reviewed by a blinded observer, who was not involved with the scanning of the patients. Practical feasibility was determined subjectively by evaluating the ability of the internists to incorporate the measurement in their outpatient work and quantifying the time spent performing the scans.

Ultrasound protocol in the vascular laboratory

In the vascular laboratory the identical ultrasound protocol was performed using an Acuson Sequoia 512 (Siemens Medical AG, Munich, Germany) high-resolution ultrasound machine with an 8LS (8MHz) linear transducer. This transducer frequency was 0.5MHz higher than the one used on the OPD but this minor difference resulted in similar screen resolution and image quality.

One sonographer (4 years of experience) who has been certified to implement this ultrasound protocol for clinical trials performed and analysed the scans. The within-subject reproducibility achieved by the sonographer, performing the same scanning protocol in a previous trial was >95% [27]. At the time of scan and analysis the observer was blinded for both the clinical data of the patient and the result of the OPD scan. The scans from the vascular laboratory were quantitatively analysed off-line with computer-aided automatic boundary detection where possible and manual adjustment where necessary, using the ASM II software package version 1.1364 (Gustavson, Chalmers, Sweden). The use of automatic boundary detection has been shown to be accurate and is advised in the recent Mannheim consensus on IMT measurement [28]. The overall gain settings during scanning were kept at 0dB when possible. The sonographer was free to adjust the gain levels if necessary, but within the limits of -7dB to 7 dB to avoid possible influences of extreme gain settings on IMT quantification. Mean IMT was defined as the average of the mean values of the far and near walls of the common carotid artery at all four angles. Mean maximum IMT was calculated from the mean of the maximum values at these sites. All quantifications were performed in cardiac diastole.

The definition of plaque was identical to the one used on the OPD (focal thickening of >1.5mm thickness or >1.5 times the surrounding IMT). In the vascular laboratory plaques were further classified as described earlier [6,28]. They were classified to be mild if only the aforementioned criteria were met. Moderate plaques were defined as lesions encroaching into the lumen. If the lesion caused stenosis the plaque was classified as severe.

Statistical analysis

All data were analyzed using the SPSS 12.0 software package. Data are expressed in means [95% confidence intervals] unless indicated otherwise. Differences between groups were analyzed with a student T-test, categorical data were compared using a Chi-squared test. P-values of <0.05 were considered statistically significant. Calculation of sensitivity, specificity, positive and negative predictive values as well as likelihood ratios for the internists to identify plaque and abnormal IMT was done as described earlier [21,29]. The retrospective analyses of the false negative and false positive OPD scans were compared to the reference standard by a Bland-Altman plot [30].

RESULTS

A total of 112 patients were included in the study. The clinical and demographic characteristics of the study population are summarized in table 1. Mean age was 53.8 yrs [51.6-56.6], 11.6% of patients had type 2 diabetes mellitus and 21.4% had a history of CVD. There was a high prevalence of hypertension and hypercholesterolemia of 49.0% and 67.6% respectively. Of our patients 52.5% was either a current smoker or had smoked in the past, and 30.4% had a family history of CVD. Mean IMT in our sample was 0.743 [0.719-0.768] and the mean of the maximum IMT was 0.847 [0.817-0.877], quantified in the vascular laboratory. Of the 112 patients 65 (58.0%) had atherosclerotic plaque in the carotid artery. Of these plaques 50% were classified in the vascular laboratory as severe, 26% as moderate and 24% as mild.

Feasibility

On average the scans on the outpatient department took 7.3 minutes to perform (range 4.5 to 16.7 minutes). This was the effective scanning time and did not include the preparation time etc. Typically, if a patient was already in supine position for blood pressure measurement or physical examination the ultrasound could be easily incorporated. In cases where the patients had to lie down specifically for the ultrasound the internists subjectively found including the scan more intrusive in their normal routine. In a half-day session on the OPD, during which time a total of approximately 15 patients were seen, the clinicians were able to include an average of 2-3 patients.

Accuracy of plaque detection

Data on plaque detection are summarized in table 2. Of the 65 plaques identified in the vascular laboratory, 51 were recognized by the internists on the outpatient and 14 were not seen, for a sensitivity of 78.5% [69.9%-86.1%]. In 3 patients the internists on the outpatient indicated the presence of a plaque where no plaques were seen in the vascular laboratory for a specificity of 93.6% [89.1%-98.1%]. The positive predictive value for plaque detection was 83.1% and the negative predictive value was 75.9%. The likelihood ratios of positive and negative tests on the OPD were 12.2 and 0.23, respectively for plaque detection.

Table 1 | Patient characteristics. CVD: cardiovascular disease; BMI: body mass index; IMT: intima-media thickness.

Variable	Total group (n=112)
Age (yrs)	53.8 [51.6-56.6]
% Diabetes	11.6%
% CVD	21.4%
% Hypertension	49.0%
% Hypercholesterolemia	67.6%
% Family History of CVD	30.4%
Smoking	
% Current smokers	21.8%
% Ex-smokers	30.7%
BMI (kg/m^2)	27.3 [26.4]
Systolic BP (mmHg)	133.1 [129.0-137.3]
Diastolic BP (mmHg)	81.9 [79.8-84.1]
Anti-platelet therapy	22.0%
Statin therapy	54.3%
Antihypertensive therapy	46.7%
Mean IMT (mm)	0.743 [0.719-0.768]
Mean max IMT (mm)	0.847 [0.817-0.877]
Plaques	65 (58.0%)
Mild	23.7%
Moderate	26.2%
Severe	50.1%

CVD: cardiovascular disease; BMI: body mass index; IMT: intima-media thickness

Table 2 | Comparison of plaque findings on the outpatient department and the vascular laboratory.

	Vascular lab plaque +	Vascular lab plaque -	total
OPD plaque +	51	3	54
OPD plaque -	14	44	58
Total	65	47	112

OPD: outpatient department. Sensitivity: 78.5% [95% CI: 69.9%-86.1%]. Specificity: 93.6% [95% CI: 89.1%-98.1%]. Plaque prevalence: of 58.0%. Positive predictive value: 94.4%. Negative predictive value: 75.9%. Likelihood ratio of a positive test: 12.2. Likelihood ratio of a negative test: 0.23

Accuracy of IMT measurement

Data regarding the measurement of IMT are summarized in table 3. Sensitivity of the internists for recognizing IMT ≥ 1mm was 46.7% [37.5%-55.9%], with a specificity of 87.6% [81.5%-93.7%]. The positive and the negative predictive values were 36.8% and 91.4% respectively. Likelihood ratios for positive and negative tests for the IMT estimation on the OPD were found to be 3.7 and 0.69, respectively.

Table 3 | Comparison of IMT findings on the outpatient department and the vascular laboratory.

	Vascular lab IMT ≥ 1mm	Vascular lab IMT < 1mm	total
OPD IMT ≥ 1mm	7	12	19
OPD IMT < 1mm	8	85	93
Total	15	97	112

OPD: outpatient department. Sensitivity: 46.7% [95% CI: 37.5%-55.9%]; Specificity: 87.6% [95% CI: 81.5%-93.7%]; Prevalence of increased IMT: 13.4%; Positive predictive value: 36.8%; Negative predictive value: 91.4%; Likelihood ratio of a positive test: 3.7; Likelihood ratio of a negative test: 0.69.

Look back at false negative and false positive findings

The distribution of false negative scans was constant over the study period and equal between the two internists. In the case of plaque detection there were two main factors that led to incorrect findings on the OPD as compared to the vascular laboratory. First, in several instances the carotid bifurcation was cranially located making it difficult for the clinicians to visualize it due to obstruction of the ultrasound transducer by the patient's mandible. In these cases, the sonographer in the vascular laboratory was better able to detect plaques by helping patients to hyperextend their neck using foam rolls or pillows. Second, due to the curvilinear anatomy of the carotid bifurcation the clinicians were not always able to adequately visualize the intima-media complex. The sonographer, having no time restraints was able to meticulously examine the curved surface.

Regarding IMT, the 20 false negative and false positive cases had a mean maximum IMT of 0.970mm [range: 0.723mm-1.380mm]. Retrospective analysis revealed that, in all cases the internists adequately visualized the intima-media complex and the image quality was sufficient to allow off-line analysis. Quantitative analysis of the OPD scans resulted in identical values as were found in the vascular laboratory (mean difference 0.001mm; p=0.939), illustrated in the Bland & Altman plot in figure 1. The main factor leading to incorrect designation

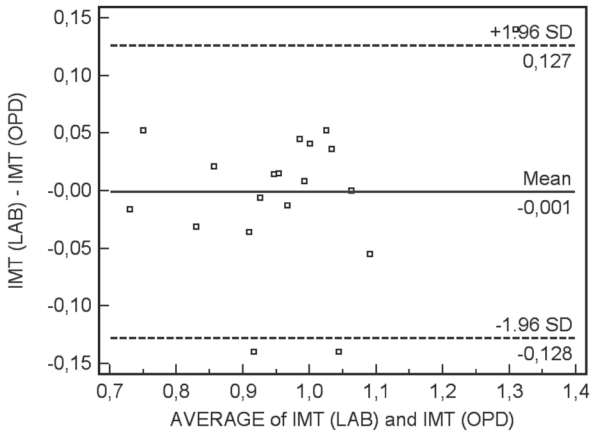

Figure 1 | Bland & Altman plot of retrospective quantitative IMT measurements of false negative and false positives.

IMT: intima-media thickness; LAB: vascular laboratory; OPD: outpatient department.

of IMT as being normal or abnormal by the internists was lack of precision of the manual calliper placement.

DISCUSSION

This study has assessed the accuracy of plaque detection and IMT measurement by internists in a routine outpatient setting. The most important finding is that internists can achieve acceptable sensitivity and high specificity for carotid plaque detection after a six-week training period, resulting in high positive and negative predictive values. We believe that the external validity of our data is strengthened by the fact that the 58% overall plaque prevalence in our patients was comparable to previous reports: Rosvall et al. [5] found 43% carotid plaques in 5163 subjects with and average age of 58 years and van der Meer et al. [31] reported 58% plaques in 6389 subjects of >55 years of age. Both these study samples were recruited from the general population and showed similar patterns of co-morbidity, although our patients were slightly younger. Our data show that 20% of plaques were not identified by the internists on the outpatient department. The look back at these false negatives revealed that it was due

to high anatomical location and curvilinear anatomy of the carotid bifurcation, making it difficult to visualize. In a normal clinical situation these patients, in whom the carotid bifurcation was not optimally visualized, could still be referred to a specialized vascular laboratory or radiology department. The high specificity of outpatient plaque detection demonstrates that when carotid abnormalities are seen using this simple test it represents true presence of plaque.

The clinical implications of our study are underlined by the results from other studies relating the presence of carotid plaque to future cardiovascular events [2,6,8]. In particular, Griffin et al. in 2002 [32] demonstrated that with ultrasound plaque detection and characterization high-risk patients could be identified, independent of other cardiovascular risk factors. Our data strongly suggest that carotid plaque detection during routine clinical practice may be a useful tool in identifying high risk patients for CVD and peripheral artery disease.

In our study it was more difficult for internists to accurately identify increased IMT. The internists could only achieve 47% sensitivity with respect to identifying abnormal IMT but a high specificity (88%) was achieved. In our look back at the reasons for incorrect estimation of IMT we found that it was mainly due to inaccurate placement of the electronic callipers. Retrospective quantitative analysis of the twenty discrepant OPD and vascular laboratory scans showed excellent agreement suggesting that inaccurate manual measurement, and not insufficient image quality, caused the false results. Based on our findings we argue that manual normal IMT measurement in clinical practice is not suitable to exclude additional cardiovascular risk and it should be followed by an IMT performed in a specialized vascular laboratory. Moreover, using a dichotomous cut-off value for IMT is a relatively crude method. Although IMT values > 1mm are associated with elevated risk it would be more accurate to statistically correct the continuous value for IMT in individual patients to age, gender and ethnicity. Such calculations and corrections however are time consuming and may not be feasible in an office-based approach. Whether the use of more advanced ultrasound equipment, including real-time IMT quantification and automatic correction for these factors may facilitate the use of IMT values for risk assessment in an outpatient setting remains to be prospectively studied.

Our study had limitations. It is possible that longer and more intense training would have improved the results achieved by the internists. We achieved a

high accuracy for plaque detection using training program that can be easily implemented. This is reflected by the constant distribution of false positive and false negative findings on the outpatient department during the inclusion period, indicating the absence of an additional learning curve during the study. Second, different ultrasound machines were used for the outpatient measurements and the scans in the vascular laboratory. The minor difference of 0.5 MHz in transducer frequency however did not lead to differences in image quality, which is supported by the high agreement in the retrospective quantitative analysis.

In conclusion, our findings demonstrate that internists can accurately detect carotid plaques using ultrasound, after a six-week training period. The limited amount of time required to perform the scans allowed the internists to incorporate the measurements in their normal clinical routine. Adequate recognition of increased IMT is more difficult and may require a specialized vascular laboratory or alternatively ultrasound equipment dedicated to IMT quantification.

Learning Points

- Internists are able to identify carotid plaques by ultrasound in routine clinical practice with high precision after a six-week training period
- In-office plaque detection may aide internists to individualize preventive cardiovascular strategies per patient
- Measurement of carotid intima-media thickness by internists is not recommended because it lacks accuracy when compared to measurement in a specialized vascular laboratory
- Errors in measuring intima-media thickness in an out-patient setting are mainly due to insufficient precision of manual estimations of thickness

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CHAPTER 3

Associations Between Cardiovascular Risk Score and Ultrasound-Determined Subclinical Carotid Atherosclerosis

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ABSTRACT

Aims

Current guidelines in cardiovascular disease prevention advocate the use of carotid ultrasound measurements in risk assessment. The additional predictive power of adding carotid ultrasound parameters to traditional risk assessment tools is thought to be limited, especially in high risk patients. In the present study we aimed to evaluate the prevalence of subclinical carotid atherosclerosis in presumed high-risk vascular outpatients in relation to calculated cardiovascular risk scores.

Methods and Results

Carotid ultrasound was performed in 112 vascular outpatients to measure intima-media thickness and detect plaques. Plaques were observed in 52% of primary prevention patients and 88% of secondary prevention patients. In a low risk statin-naïve primary prevention subgroup (mean calculated risk score: 3.3%/10years) 48% of the patients were found to have plaques present. Intima-media thickness was explained (r^2=0.575; p<0.001) by traditional risk factors in a multiple regression model. Plaque presence only related (r^2=0.372; p<0.001) to hypertension (β=0.347; p=0.036), a positive family history for cardiovascular disease (β=0.396; p=0.016) and fasting glucose levels (β=0.158; p=0.047). Adding carotid ultrasound parameters to risk assessment strategies would result in an additional 23% (plaque presence alone) to 36% (plaque and/or increased intima-media thickness) of our primary prevention patients being reclassified as higher risk patients.

Conclusion

Our findings show that subclinical carotid atherosclerosis is highly prevalent in a vascular clinic setting and is frequently observed in patients with low calculated risk scores. Carotid ultrasound can potentially improve risk assessment strategies in these patients.

INTRODUCTION

Calculation of absolute risk of future cardiovascular events is an important tool in cardiovascular disease prevention strategies. The algorithms for such calculations are based on data from large epidemiological studies, such as the Framingham study (55), the Münster heart study (56) and the SCORE project (57) However, up to 40% of incident myocardial infarctions are not explained by the presence of traditional risk factors on which risk calculations are based, illustrating that significant limitations exist in predicting cardiovascular events. (58) It remains difficult to pinpoint individual patients that may require early or intensive pharmacological interventions, especially in an intermediate risk group.

Ultrasound determined carotid intima-media thickness (IMT) and the presence of carotid plaques are independently associated with incident cardiovascular events, even after adjustment for traditional cardiovascular risk factors (59) (60) (7) (8) (63) (64). The European Society of Cardiology (65), The European Society of Hypertension (66) and the American Heart Association (67) have added recommendations for the use of carotid ultrasound in cardiovascular risk stratification to their guidelines. Large meta-analyses have recently questioned the additional value of carotid ultrasound. There does appear to be an improvement in the predictive potential of risk assessment models containing carotid ultrasound findings but the benefit may be so marginal that it is not clinically relevant, (68) (69) . Although there is no broad consensus on whether to use carotid ultrasound most authors agree that the potential target population is the intermediate risk group. Patients who are already at high cardiovascular risk should receive an intensive risk management strategy regardless of any findings at carotid examination therefore rendering the investigation unnecessary. In the Netherlands a substantial part of the initial cardiovascular risk management is performed by primary healthcare providers, Access to carotid artery imaging is less readily available in this setting than it is in a dedicated vascular unit of a referral hospital. However, patients referred to a vascular clinic have different characteristics compared to a general population. The prevalence of carotid atherosclerosis and correlation to calculated risk in such a specific, presumably high-risk population is not known. In the present study we therefore investigated the prevalence of subclinical carotid atherosclerosis (SCA) defined as increased

IMT and/or plaque presence in vascular outpatients and related SCA to a traditional risk factor scoring system, with a secondary prevention population as reference.

PATIENTS AND METHODS

Patients

Patients were recruited from the vascular outpatient department of our hospital. The population included patients newly referred by their primary healthcare providers as well as patients already under evaluation. Oral and written informed consent was obtained from all patients; the study protocol was approved by the Institutional Review Board. The primary focus of the inclusion were subjects free of clinically manifest cardiovascular disease (CVD), defined as prior myocardial infarction or stroke, coronary revascularization or known peripheral vascular disease. As a reference, a group of patients with a history of CVD were included. There were no predefined exclusion criteria to ensure a representative cross-section of patients. The presence of hypertension was defined as an average of 3 measurements over 140/90 mmHg or the use of anti-hypertensive medication. Subjects were designated as having a positive family history if at least one first degree relative had a cardiovascular event or revascularization intervention before the age of 60 years. Smoking habits were recorded, and patients who had not smoked in the last 12 months were designated as ex-smokers. Absolute risk of a fatal cardiovascular event over 10-years was calculated using the SCORE algorithms as described earlier (57), and values of more than 5% per 10-years risk of a fatal cardiovascular event were considered treatment indications, as recommended by the European Society of Cardiology guidelines (65).

Study design

Carotid ultrasound measurements were performed in a specialized vascular laboratory. Distribution of plaque prevalence and IMT values was assessed in statin-naïve and statin-treated primary prevention patients as well as secondary prevention patients. Subsequently associations of carotid parameters and traditional risk factors were assessed. In the untreated primary prevention group the number of patients with a treatment indication based on calculated

risk scores was compared to the number of patients with potential treatment indications if risk scores were to be augmented by vascular imaging. The presence of moderate to severe carotid plaques and/or increased IMT was arbitrarily used as potential treatment indications for this analysis.

Ultrasound protocol

Carotid ultrasound scans were performed using an Acuson Sequoia 512 (Siemens Medical AG, Munich, Germany) high-resolution ultrasound machine with an 8LS (8MHz) linear transducer. One sonographer (4 years of experience) who has been certified to implement the ultrasound protocol for clinical trials performed and analysed the scans. At the time of scan and analysis the sonographer was blinded for the clinical data of the patient. Subjects were examined in prone position with the head tilted to the side counter lateral to the site of examination. The left and right carotids were both scanned. First, a transverse scan was performed for orientation, starting at the clavicle and moving cranially up to the mandible, hereby locating the height of the carotid bifurcation. Subsequently, longitudinal images were obtained. This technique allows visualization of two echogenic lines, separated by an anechoic space. It has previously been established that these lines indicate the blood-intima and the media-adventitia interfaces, and that the distance between the lines represents a histologically reliable measure for the thickness of the lamina intima and media (70). The caudal tip of the flow divider (i.e. the wall structures between the internal and external carotid arteries) was used as the anatomical landmark to localize the most distal 1cm of the common carotid artery. The artery was visualized at four angles (anterior, two antero-lateral projections and lateral). Subsequently, the carotid bifurcation and internal carotid artery were visualized and assessed for the presence of plaque. The overall gain settings during scanning were kept at 0dB when possible. The sonographer was free to adjust the gain levels if necessary, but within the limits of -7dB to 7 dB to avoid possible influences of extreme gain settings on subsequent IMT quantification. It has been recently shown that a similar ultrasound protocol, limited to plaque detection and IMT measurement in the common carotid artery, produces the same results as more comprehensive protocols that include quantification of IMT in the carotid bifurcation and internal carotid artery (17). The scans were recorded on sVHS video tapes and quantitatively analysed off-line with computer-aided automatic boundary detection where possible and

manual adjustment where necessary, using the ASM II software package version 1.1364 (Gustavson, Chalmers, Sweden). IMT was defined as the average of the maximum values of the far and near walls of the left and right common carotid artery at all four angles. Plaques were defined as focal thickening of >1.5mm or >1.5 times the surrounding IMT. If present the plaques were classified to be mild if only the aforementioned criteria were met. Moderate plaques were defined as lesions encroaching into the lumen. If the lesion caused stenosis the plaque was classified as severe. Subclinical carotid atherosclerosis (SCA) was defined as presence of moderate to severe plaque and/or an IMT of ≥0.9mm, considered the clinically relevant cut-off value (65). All quantifications were performed in cardiac diastole.

Statistical analysis

All data were analyzed using the SPSS 12.0 software package. Data are expressed in means [95% confidence intervals] unless indicated otherwise. All continuous variables followed normal distribution. Differences between groups were analyzed with an independent Student *t*-test; categorical data were compared using a Chi-squared test. Pearson correlation coefficients were calculated to assess associations between vascular parameters and risk scores. Stepwise multiple regression analysis was performed to assess explanatory parameters for plaque presence and variability in IMT values.

RESULTS

Patient characteristics

A total of 112 patients (60 (54%) males) were included in the study of which 88 (79%) were seen in a primary prevention setting and 24 (21%) had a prior history of CVD. The characteristics of the patients are shown in table 1. The secondary prevention patients were significantly older and had smoked more packyears. Diastolic blood pressure was significantly lower in the secondary prevention group. Secondary prevention patients were more frequently treated with anti-platelet therapy, anti-hypertensive medication and statins, possibly partly explaining the lower blood pressure and lipid values.

Of the primary prevention patients 50% were receiving statin therapy for

Table 1 | Characteristics of primary and secondary prevention patients

Variable	Primary prevention (n=88)	Secondary prevention (n=24)	p-value
Age (yrs)	52.1 [49.8-54.4]	58.7 [54.2-63.2]	0.01
Male Gender	49 (54.5%)	13 (54.2%)	-
Waist circumference (cm)	97.8 [95.3-100.3]	98.7 [94.4-103.0]	0.74
Systolic BP (mmHg)	134.1 [130.2-138.1]	132.2 [132.2-123.6]	0.66
Diastolic BP (mmHg)	83.21 [81.0-85.1]	78.2 [74.7-81.7]	0.03
Diabetes Mellitus	12 (13.6%)	2 (8.3%)	0.54
Hypertension	37 (42.0%)	16 (66.7%)	0.02
Hypercholesterolemia	61 (69.3%)	14 (58.3%)	0.42
Family history of CVD	25 (28.4%)	10 (41.7%)	0.20
Current smokers	21 (23.8%)	3 (12.5%)	0.27
Packyears of smoking	8.37 [5.2-11.6]	19.85 [7.7-32.0]	0.01
Anti-platelet therapy	10 (11.4%)	14 (58.3%)	<0.001
Statin therapy	44 (50.0%)	18 (75.0%)	0.13
Anti-hypertensive therapy	33 (37.5%)	14 (58.3%)	0.01
HbA1c (%)	5.3 [5.1-5.4]	5.6 [5.2-5.9]	0.12
Fasting glucose (mmol/L)	5.4 [5.1-5.6]	5.3 [5.1-5.7]	0.90
Total cholesterol (mmol/L)	5.7 [5.4-5.9]	5.2 [4.7-5.7]	0.06
Triglycerides (mmol/L)	1.9 [1.6-2.2]	1.5 [1.2-1.7]	0.02
HDL-cholesterol (mmol/L)	1.6 [1.4-1.7]	1.6 [1.4-1.8]	0.66
LDL-cholesterol (mmol/L)	3.7 [3.4-3.9]	3.1 [2.6-3.7]	0.07
Plaques	46 (52.3%)	21 (87.5%)	0.006
IMT (mm)	0.830 [0.797-0.863]	0.917 [0.873-0.960]	0.01
IMT≥0.9mm	29 (32.9%)	12 (52.2%)	0.56

previously diagnosed hyperlipidemia at time of inclusion. The characteristics of the statin-treated and statin-naïve primary prevention patients are shown in table 2. Plasma levels of LDL cholesterol were significantly lower in the statin-treated group and there was a higher prevalence of type 2 diabetes mellitus and hypertension. Levels of HbA1c and fasting glucose were significantly higher in the statin-treated group.

Table 2 | Characteristics of primary prevention patients

Variable	Statin naïve (n=44)	Statin treated (n=44)	p-value
Age (yrs)	50.0 [46.2-53.1]	54.1 [51.3-56.9]	0.08
Male gender	24 (54.5%)	25 (56.8%)	-
Waist circumference (cm)	95.9 [92.5-99.3]	99.6 [95.8-103.3]	0.15
Systolic BP (mmHg)	132.9 [127.1-138.7]	135.3 [129.7-140.9]	0.55
Diastolic BP (mmHg)	81.5 [78.6-84.4]	84.5 [81.7-87.4]	0.14
Diabetes Mellitus	2 (4.5%)	10 (22.7%)	0.02
Hypertension	13 (29.5%)	24 (54.5%)	0.02
Hypercholesterolemia	19 (43.2%)	44 (100%)	<0.001
Family history of CVD	10 (26.3%)	15 (34.1%)	0.45
Current smokers	12 (27.3%)	9 (20.5%)	0.45
Packyears of smoking	6.9 [3.2-10.6]	10.5 [4.7-16.4]	0.29
Anti-platelet therapy	6 (13.6%)	10 (22.7%)	0.50
Anti-hypertensive therapy	12 (27.3%)	21 (47.7%)	0.05
HbA1c (%)	5.1 [4.9-5.3]	5.4 [5.2-5.7]	0.05
Fasting glucose (mmol/L)	5.1 [4.7-5.4]	5.7 [5.3-6.1]	0.01
Total cholesterol (mmol/L)	6.1 [5.7-6.4]	5.3 [4.9-5.6]	0.02
Triglycerides (mmol/L)	2.0 [1.5-2.3]	1.8 [1.4-2.3]	0.85
HDL-cholesterol (mmol/L)	1.5 [1.3-1.7]	1.6 [1.4-1.7]	0.62
LDL-cholesterol (mmol/L)	4.2 [3.8-4.5]	3.2 [2.8-3.5]	<0.001
SCORE-risk (% per 10 yrs)	3.3 [2.1-4.5]	-	-
Plaques	21 (47.7%)	26 (59.1%)	0.34
IMT (mm)	0.787 [0.743-0.830]	0.875 [0.829-0.919]	0.006
IMT≥0.9mm	12 (27.3%)	18 (40.1%)	0.20

Ultrasound findings

In the primary prevention group 52% had evidence of atherosclerotic plaque in the carotid artery, whereas 88% of secondary prevention patients had plaques (p=0.006, table 1). Within the primary prevention group there was no difference in plaque prevalence between the statin-naive patients and the statin-treated group (48% vs. 59%, respectively; p=0.34). Carotid IMT was significantly higher in

the secondary prevention group (0.830mm for primary prevention patients vs. 0.917mm for secondary prevention patients; p=0.01). The percentage of patients above the clinically relevant cut-off value of 0.9mm did not significantly differ between the groups (33% vs. 52%, respectively; p=0.56). Within the primary prevention group the statin-treated patients had significantly higher IMT values (0.787mm for untreated patients vs. 0.875mm for statin treated patients; p=0.006), but the number of patients above the clinical cut-off value was comparable between the groups (27% vs. 40%, respectively; p=0.20). The distribution of carotid plaque prevalence and increased IMT values is illustrated in figure 1.

The statin-naïve patients had a mean calculated risk of a fatal cardiovascular event (SCORE system) of 3.3% per10yrs [95% CI: 2.1-4.5]. Risk scores correlated with carotid IMT (r=0.647; p<0.001, illustrated in figure 2) and plaque presence (r=0.37; p<0.05). Significant multiple regression models could be made using the following co-variates: age, gender, waist circumference, body mass index, systolic and diastolic blood pressure, blood lipid levels, fasting glucose and HbA1c, the presence of diabetes mellitus, hypertension, hypercholesterolemia, family history of CVD and smoking status. Variability in IMT values was significantly explained (r^2=0.575; p<0.001) by age (β=0.005; p=0.002), systolic blood pressure (β=0.004; p=0.003) and waist circumference (β=0.003; p=0.012). Plaque presence was explained (r^2=0.372; p<0.001) by the presence of hypertension (β=0.347; p=0.036), a positive family history for CVD (β=0.396; p=0.016) and fasting glucose levels (β=0.158; p=0.047). None of the other traditional risk factors significantly contributed to this model.

Of the statin-naïve primary prevention patients 11/44 (25%) had a treatment indication on the basis of a calculated risk of above 5% per 10yrs. The distribution of carotid parameters in patients above and below this treatment threshold is summarized in table 3. Adding of the presence of moderate to severe carotid plaque to the decision process would result in an additional 10/44 (23%) of patients being reclassified in a higher risk group. When taking into account increased IMT values as well as plaque presence, a further 6/44 (14%) patients would be identified as higher risk patients.

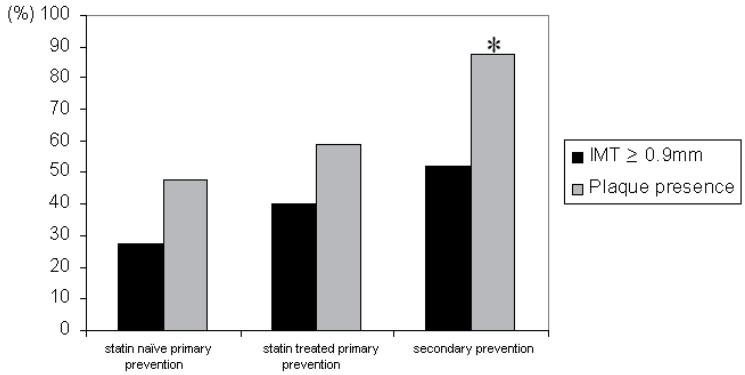

Figure 1 | Distribution of carotid plaques and increased intima-media thickness.
(*) indicates that secondary prevention group had significantly higher plaque prevalence compared with primary prevention patients

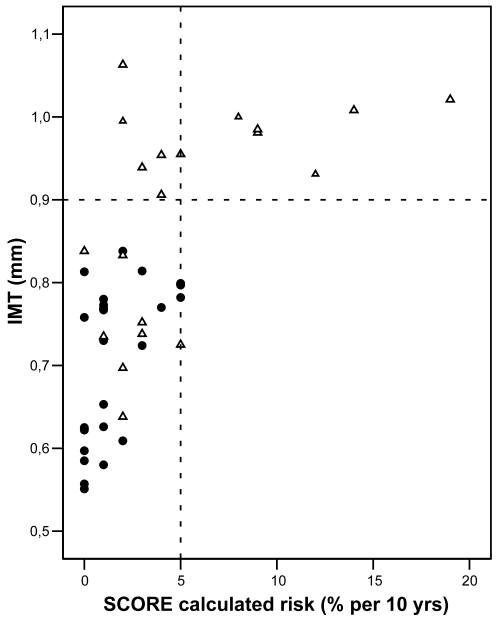

Figure 2 | Relation between subclinical carotid atherosclerosis and calculated risk score in statin naïve primary prevention cases. Dotted lines indicate the treatment threshold advised in ESC guidelines (SCORE ≥ 5% per 10 yrs; x-axis) and a proposed treatment threshold using ultrasound (IMT ≥ 0.9mm; y-axis).

● patients with no treatment indication based on ultrasound parameters
Δ patients with carotid plaque and/or IMT ≥ 0.9mm

Table 3 | Distribution of plaques alone and subclinical carotid atherosclerosis (SCA) defined as moderate to severe plaque and/ or IMT ≥ 0.9mm over risk strata in statin naïve primary prevention patients.

	Analysis based on plaque		Analysis based on plaque and/or IMT		
	No/mild plaque	Moderate/severe plaque	SCA -	SCA +	total
SCORE < 5	23	10	17	16	33
SCORE ≥ 5	5	6	3	8	11
Total	28	16	20	24	44

DISCUSSION

The main findings of our study are that subclinical carotid atherosclerosis is highly prevalent in a vascular outpatient setting and that it is frequently observed in patients with low calculated cardiovascular risk scores.

Carotid ultrasound imaging identified plaques in approximately 50% of our primary prevention patients, whereas nearly all (88%) of the secondary prevention patients had evidence of atherosclerotic carotid plaque. These figures are comparable to prevalence numbers reported in population based studies. Rosvall et al. (72) found 43% carotid plaques in 5163 subjects with and average age of 58 years and van der Meer et al. (19) reported 58% plaques in 6389 subjects of >55 years of age. Both these study samples were recruited from the general population and showed similar patterns of co-morbidity as our patients, although our patients were younger. This is an indication that a population in a referral center such as a vascular outpatient department may reach comparable levels of subclinical atherosclerosis at an earlier age than a general population.

A substantial number of plaques and increased IMT values were found in patients with low calculated risk in our study. Both plaque presence and IMT values correlated with calculated risk scores, but traditional risk factors could only partly explain plaques presence and IMT values. Besides the traditional cardiovascular risk factors, carotid ultrasound parameters have been associated with many factors not included in algorithms for risk calculation. Amongst these are levels of homocysteïne (74), lipoprotein(a) (75) C-reactive protein (76), fibrinogen (23), markers of oxidative stress (24) and endothelial dysfunction (25). The impact of these non-traditional risk factors on SCORE-

calculated risk has been acknowledged in the European Society of Cardiology guidelines (65), stating that patients exhibiting these characteristics may be at higher actual risk than their calculated SCORE results indicate. In addition, the development of atherosclerosis is subject to individual genetic susceptibility. Current guidelines recommend screening for ultrasound measured subclinical carotid atherosclerosis. The presence of SCA should be interpreted as target organ damage rather than a risk factor. It could in this regard be viewed as an easily obtained *in vivo* marker for the cumulative effect of traditional and non-traditional risk factors on the arterial wall. Importantly, both carotid plaque and IMT have been associated with atherosclerotic changes in other vascular beds, including the coronary arteries (80) (27) (82) (29), where these risk factors may also promote atherosclerosis formation.

Previous reports have addressed the added value of carotid ultrasound in risk assessment. The addition of carotid parameters to risk prediction models does appear to improve their performance (84). In a large population-based study as well as recent systematic reviews however the positive effect on predictive power has been shown to be marginal (68) (69) It is therefore still questionable whether the measurements offer clinically relevant benefits in risk assessment. Not all the scanning protocols in these studies included carotid plaques in the analysis. In several earlier reports, plaque presence has been associated with higher risk of future cardiovascular events (8) (85) (32) (33) (34). Therefore, adding plaque detection may improve the predictive value of carotid ultrasound results. The current recommendations therefore advise prudent use of carotid ultrasound in clinical practice by carefully selecting patients in whom the findings offer additional information. Plaque detection combined with IMT measurements appears to offer the most relevant information. It can be argued that patients referred to a specialized vascular clinic where carotid ultrasound is readily available may not be comparable to the general public. The added value of carotid imaging has not been prospectively studied in such a setting. Our study provides data exploring carotid ultrasound in risk assessment in the setting of a vascular referral clinic. The consequences regarding treatment decisions may be considerable. In untreated subjects, imaging may identify a significant number of high risk primary prevention patients (23%-36%, according to our cross-sectional data) with subclinical atherosclerosis. These subjects are not identified as high risk by current risk assessment algorithms. This potential

should be explored further, as the lack of outcome studies poses a critical factor limiting routine use of carotid ultrasound in clinical practice. Although subclinical carotid atherosclerosis has consistently been associated with higher cardiovascular risk, a prospective study incorporating carotid ultrasound in treatment decisions is warranted.

Our study has limitations. We evaluated a small cross-sectional sample. Larger studies in the referral centers where the carotid ultrasounds are available in clinical practice are needed. We defined the presence of moderate to severe plaque and/or increased IMT values as subclinical carotid atherosclerosis. More elaborate plaque classifications are available, including plaque size, echogenicity and regularity of the plaque surface. We did not evaluate these parameters.

Furthermore, our patients were recruited from a vascular referral center in the Netherlands. This limits the possibilities to extrapolate our findings to populations in other countries where the role of primary health care providers in CVD risk management and thresholds for referral may be different.

In conclusion, our findings demonstrate that subclinical carotid atherosclerosis is highly prevalent in a vascular outpatient setting in the Netherlands. It was frequently observed in patients with low calculated risk scores. Our data suggest that utilization of carotid ultrasound may be useful, even in a setting of presumed high-risk patients.

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CHAPTER 4

Carotid Artery Diameter, Wall Thickness and Wall Area by MRI at 3-Tesla: Comparison with Ultrasound

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ABSTRACT

Purpose

To compare MRI based measurements of the lumen diameter, vessel wall thickness and wall area of the carotid artery to ultrasound based measurements of the lumen diameter and intima-media thickness (IMT) in asymptomatic young adult and middle aged subjects.

Subjects and Methods

Ultrasound and MRI at 3-Tesla of the left common carotid artery were performed in 39 healthy subjects. The mean age in group 1 (n=28) was 51 years and 25 years in group 2 (n=11). The lumen diameter, mean wall thickness and wall area were measured in a predefined segment of the carotid artery using a black blood fast gradient MRI sequence. The lumen diameter and IMT were measured by ultrasound in the same vessel segment. Correlation analysis and Bland & Altman plots were used to compare MRI and ultrasound measurements.

Results

Measurements of the carotid lumen diameter by ultrasound and MRI showed high agreement (intraclass correlation coefficient 0.882). Highly significant correlations were found between IMT and MRI measurements of mean wall thickness and wall area ($r=0.84$, $p<0.001$ and 0.74, $p<0.001$, respectively). Middle aged asymptomatic subjects had significant higher IMT, wall thickness and wall area values as compared to healthy young adults ($p<0.001$).

Conclusion

MRI and ultrasound show excellent agreement for measuring the carotid lumen diameter. MRI measurements of wall thickness and wall area correlate well with IMT. Asymptomatic subjects reveal an age-dependent increase in IMT, wall thickness and wall area.

INTRODUCTION

Carotid intima-media thickness (IMT) by ultrasound is a widely accepted surrogate marker for cardiovascular disease. It has been extensively documented that carotid atherosclerosis is related to cardiovascular risk, coronary and cerebrovascular atherosclerosis (89) (90) (91) (92). Large epidemiological studies have shown that the presence of carotid atherosclerosis as measured by high resolution ultrasound is related to the occurrence of future cardiovascular events (5) (6) (7) (8)((9). These data have prompted the American Heart Association (93), The European Society of Hypertension (94) and the European Society of Cardiology (9) to advocate the use of carotid imaging in cardiovascular risk assessment and as an intermediate endpoint in clinical trials. Both applications of carotid ultrasound have limitations. Additional predictive value of IMT measurement above risk assessment algorithms based on traditional risk factors seems to be a consistent finding, however the magnitude of the improvement may be marginal. (95) (96) (97) (98). These data make its clinical relevance for risk restratification in individual patients questionable. Studies using carotid ultrasound as an endpoint require relatively large sample sizes partly due slow progression rates of IMT and the cross-sectional images ultrasound provides limiting full quantification of the vessel wall geometry. Moreover many ultrasound protocols do not take carotid plaques into account.

Magnetic resonance imaging (MRI) has recently emerged as a reproducible and reliable imaging modality for the assessment of atherosclerosis (17) (100) (101). Serial MRI of the vessel wall allows for monitoring of carotid atherosclerosis in relatively small samples of the population owing to the lower interscan variability of the MRI measurements as compared to those by ultrasound. (40) (41) Furthermore, MRI provides a circumferential image of the vessel, which may potentially better represent wall pathology such as eccentric arterial remodelling as compared to luminography and ultrasound.

The role of 3T carotid MRI as a predictor of future vascular events is still unknown due to a lack of clinical outcome studies. There are studies associating MRI-based carotid plaque presence and characteristics to stroke risk (101) but these findings are all in high-risk patients where carotid plaques are already present. Data on vessel wall geometry parameters in early atherosclerosis (i.e. no plaque presence) and future cardiovascular events are lacking. Regarding

ultrasound-based IMT measurements there is a broad consensus on threshold values for higher cardiovascular risk (usually >0.9mm at the level of the common carotid artery). Its equivalent for MRI based measurements is yet not defined

The purpose of the current study is to evaluate the potential of 3T carotid MRI as a biomarker for early atherosclerosis in absence of carotid plaque. To this end, a head-to-head comparison was performed of measurements of the carotid lumen diameter and IMT by ultrasound and the lumen diameter, wall area and wall thickness by black blood fast gradient MRI at 3-Tesla in an population with no evidence of carotid plaque.

Furthermore, direct comparison of the two imaging modalities could offer insight on cut-off values for higher cardiovascular risk.

METHODS

Study Design & Subjects

A series of 41 subjects were prospectively included in the study over a 1 year period. The time between ultrasound (US) and MRI examination was never more than 2 months. In 2 cases the MRI was not interpretable due to insufficient quality leaving 39 subjects for analysis. Informed consent was obtained and the study protocol was approved by the hospital ethics committee. The sample was comprised of 28 older male subjects with a broad range in cardiovascular risk (group 1), recruited from a separate ongoing randomised clinical trial. The inclusion criterion was the presence of visceral obesity (>94 cm waist circumference). The use of statins or non-steroidal anti-inflammatory drugs, as well as the presence of diabetes mellitus was considered exclusion criteria. In addition, we recruited 11 young healthy subjects (group 2). All subjects were free of clinically manifest cardiovascular disease and the 10-year risk of a coronary event was calculated for group 1 using the Framingham risk score, described in detail elsewhere. (102) Based on age, medical and family history it is assumed all subjects in group 2 are at low cardiovascular risk. The US and MRI scans were performed by fully trained single observers. Both observers were blinded for the results of the scan with the other imaging modality.

Magnetic Resonance Imaging Protocol

Magnetic resonance imaging was performed on a 3-Tesla scanner (Philips, Achieva, Best, The Netherlands). The reproducibility of the technique has been previously reported. (100) In brief, a standard Philips SENSE-flex-M surface coil was used for imaging. The left carotid artery was examined in all subjects. Three fast gradient echo sequence surveys were performed to localize the course of the common carotid artery. Subsequently, five contiguous transverse slices with 2mm slice thickness were acquired, starting from 1cm proximal to the flow divider, thereby covering 1 cm of the common carotid artery. A dual inversion recovery (black-blood), spoiled segmented k-space fast gradient echo sequence with spectral selective fat suppression was used for the acquisition of transverse slices. Images were acquired in cardiac end-diastole at each RR interval using ECG triggering. The following imaging parameters were used: echo time 3.6ms, repetition time (TR) 12ms, flip angle 45 degrees, and 2 signal averages were performed. A re-inversion slice thickness of 3mm was used. The field of view was 140mm. When using a matrix size of 306 the resulting voxel size was 0.46mm x 0.46mm x 2mm. Each MRI study took approximately 30 minutes depending on the cardiac frequency. All images were analyzed by a single observer with 4 years' experience, using the VesselMASS software package, allowing manual tracing of vessel boundaries and automated quantification of lumen diameter, wall area and mean wall thickness. Mean wall thickness (MWT) was calculated by averaging the mean thickness at all 5 slices of the common carotid artery. Vessel wall area (VWA) was quantified by extracting the luminal area from the detected outer vascular boundary at each slice. The sum of these 5 area values was used the outcome parameter VWA. Figure 1 shows a representative example of an MRI scan of the carotid artery.

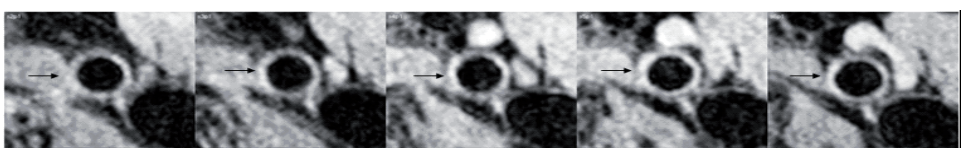

Figure 1 | Representative example of 3-Tesla MRI image of the carotid artery of a 62-year old male subject. Black arrows indicate the carotid artery. Five contiguous transverse slices of 2mm, covering the most distal 1cm of the common carotid artery.

Ultrasound protocol

IMT measurement was performed using an Acuson Sequoia 512 (Siemens Medical Solutions, CA, USA) high-resolution ultrasound machine with an 8MHz linear transducer. One IMT-certified sonographer (4 years of experience) performed all the ultrasounds. First, a transverse scan was performed for orientation, starting at the clavicle and moving cranially up to the mandible, hereby locating the height of the carotid bifurcation. Subsequently, longitudinal images were obtained. This technique allows visualization of two echogenic lines, separated by an anechoic space. It has previously been established that these lines indicate the blood-intima and the media-adventitia interfaces, and that the distance between the lines represents a reliable measure for IMT. (23) The scan included visualization of the near and far walls of the left common carotid artery, at four angles of insonation (anterior, two antero-lateral projections and lateral). The caudal tip of the flow divider was used as the anatomical landmark to localize the most distal 1cm of the common carotid artery. Overall gain settings were kept at 0dB when possible. The sonographer was free to adjust the gain levels if necessary, within the limits of -7dB to 7 dB. The scan was recorded on sVHS video cassettes and digitalized for off-line analysis. IMT values were quantified using computer aided automatic boundary detection where possible and manual adjustment where necessary. Analyses were done by a trained analyst, using the ASM II software package version 1.1364. IMT was defined as the average of the mean values of the common carotid artery, at all four angles. Lumen diameter was also determined at four angles and defined as the distance between the intima-lumen interface of the near wall and the lumen-intima interface of the far wall. Quantification of all parameters was timed to coincide with cardiac end-diastole using an on-screen 3-lead ECG.

Statistical analysis

All data followed normal distribution. The primary analysis was aimed at a comparison of lumen diameter and wall thickness by US and MRI, in which the same geometric parameters (mm) could be compared. The comparison between US and MRI-based quantification of lumen diameter was included to provide data on the technical agreement between the two imaging modalities. A secondary analysis compared US-based thickness (mm) to MRI-based area (mm^2) measurement. Vessel wall area is an important outcome parameter

in 3T carotid MRI and its relation to US-based IMT values was deemed to be relevant for interpretation of future studies using 3T carotid MRI. Within-subject differences between US and MRI were evaluated with a T-test. Correlations between US and MRI were calculated using Pearson's correlation coefficients. Extrapolation of normal and high-risk values for MRI parameters was based on linear estimation functions from the US- IMT regression lines. Bland-Altman analyses (24) and intraclass correlations coefficients (ICC) were used to assess agreement of the two imaging modalities, regarding measurement of thickness and lumen diameter within subjects. Two-sided p-values of <0.05 were considered statistically significant. All data are expressed as means and 95% confidence intervals, except in table 1 in which the range is given in parentheses to illustrate the diversity of vascular parameters in the study population.

RESULTS

The technical success rate for completing the MRI examinations was high (39/41 subjects; 95%). Subject characteristics and results are summarized in table 1. The mean lumen diameter measured by US and MRI showed very high agreement,

Table 1 | Values are expressed in means [range].

Variable	Total sample	Group 1	Group 2
N	39	28	11
age (yrs)	51.3 [20-73]	61.8 [54-73]	24.7 [20-39]*
Framingham CHD risk score (%/10yrs)	-	15.8 [6-29]	-
mean IMT; US (mm)	0.76 [0.45-1.36]	0.86 [0.65-1.36]	0.50 [0.45-0.70]*
MWT; MRI (mm)	1.25 [0.72-2.29]	1.40 [0.98-2.29]	0.87 [0.72-1.27]*
VWA; MRI (cm^2)	1.64 [0.67-3.77]	1.90 [1.11-3.77]	0.96 [0.67-1.45]*
lumen diameter; MRI (mm)	6.71 [5.08-8.99]	7.00 [6.68-7.30]	6.00 [5.73-6.28]
lumen diameter; US (mm)	6.71 [5.28-9.00]	7.00 [5.53-9.10]	5.96 [5.28-6.37]

Group1=older subset; group 2=young healthy volunteers. Data show the expected differences between the younger and the older group in vessel wall parameters. No difference was observed between MRI and US in measurement of lumen diameter, confirming dimensional compatibility between the techniques. Asterisk (*) indicates statistically significant difference between group 1 and 2 (p<0.001). CHD=coronary heart disease; IMT=intima-media thickness; MWT=mean wall thickness; VWA=common carotid vessel wall area.

with a mean difference of 0.03mm [-0.16 to 0.11]; p=0.669 and a high intraclass correlation for absolute agreement of 0.882 (p<0.001). The Bland-Altman plot (figure 2) showed no bias, with the mean line corresponding exactly with 0.00 with an acceptable spread around the mean (within 1.96 standard deviations).

IMT values in the total sample ranged from 0.45mm to 1.36mm encompassing subjects with a broad range of the Framingham risk score. The younger subjects (group 2) had a mean IMT of 0.50mm [range: 0.46 to 0.55]. The corresponding mean values for MWT and VWA were 0.87mm [range: 0.72 to 1.27] and 0.96cm^2 [range: 0.67 to 1.45], respectively. Mean IMT, MWT and VWA in group 1 subjects were significantly higher than in group 2 subjects (table 1).

The bivariate correlations between the US and MRI parameters are shown in Table 2 and illustrated in figures 4 & 5. Highly significant correlations were observed for all measures. The statistically significant regression lines between IMT and MRI parameters suggest that the clinical cut-off for IMT of 0.9mm corresponds approximately with a MWT value of 1.45mm (figure 4) and VWA of 2.00cm^2 (figure 5).

The ICC between measurements of MWT and IMT (0.347, p<0.001) was statistically significant but lower than the ICC observed for luminal diameter, due to consistently higher values of the MWT compared to IMT (paired sample T-test p<0.001). Furthermore, an upward trend in this difference was observed when wall thickness was increasing [r=0.72 (p<0.001); (figure 3)].

Table 2 | The high correlations with IMT validate the use of these MRI parameters as surrogate markers for atherosclerosis.

Variable	Pearson's coefficient (r)	P-value
MWT vs. IMT	0.84	<0.001
VWA vs. IMT	0.78	<0.001
lumen diameter (MRI vs. US)	0.88	<0.001

IMT=intima media thickness; MWT=mean wall thickness; VWA=vessel wall area.

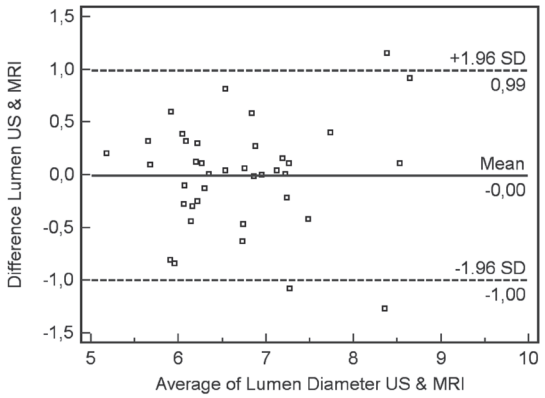

Figure 2 | Bland-Altman plot of ultrasound and MRI values for lumen diameter.
Data indicate a very high agreement between the two imaging modalities, shown by the mean line coinciding exactly with 0.0 and acceptable distribution within a range of 1.96 standard deviations (SD). No trend is seen in the distribution around the mean.

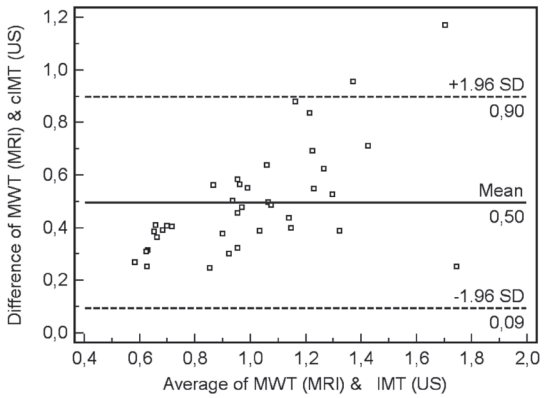

Figure 3 | Bland-Altman plot of ultrasound and MRI values for carotid vessel wall thickness.
Data indicates that MRI findings for MWT are systematically higher than US values of IMT, shown by the mean line coinciding with 0.50. A significant upward trend is seen in the distribution around the mean (r=0.72, p<0.001), implying the difference between US and MRI evaluation is more pronounced with increasing vessel wall thickness.

DISCUSSION

The main findings of the current study are that 3-Tesla MRI measurement of the lumen diameter of the carotid artery almost perfectly agrees with ultrasound measurement, that the MRI measurements of wall thickness and wall area closely correlate with ultrasound IMT measurements, but that MRI systematically finds

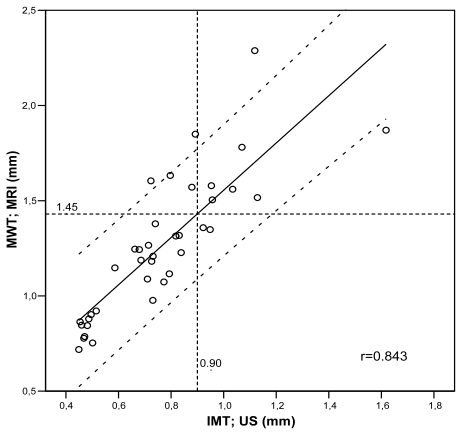

Figure 4 | Scatterplot of mean thickness; IMT (US) vs. MWT (MRI).

Data demonstrate strong correlation between these thickness parameters (r=0.84). The dotted extrapolation lines indicate that the clinically relevant cut-off value of 0.9mm for IMT corresponds with 1.45mm for MWT
IMT=intima media thickness
MWT=mean wall thickness
(——) indicates linear regression line
(- - - -) indicates 95% confidence interval
(------) indicates linear extrapolation lines

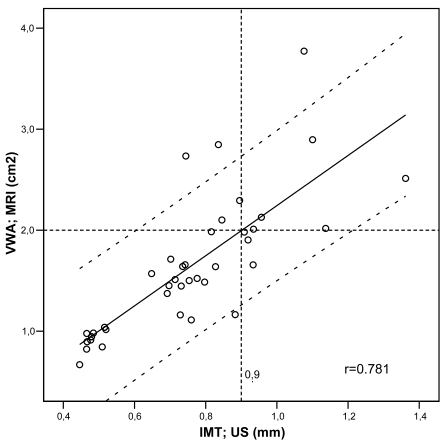

Figure 5 | Scatterplot of IMT (US) vs VWA (MRI).

Data demonstrate strong correlation between the parameters (r=0.78). The dotted extrapolation lines indicate that the clinically relevant cut-off value of 0.9mm for IMT corresponds with 2.00cm^2 for VWA.
IMT=intima media thickness
VWA=vessel wall area.
(——) indicates linear regression line
(- - - -) indicates 95% confidence interval
(------) indicates linear extrapolation lines

higher values for wall thickness than ultrasound. Furthermore, preliminary data are provided to extrapolate IMT cut-off values for higher cardiovascular risk to MRI measurements of wall thickness and wall area.

The high level of agreement in quantifying carotid lumen diameter indicates the dimensional accuracy of MRI as compared to ultrasound. Apparently, the inner vessel boundaries are defined by both techniques adequately without systematic differences allowing very accurate measurements of the lumen diameter.

Previous studies have evaluated the use of black blood fast spin echo MRI at 1.5-Tesla for assessing mean wall thickness of the carotid artery as compared to IMT values. Underhill et al. (25) used a statistical shape modelling technique for automated measurement of mean wall thickness in the common carotid artery by MRI. Patients with a range of carotid artery stenoses were included in that study and not only thin-walled segments as was done in our study. A very high correlation coefficient between MRI measured wall thickness and IMT was reported (r=0.93). In that study 28 out of 43 patients were successfully evaluated by both MRI and ultrasound.

Mani et al. (26) compared MRI based measurements of wall thickness and wall area with IMT in 17 patients with intermediate to high Framingham risk score. In that study a somewhat lower correlation between MRI measurements of mean wall thickness and IMT was reported (r=0.71).

In our study the correlation for measuring wall thickness by MRI and ultrasound is 0.84 and therefor e comparable to the findings in the abovementioned studies. The technical success ratio for completing the MRI study was 95% in our population, higher than reported in previous studies, predominantly done with 1.5Tesla MRI.. It is expected that higher spatial resolution at 3-Tesla may help to better define vessel boundaries and thereby improving accuracy. However, several factors may have contributed to the accuracy of MRI when contrasting the results with those of other studies. The acquisition protocol was different from previously reported protocols. We used a black blood fast gradient echo sequence that has shown good reproducibility in a previous study. (100) Furthermore, we measured relatively thin-walled vascular segments as opposed to stenosed vessels with significant atherosclerosis.

We observed systematically higher MRI-based wall thickness measurements when compared to ultrasound. In addition, we observed a more pronounced

difference with increasing wall thickness. This observation has been noted previously (25) (26). Other researchers have provided several explanations for this finding. A factor that contributes to this overestimation is that MRI measurements include the lamina adventitia, whereas ultrasound measures only the combined thickness of the intima and media. Therefore, we speculate that there is increasing adventitial thickening commensurate with overall wall thickening, as is observed in experimental studies. (27) (28) (29) Of note, the degree of overestimation (0.4 mm) is consistent with the thickness of the adventitia and residual media that comprise the artery wall following endarterectomy. Further studies are required to assess the contribution of the adventitia to the wall thickness and to explore its potential clinical significance.

Although the subjects included in the present study were asymptomatic, we observed significant differences in IMT, wall thickness and wall area by MRI when comparing a relative young age group and an older age group. The older group did not exhibit plaque formation. These findings confirm that the carotid artery thickens with advancing age. The data also suggest that it is possible to visualize early stage atherosclerosis by means of MRI, although we cannot make a distinction between physiological vascular adaptation and early atherosclerosis. We have compared the cut-off value of the IMT of 0.9 mm to the MRI measurements of mean wall thickness and wall area. Our preliminary data indicate that the corresponding wall thickness is 1.45 mm and wall area 2.00 cm^2, respectively. Further study is required to assess appropriate threshold values for MRI measurements of wall thickness and wall area as compared to clinically meaningful threshold values by ultrasound.

This study has several limitations. The coverage of the common carotid artery was limited to 1 cm. Further technical improvements by using different acquisition schemes and surface coils may be anticipated to improve spatial resolution. Furthermore, a limited number of subjects of two age groups were included. To assess the clinical relevance of the observations further study is required in larger cohorts with different risk profiles. Moreover, the data for the threshold values for wall thickness and wall area by MRI are only preliminary and have to be explored in follow-up studies with clinical end-points for further validation. Despite these limitations, we believe that our current protocol is optimized for 3-Tesla imaging of the common carotid artery and provides an insight on what

can be achieved using this technology.

In conclusion, 3-Tesla imaging of the carotid artery provides excellent dimensional accuracy for measuring the lumen diameter. Close correlations with IMT is achieved for measuring wall thickness and wall area. Further study is required to correlate the MRI findings with different risk profiles to assess the clinical utility for risk stratification and outcomes.

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CHAPTER 5

In vivo estimation of carotid artery adventitia thickness in relation to Cardiovascular Risk Factors and Circulating Serum Vascular Endothelial Growth Factor: a combined Three Tesla MRI - Ultrasound approach

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ABSTRACT

Background

Carotid artery magnetic resonance imaging (MRI) may provide additional information to ultrasound-measured carotid intima-media thickness (IMT) by including the lamina adventitia. The relation between adventitial angiogenesis and development of atherosclerosis is subject to intensive research but has not been fully elucidated. Vascular endothelial growth factor (VEGF) may be relevant for vasa vasorum angiogenesis.

Purpose

To assess the association between estimated adventitial thickness cardiovascular risk factors and circulating VEGF levels.

Subjects and Methods

High resolution ultrasound to determine IMT and 3-Tesla MRI scans to determine total vessel wall thickness (TWT) were performed at identical locations in the left common carotid artery of 51 male subjects (mean age: 60.7yrs; range 51yrs-73yrs) free of clinically manifest cardiovascular disease. Estimation of adventitial thickness was defined as the difference between TWT and IMT and is referred to as carotid extramedial thickness (cEMT).

Results

cIMT was not related to cardiovascular risk factors in contrast to IMT and TWT. IMT correlated significantly with age ($r=0.41$; $p<0.01$), , systolic blood pressure ($r=0.41$; $p<0.01$), waist circumference ($r=0.29$; $p<0.01$); total cholesterol ($r=0.30$; $p<0.05$), C-reactive protein (0.29; $p<0.01$) and Framingham risk score ($r=0.55$; $p<0.01$). TWT correlated significantly with age ($r=0.31$; $p<0.05$) and Framingham risk score ($r=0.42$; $p<0.01$). cEMT significantly correlated with circulating VEGF levels ($r= -0.38$; $p<0.01$); this was not observed for IMT or TWT.

Conclusion

cEMT was not associated with traditional cardiovascular risk factors but did correlate with VEGF. The combination of MRI and ultrasound-based vascular imaging carries the potential to study the pathophysiology of the lamina adventitia in human subjects *in vivo*.

INTRODUCTION

The role of the lamina adventitia in the progression of atherosclerosis is currently under active research and has been comprehensively reviewed (109) (110) (3) (112). Thickening of the adventitia in the setting of atherosclerosis is associated with neovascularisation due to angiogenesis of the vasa vasorum. Increased vascular endothelial growth factor (VEGF) expression due to hypoxia at the luminal site of the thickened atherosclerotic intima is regarded as a pathophysiological stimulus for angiogenesis. Vasa vasorum angiogenesis amongst other processes is thought to be a driving force of adventitial thickening. (5) (114) (7) (8) (9) Consequently, VEGF is associated with the pathophysiological changes occurring at the level of the adventitia (114).

Intima media thickness (IMT) of the carotid artery by high-resolution ultrasound (US) is an established vascular imaging tool used in clinical practice and cardiovascular research. IMT has been linked to cardiovascular risk (10) (119) (120) (121) (14) (123) (124) and coronary atherosclerosis (125) (18) (19) (20). Histologic studies have shown IMT reflects the combined thickness of the lamina intima and media (21). Carotid magnetic resonance imaging (MRI) has now emerged as an alternative method of carotid artery imaging. It can be performed with high precision and reproducibility (22) (23) (24). In recent years carotid MRI protocols have been developed that are able to accurately characterize carotid plaques. As opposed to ultrasound, MRI-images of carotid arteries do not distinguish the lamina intima, media and adventitia. Comparative studies between US and MRI imaging in the same segment of the carotid artery have therefore consistently shown higher thickness values in the MRI based images (133) (26). It has been suggested that the difference between TWT and IMT is an in vivo reflection of the thickness of the lamina adventitia (27). To date there has been no histological confirmation of the accuracy of such imaging techniques. The estimated thickness of the carotid adventitia is referred to as carotid extramedial thickness or cEMT. Several different imaging modalities are being utilized to visualize and quantify adventitial thickness (28).

The aim of the current study is to explore a novel method for measuring cEMT by combining US and MRI images of the same segment of the carotid artery. The correlation between cEMT, cardiovascular risk factors and serum VEGF-levels are also reported. IMT and TWT were used as reference. We hypothesized that the

relation between cEMT and cardiovascular risk factor may differ from IMT and TWT. In addition, VEGF was expected to correlate with cEMT due to its effects on vasa vasorum angiogenesis.

METHODS

Study Design & Subjects

Fifty-three patients were prospectively included in the study over a 1 year period. Informed consent was obtained and the study protocol was approved by the hospital ethics committee. Exclusion criteria were the use of statins or non-steroidal anti-inflammatory drugs and the presence of diabetes mellitus. All subjects were free of clinically manifest cardiovascular disease. Physical examination was performed according to standardized methods and venous blood samples were drawn after a 12 hour fasting period. Carotid MRI and US examinations were performed within a two week period. Values for IMT, TWT and non-IMT (definitions described below) were correlated to cardiovascular risk factors and serum levels of cytokines, adhesion molecules and growth factors.

Laboratory measurements.

The CRP measurements were performed with the Tinaquant CRP (latex) high-sensitive assay from Roche. This particle enhanced immunoturbidimetric assay was carried out on a Roche Module P using serum. The serum levels of VEGF were measured using a Randox Evidence Investigator and the Cytokine & Growth Factors Biochip Array and Adhesion Molecules Biochip Array. The light signal generated from the test region on the Biochip with antibodies labelled with Horse Radish Peroxidase is detected using a super cooled charge coupled device camera and compared to that from a stored calibration curve. Sample preparation in short: the sample is diluted with assay buffer or diluent and applied to a biochip (well). The biochip (carrier) is incubated at 37 ^{o}C and shaken at 370 rpm at a thermoshaker for 60 min. After washing the conjugate (HRM labelled antibodies) is added and again incubated at 37^{o}C and shaken at 370 rpm at a thermoshaker for 60 min. After washing 250 ml of a 1:1 mix of luminol and peroxide is added and incubated for 2 minutes. Finally the carrier is imaged using an Investigator System conform the manufacturers instruction.

Magnetic Resonance Imaging Protocol

Magnetic resonance imaging was performed on a 3-Tesla scanner (Philips, Achieva, Best, The Netherlands). The reproducibility of the technique has been previously reported (22). In brief, a standard Philips SENSE-flex-M surface coil was used for imaging. The left carotid artery was examined in all subjects. Three fast gradient echo sequence surveys were performed to localize the course of the common carotid artery. Subsequently, five contiguous transverse slices with 2mm slice thickness were acquired, starting from 1cm proximal to the flow divider, thereby covering 1 cm of the common carotid artery. A dual inversion recovery (black-blood), spoiled segmented k-space fast gradient echo sequence with spectral selective fat suppression was used for the acquisition of transverse slices. Images were acquired in cardiac end-diastole at each RR interval using ECG triggering. The following imaging parameters were used: echo time 3.6ms, repetition time (TR) 12ms, flip angle 45 degrees, and 2 signal averages were performed. A re-inversion slice thickness of 3mm was used. The field of view was 140mm. When using a matrix size of 306 the resulting voxel size was 0.46mm x 0.46mm x 2mm. Each MRI study took approximately 30 minutes depending on the cardiac frequency. All images were analyzed by a single observer with 4 years' experience, using the VesselMASS software package, allowing manual tracing of vessel boundaries and automated quantification of lumen diameter, wall area and mean wall thickness. Total wall thickness (TWT) was calculated by averaging the maximum thickness at all 5 slices of the common carotid artery. Figure 1 shows a representative example of an MRI scan of the carotid artery.

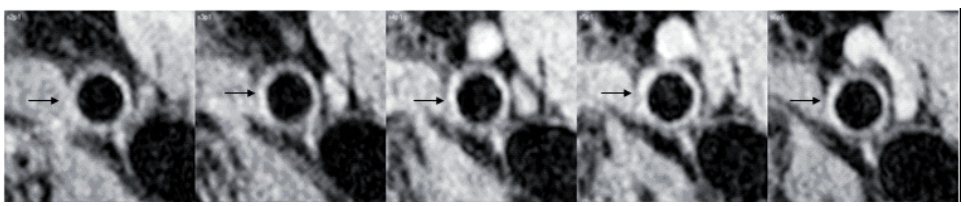

Figure 1 | Representative example of 3-Tesla MRI image of the carotid artery of a 62-year old male subject. Black arrows indicate the carotid artery. Five contiguous transverse slices of 2mm, covering the most distal 1cm of the common carotid artery.

Ultrasound protocol

IMT measurement was performed using an Acuson Sequoia 512 (Siemens Medical Solutions, CA, USA) high-resolution ultrasound machine with an 8MHz linear transducer. One IMT-certified sonographer (4 years of experience) performed all the ultrasounds. First, a transverse scan was performed for orientation, starting at the clavicle and moving cranially up to the mandible, hereby locating the height of the carotid bifurcation. Subsequently, longitudinal images were obtained. This technique allows visualization of two echogenic lines, separated by an anechoic space. It has previously been established that these lines indicate the blood-intima and the media-adventitia interfaces, and that the distance between the lines represents a reliable measure for IMT (21). The scan included visualization of the near and far walls of the left common carotid artery, at four angles of insonation (anterior, two antero-lateral projections and lateral). The caudal tip of the flow divider was used as the anatomical landmark to localize the most distal 1cm of the common carotid artery. Overall gain settings were kept at 0dB when possible. The sonographer was free to adjust the gain levels if necessary, within the limits of -7dB to 7 dB. The scan was recorded on sVHS video cassettes and digitalized for off-line analysis. IMT values were quantified using computer aided automatic boundary detection where possible and manual adjustment where necessary. Analyses were done by a trained analyst, using the ASM II software package version 1.1364. IMT was defined as the average of the maximum values of the common carotid artery, at all four angles. Lumen diameter was also determined at four angles and defined as the distance between the intima-lumen interface of the near wall and the lumen-intima interface of the far wall. Quantification of all parameters was timed to coincide with cardiac end-diastole using an on-screen 3-lead ECG.

Definition of cEMT

For the calculation of cEMT only the far wall values were used, because US quantification of near wall IMT is known to result in a systematic underestimation of up to 20% compared to histology (29). The MRI quantifications of TWT were done at four points in the arterial wall (posterior, two postero-medial projections and medial), closely approximating the sites of US-based IMT quantification (figure 2). At these four locations the IMT values were subtracted from the TWT values. The average of the difference between IMT and TWT at the four sites was defined as cEMT.

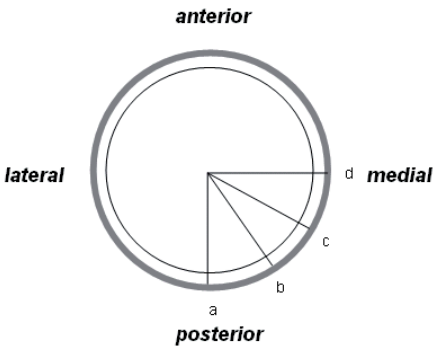

Figure 2 | Schematic illustration of the left common carotid artery. The inner black circle represents the lumen-intima border. The white area of the vessel wall indicates the intima-media complex, which is quantified by high-resolution ultrasound. The outer grey circle represents the lamina adventitia. MRI measurement of total wall thickness quantifies both the white and the grey portions of the vessel wall. Carotid extramedial thickness was defined as the difference between total wall thickness and intima-media thickness at sites a, b, c, and d.

Carotid extramedial thickness = $\underline{\Sigma a\text{-}d(TWT_{mri}\text{-}IMT_{us})}$ **4**

Statistical analysis

All data followed normal distribution and are expressed as means and 95% confidence intervals unless stated otherwise. Independent Student *t* test was used to compare means. Vessel wall thickness measures were correlated to cardiovascular risk factors and serum parameters using Pearson's correlation coefficients. Univariate analysis with eEMT as dependent variable was used to address the issue of interaction between the covariates VEGF (as category and subsequently as continuous variable after log transformation) and IMT. P-values of <0.05 were considered statistically significant.

RESULTS

A total of 51 male patients were included in the analysis. In 2 cases the MRI was of insufficient quality. The success rate of MRI scans was 96%. Patient characteristics are summarized in table 1. The mean age of the patients was 61 years [59.3-60.2], their mean calculated Framingham cardiovascular risk score was 13.2%/10 years [11.4-15.0]. The mean IMT was 0.98mm [0.93-1.02], mean

Table 1 | Subject characteristics and vessel wall parameters

Variable	N=51
age (yrs)	60.7 [59.3-60.2]
BMI (kg/m^2)	29.0 [28.1-29.8]
Waist circumference (cm)	106.9 [104.6-109.1]
Systolic blood pressure (mmHg)	146.4 [141.1-151.8]
Diastolic blood pressure (mmHg)	88.9 [86.6-91.3]
Fasting blood glucose (mmol/L)	5.2 [5.0-5.4]
HbA1c (%)	5.0 [4.9-5.2]
Insulin (µU/mL)	9.5 [7.7-11.4]
HOMA-index	1.3 [1.0-1.5]
Total Cholesterol (mmol/L)	5.7 [5.4-6.0]
HDL-cholesterol (mmol/L)	1.4 [1.3-1.5]
LDL-cholesterol (mmol/L)	3.4 [3.2-3.7]
Triglycerides (mmol/L)	1.9 [1.6-2.2]
C-reactive protein (mg/L)	2.0 [1.6-2.5]
VEGF (pg/mL)	181.7 [140.2-223.2]
Framingham CHD risk score (%/10yrs)	13.2 [11.4-15.0]
Vascular parameters	
cEMT (mm)	0.49 [0.42-0.55]
IMT; US (mm)	0.98 [0.93-1.02]
TWT; MRI (mm)	1.54 [1.46-1.63]

Values are expressed in means [95% confidence interval].

TWT 1.54mm [1.46-1.63] and mean non-IMT 0.49 [0.42-0.55]. The diastolic lumen diameter was very similar when assessed by MRI and US: 7.03mm [6.79-7.27] vs. 6.92mm [6.70-7.14], respectively; p=NS.

Vascular wall and CVD risk factors

cEMT did not correlate with age or cardiovascular risk factors, including total cholesterol, systolic blood pressure, waist circumference and CRP as measure of inflammation (table 2). This was in contrast to IMT and TWT . For IMT correlations with age and several risk factors were observed: total cholesterol ($r=0.30$; $p<0.05$), systolic blood pressure ($r=0.41$; $p<0.01$), waist circumference ($r=0.29$; $p<0.01$), C-reactive protein (0.29; $p<0.01$) and Framingham risk score ($r=0.55$; $p<0.01$). TWT significantly correlated with age ($r=0.31$; $p<0.05$) and Framingham risk score

Table 2 | Bivariate correlations between cardiovascular risk factors and measures of carotid wall thickness

Variable	Non-IMT	IMT (US)	TWT (MRI)
age (yrs)	0.125	0.406**	0.308*
BMI (kg/m^2)	-0.065	0.320*	0.115
Waist circumference (cm)	-0.069	0.285*	0.101
Systolic blood pressure (mmHg)	-0.088	0.407**	0.269
Diastolic blood pressure (mmHg)	-0.165	0.113	-0.004
Fasting blood glucose (mmol/L)	-0.046	0.241	0.105
Insulin (µU/mL)	-0.163	0.161	-0.016
HOMA-index	-0.171	0.180	-0.008
Total Cholesterol (mmol/L)	0.068	0.295*	0.244
HDL-cholesterol (mmol/L)	0.064	-0.022	-0.067
LDL-cholesterol (mmol/L)	0.030	0.294*	0.214
Triglycerides (mmol/L)	0.035	0.092	0.158
C-reactive protein (mg/L)	-0.106	0.288*	0.135
VEGF (pg/mL)	-0.375**	0.203	-0.175
Framingham CHD risk score (%/10yrs)	-0.032	0.551**	0.420**

Values are expressed as Pearson's correlation coefficients (r).* p<0.05; ** p<0.01.

(r=0.42; p<0.01), the correlations with the other factors were in general weaker than for IMT and did not reach statistical significance (table 2).

Vascular wall and VEGF

cEMT significantly correlated with circulating serum VEGF levels (r=-0.375; p<0.01). In contrast, circulating VEGF levels did not correlate with IMT or TWT (table 2). Post-hoc exploration revealed that cEMT was 0.556±0.241 in patients with VEGF levels below the median compared to 0.422±0.183 in patients with VEGF levels above the median (p=0.03). The relation between cEMT and VEGF was influenced by differences in IMT (figure 3): cEMT values were significantly lower in the high-IMT high-VEGF group (0.310±0.191) compared to the low-IMT high-VEGF group (0.478±0.155, P<0.022) and compared to the high-IMT low-VEGF group (0.622±0.258, p<0.018).

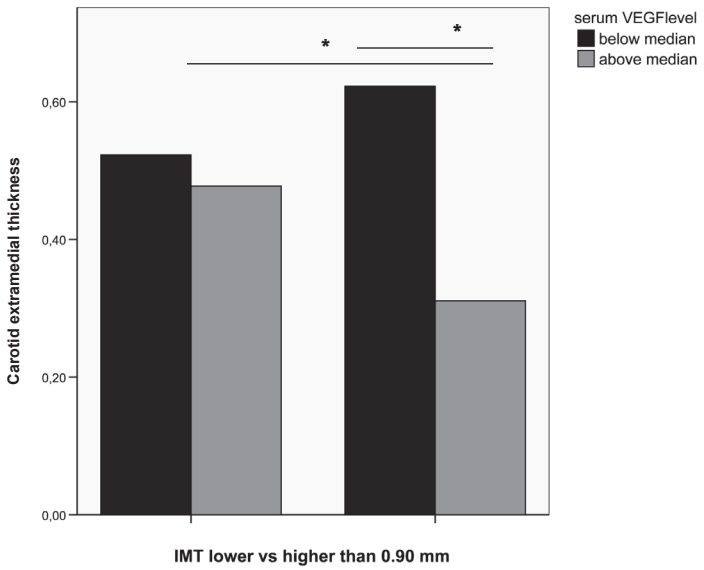

Figure 3 | cEMT values were significantly lower in the patients with elevated IMT levels and VEGF levels above the median (* $p<0.05$)

DISCUSSION

The main findings of the current study are that the combination of MRI and US images may be useful in estimating cEMT and thereby adventitial thickness. cEMT did not correlate with cardiovascular risk factors but does correlate with circulating VEGF levels.

Several imaging techniques are being developed to visualize and quantify adventitial thickness. Our approach was to combine the information yielded by two different carotid artery imaging techniques: 3 tesla MRI and ultrasonography. The accuracy of both individual techniques has been validated by comparison to the gold standard of thickness measurements by light microscopy. For IMT the far wall is the sampling area of choice as it has been shown that the medial-adventitial signal closely mirrors histology in contrast to signal of the near wall (29). Consequently, we used far wall IMT values only and the corresponding MRI images. Using a combined imaging approach requires that measurement location should be identical and the sampling should be performed within a reasonable time span. Ultrasound provides cross-sectional images in a longitudinal plane

whereas MRI images yield a transverse image. We approached this challenge by using an ultrasonography protocol with 4 angles of investigation to get a more accurate estimate of the average wall thickness.

In our study cEMT did not correlate with traditional cardiovascular risk factors as opposed to IMT and TWT. This may well be due to the limited sample size. A previous study showed that elevated ultrasound-based cEMT values were associated with hypertension, diabetes and dyslipidemia, indepandant of the intima-media value (28). In our sample cEMT did correlate inversely with circulating VEGF levels. This preliminary finding seems at odds with experimental studies showing that increased local VEGF expression promotes adventitial growth (5) (114) (30). Further study on the role of vasa vasorum angiogenesis will be required, a combined ultrasound and MRI approach may allow for such pathophysiological studies in vivo.

Our study has limitations. We included a small sample of subject and further validation of cEMT measurements is required. The study was cross-sectional and though the association with VEGF levels are potentially interesting our findings are preliminary.

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CHAPTER 6

The role of inflammation on atherosclerosis, intermediate and clinical cardiovascular endpoints in type 2 diabetes mellitus

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ABSTRACT

Background

Type 2 diabetes mellitus (T2DM) is associated with increased cardiovascular morbidity and mortality. Sub-clinical systemic inflammation is often present in T2DM patients. Systemic inflammation has also been implicated in the pathophysiology of atherosclerosis.

This review investigates the direct evidence present in literature for the effect of inflammation on atherosclerosis, specifically in the setting of T2DM. Special emphasis is given to the pathogenesis of atherosclerosis as well as intermediate and clinical cardiovascular endpoints. The important role of deteriorated endothelial function in T2DM was excluded from the analysis.

Methods

Extensive literature searches were performed using the PubMed and Web of Science databases. Articles were identified, retrieved and accepted or excluded based on predefined criteria.

Results

Substantial evidence was found for an important inflammatory component in the pathogenesis of atherosclerosis in T2DM, demonstrated by inflammatory changes in plaque characteristics and macrophage infiltration. Most epidemiologic studies found a correlation between inflammation markers and intermediate cardiovascular endpoints, especially intima-media thickness. Several, but not all clinical trials in T2DM found that reducing sub-clinical inflammation had a beneficial effect on intermediate endpoints. When regarding cardiovascular events however, current literature consistently indicates a strong relationship between inflammation and clinical endpoints in subjects with T2DM.

Conclusion

Current literature provides direct evidence for a contribution of inflammatory responses to the pathogenesis of atherosclerosis in T2DM. The most consistent relation was observed between inflammation and clinical endpoints.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is an independent risk factor for cardiovascular morbidity and mortality. Relative risk of cardiovascular disease is increased 2-3 times in diabetic men and 3-4 times in diabetic women compared to non-diabetic controls.[1-5] Atherosclerosis is the major causal factor for these cardiovascular events. Atherosclerotic plaque formation precedes the clinical signs and symptoms of cardiovascular disease. When the plaque ruptures, thrombus-formation causes rapid vascular occlusion and subsequent myocardial infarction, stroke or death.

Atherosclerosis is now considered an inflammation-driven process. In non-diabetic subjects, the presence of atherosclerosis is linked to a state of chronic systemic sub-clinical inflammation and local inflammatory mediators play a key role in the formation and eventual rupture of plaques.[6-8] The presence or development of T2DM is also associated with sub-clinical systemic inflammation. [9] In this review several examples of inflammatory pathways in T2DM are used to illustrate this association. Therefore it is likely that inflammation is an important component in the development of atherosclerosis specifically in the setting of T2DM. This paradigm seems to be generally accepted. However, due to potential differences in the pathogenesis of atherosclerosis between T2DM and non-diabetic patients, it may not be prudent to extrapolate the contribution of inflammation to atherosclerosis in T2DM from studies in non-diabetic models or patients. The strength of the evidence originating from original research directly supporting the abovementioned theory has to date not been reviewed.

Therefore the aim of the current review was to investigate the evidence present in original research publications regarding the role inflammation on atherosclerosis and its sequelae in T2DM. Special emphasis was given to the pathogenesis of atherosclerosis in diabetic patients and preclinical models, using the pathways of advanced glycation end products and the ubiquitin-proteasome system as illustrative examples. The effects of inflammation on intermediate and clinical cardiovascular endpoints in T2DM are reviewed in the final two paragraphs. The important role of endothelial dysfunction and the nitrous oxide system in the pro-inflammatory profile of diabetes have been reviewed elsewhere.[10]

METHODS

Literature search was performed in the PubMed and Web of Science databases. Table 1 summarizes the keywords that were used for each of the three paragraphs, the number of references found (both original papers and reviews) and the number of original papers deemed relevant for citation in this review. Articles were identified, retrieved and accepted or excluded using predefined criteria: original articles had to be primarily designed to address the effect of inflammation on atherosclerosis or cardiovascular endpoints, in the setting of diabetes mellitus. If this criterion was not fulfilled, papers were eligible if inflammation was included the article as a predefined parameter of contrast in the study design, or if the role of inflammation on study endpoints has been emphasized in the publication. Several articles were included in which the effects of inflammation on atherosclerosis were evaluated in both diabetic and non-diabetic subjects. However, if the results in diabetic subgroups were not mentioned separately the articles were rejected. Review articles were excluded from the current work, although they have occasionally been used to obtain additional relevant references.

Table 1 | Keywords used and number of references found per topic of review.

section	Keywords used for search
I	atherosclerosis; inflammation; diabetes mellitus
II	diabetes mellitus; inflammation; CRP OR c-reactive protein; fibrinogen; interleukin-6; IMT OR intima-media thickness; FMD OR flow mediated dilatation OR endothelial function OR endothelial dysfunction; PWV OR pulse wave velocity OR arterial stiffness; cardiovascular magnetic resonance; cac OR coronary artery calcification
III	diabetes mellitus; inflammation; CRP OF c-reactive protein; cardiovascular events; cardiovascular disease; cardiovascular mortality; ARIC; Rotterdam; Procam; Monica

section	Total search	Review	Original	Included
I	428	219	209	12
II	400	142	258	14
III	2045	620	1425	11

Original articles were included if data were given on inflammation and atherosclerosis in the setting of type II diabetes mellitus. Roman numerals indicate the following topics: I) pathophysiology; II) intermediate cardiovascular endpoints; III) cardiovascular events

RESULTS

The Pathogenesis of Atherosclerosis in Diabetes Mellitus

Twelve papers were retrieved that could be used to review the inflammatory processes involved in the pathogenesis of atherosclerosis in the setting of T2DM. Atherosclerotic coronary plaques of diabetic subjects were found to show different characteristics with more inflammatory cell infiltration and significantly larger necrotic core size compared to non-diabetic subjects.[11;12] Macrophage plaque area, T-cell infiltration and HLA-DR expression were significantly increased in diabetic patients. The pro-inflammatory characteristics of the plaque in T2DM were also illustrated by an increase of TNF-α expression.[13] Furthermore, an increased expression of the receptor for advanced glycation end products (RAGE), which will be discussed in more detail later, was observed in diabetic plaques. The inflammatory changes observed in atherosclerotic plaques in human diabetic subjects are paralleled by very similar plaque characteristics observed in diabetic animal models. In murine models, an increase in plaque area was found in the aorta of diabetic versus non-diabetic mice. In addition, these larger plaques had qualitatively changed in an inflammatory fashion as was shown by increased expression of platelet-derived growth factor B, platelet-derived growth factor receptor, vascular cell adhesion molecule-1 and marked macrophage infiltration.[14;15] In the atherosclerotic plaques of mice with induced diabetes higher numbers of inflammatory cells and pro-atherogenic proteins were found.[15;16]

Thus, several observations point to the presence of an exaggerated inflammatory component in the diabetic atherosclerotic plaque. In contrast, no inflammatory changes but more fibrosis and thrombotic complications were observed by Sommeijer et al., in 11 carotid atherectomy specimens from patients with diabetes compared to 12 specimens from a matched patient group without T2DM.[17] In this study no differences were observed in the amount of SMCs, macrophages, T-cells and AGEs. Furthermore, no differences were found in the presence of tissue factor, endothelial protein C receptor, nuclear factor kappa B (NF-$_{\kappa}$B), and carboxymethyl-lysine-staining between lesions from T2DM patients and controls. Several explanations for this lack of relationship were suggested by the authors. The lesions were all obtained from symptomatic plaques and the

population in this study had a mean age of 70 years. AGE accumulation in tissue is more pronounced in end-stage plaques and advanced age, both in diabetics and non-diabetics.[18] Therefore, differences in inflammatory characteristics of atherosclerotic plaques between diabetics and non-diabetic controls may be more evident in younger subjects with early-stage plaques.

Further exploration revealed several pathways linking inflammation to atherosclerosis in T2DM . It is beyond the scope of the current work to review all these pathways. However, we will discuss two important mechanisms currently under active investigation and showing promise as future therapeutic targets. The receptor for advanced glycation end products (RAGE)-pathway and the ubiquitin-proteasome pathway are related to altered intimal inflammatory responses, especially in the setting of T2DM. Although the current review focuses mainly on the clinical cardiovascular implications of inflammation in T2DM the following paragraph includes several pre-clinical animal studies to clarify and illustrate the cellular mechanisms linking inflammation, atherosclerosis and T2DM.

RAGE

Advanced Glycation End products (AGEs) are formed intra- and extracellularly by non-enzymatic reduction of glucose, lipids and amino acids on proteins and nucleic acids. This reaction is partly driven by hyperglycemia, resulting in higher circulating- and tissue-levels of AGE in diabetic subjects. (20-30% higher in patients with uncomplicated T2DM[19] and 40-100% higher in patients with coronary[20] and renal[21] complications associated with T2DM). In T2DM, higher levels of AGEs are associated with overexpression of receptors for advanced glycation end products (e.g. AGE-R1 and RAGE). The positive role of these receptors (especially AGE-R1) is to clear AGEs from the circulation and mitigate their deleterious oxidative and inflammatory effects[22;23]. In contrast, RAGE appears to trigger a pro-inflammatory stress response, leading to cellular dysfunction[24]. RAGE is expressed in several tissues, but also in atherosclerotic plaques, more specifically in macrophages and around necrotic cores[25-27]. Non-diabetic subjects also express RAGE, but to a lesser extent and at a later age. The expression of RAGE has implications for plaque biology including influx of inflammatory cells. This may be due to the induction of numerous cytokines and adhesion molecules such as IL-6, TNF-a, MCP-1, ICAM-1 and VCAM-1. Furthermore, RAGE induced activation of cyclo-oxygenase 2 (COX-2), prostaglandine E

synthase-1 (mPGES-1), matrix metalloproteinase 2 (MMP-2) and MMP-9[28-32] may adversely affect plaque biology. In addition, it has been shown that RAGE is associated with enhanced NF- κB activity,[31-33] which directly links RAGE to the main regulator of the cellular inflammatory response. Moreover several studies have demonstrated that blocking RAGE action leads to attenuation of inflammation and plaque stabilisation.[33;34] Thus, increased RAGE-mediated cellular responses may link the biochemical consequences of hyperglycemia in T2DM to inflammatory atherogenesis and vulnerable plaque formation, by different pathways. Blockade of RAGE action is an important potential target for plaque stabilization in diabetic patients.

Ubiquitin-Proteasome system

There is emerging evidence that the ubiquitin-proteasome system, a major protein degradation pathway in eukaryotic cells, induces inflammation during the initiation and progression of atherosclerosis.[35] The pathway is required for activation of NF-κB, by degradation of its inhibitory IκB proteins.[36] Marfella et al.[13] studied the role of the ubiquitin-proteasome system in human carotid endarterectomy samples, and compared diabetic subjects with non-diabetic controls. Notably, ubiquitin-proteasome activity was found to be enhanced in diabetic atherosclerotic lesions, especially in the inflammatory cells therein. Activation of this system was associated with higher NF-κB and MMP activity, thereby leading to an increase in inflammation and potential destabilization of the plaques. Administration of the PPAR gamma agonist rosiglitazone inhibited the ubiquitin-proteasome pathway and partially attenuated the inflammatory changes in plaque composition. This effect was seen in vivo when rosiglitazone was given orally to subjects before endarterectomy and in vitro when rosiglitazone was added to incubated monocytes from the carotid specimens. Upregulation of the ubiquitin-proteasome pathway could be one of the mechanisms contributing to the increased inflammatory response in diabetic atherosclerotic plaques. Inhibition of this system may be a useful therapeutic target in the treatment of vulnerable diabetic plaques.

To summarize, original research articles show that the diabetic atherosclerotic plaque has prominent inflammatory characteristics, including high macrophage and T-cell content. These pro-inflammatory aspects are observed in human

subjects and experimental models. The observations in human subjects are not unequivocal and could not be demonstrated in carotid endarterectomy specimens in one study. Among the proposed mechanisms involved at the level of the intima, RAGE and the ubiquitin-protease system seem to be potential links between diabetes and the inflammatory processes of atherosclerosis.

Intermediate Endpoints

Intermediate endpoints are surrogate markers which reflect the presence and the progression of a disease. Examples of intermediate endpoints for cardiovascular disease are Intima-Media thickness (IMT), Pulse Wave Velocity (PWV) and Flow Mediated Dilatation (FMD). There are ample epidemiological and trial data to support the use of these intermediate endpoints to provide us with valuable information regarding atherosclerosis-related processes and clinical outcomes. [37;38]

Epidemiological studies

Major studies in which intermediate endpoints were assessed include the Atherosclerosis Risk in Communities (ARIC) study, the Framingham study and The Rotterdam Study. Although no published papers from these studies were set up to directly address the role of inflammation on the endpoints specifically in T2DM patients, they all included a subpopulation of diabetics. Only two large studies assessed the correlation between inflammation and intermediate endpoints in T2DM. Metcalf et al.[39] did a post hoc analysis in a part of the ARIC-population and compared a subpopulation of 921 patients with T2DM to 11.964 non-diabetic controls. Several hemostatic proteins were included in the analysis, but only fibrinogen correlated significantly with IMT in both groups. Correlation coefficients between fibrinogen and IMT are not reported separately but the relationship was significant and independent of other CVD risk factors. As a part of the INVADE-study, associations between CRP and IMT were assessed in 3,534 people, aged >55 years old, of which 882 had T2DM. [35] Multiple regression analysis revealed a positive correlation between CRP and IMT progression in T2DM patients (β=0.08; p=0.01). In contrast, in the non-diabetics CRP levels were not significantly associated with IMT progression (β=0.029; p=0.29). These data suggest that low grade systemic inflammation is relevant for the progression of atherosclerotic changes in the vessel walls of

diabetic subjects. Five smaller studies used CRP as a marker for inflammation and showed significant correlations with either IMT [40-42], FMD [43] or arterial stiffness [43;44]. Two studies found a correlation between fibrinogen and IMT [39;42], one study found a correlation between fibrinogen and arterial stiffness [44]. The results of these studies are summarized in table 3. Studies using other inflammation markers, besides the more commonly used inflammatory markers CRP and fibrinogen, have shown similar results. Leukocyte count, amyloid A protein and sialic acid significantly correlated with arterial stiffness. [45] IL-18, which stimulates release of interferon-γ, significantly correlated with IMT(r=0.224; p=0,042) and arterial stiffness (r=0.232, p=0.040) in T2DM.[46] These studies clearly show positive associations between markers for inflammation and intermediate endpoints in T2DM. However, some studies are at variance with these observations. Takebayashi et al. found no correlation between CRP or fibrinogen and IMT in 73 patients with T2DM and poor metabolic control.[47] Leionenen et al. did not find CRP as a determinant of IMT in 239 T2DM patients with cardiovascular disease.[48]. Sigurdardotter et al. concluded that there is no independent relationship between CRP and IMT in Caucasian men with either newly diagnosed or established T2DM [49]. Dullaart et al. found no correlation between CRP and IMT in 84 T2DM patients nor in the 85 controls included in their population[50]. The T2DM patients were very well controlled with use of blood glucose lowering and antihypertensive medication.

Thus, although the majority of data point toward a positive association between low grade systemic inflammation and surrogate cardiovascular endpoints in T2DM, these observations are not unequivocal. Further clarification may come from prospective studies, including clinical trials.

Clinical trials

Several clinical trials have been carried out to study the effect of pharmacological interventions on subclinical inflammation in T2DM. In four different studies IMT or FMD were used as intermediate markers. Medication in these different studies included metformin, atorvastatin, pioglitazone and rosiglitazone. In all studies, CRP levels were measured and related to IMT or FMD.

Rosiglitazone, compared to metformin, induced a prompt and marked reduction in CRP levels in diabetics.[51] This change was associated with regression of carotid IMT, independent of the blood glucose lowering effect of

the medication. This suggests that there is a direct correlation between CRP and IMT in T2DM. Another study found significant beneficial effects of rosiglitazone on both subclinical inflammation markers (CRP, MMP-9 and fibrinogen) and IMT in diabetics. However, after secondary analyses no correlation was found between the changes in inflammation markers and IMT regression.[52] Atorvastatin, compared with placebo, significantly decreased CRP levels and improved FMD.[53] Notably, there was no correlation between the percentage change in LDL-C and improvement of FMD, whereas the reduction of CRP levels was significantly associated with improvement of FMD. Pioglitazone treatment resulted a significant decrease in CRP concentrations and improvement of FMD irrespective of metabolic changes[54]. However, a correlation between CRP en FMD was not found, possibly due to the small sample size of the study.

To summarize, most data found about the effect of inflammation on intermediate cardiovascular endpoints support the concept of a positive relation between inflammation and atherosclerosis in the setting of T2DM. However, these observations are not unequivocal and some studies and clinical trials raise questions by demonstrating a lack of association between inflammatory parameters and surrogate markers of atherosclerosis. Moreover, the accuracy of these intermediate endpoints to predict future cardiovascular events is not undisputed, especially in T2DM populations. This point was recently illustrated by the discussion about the effects of rosiglitazone. Despite encouraging results from studies using intermediate endpoints, a recent meta-analysis questions the beneficial effect of rosiglitazone on the occurrence clinical cardiovascular events. Therefore the final paragraph will review the evidence in T2DM for associations between inflammatory markers and clinical cardiovascular endpoints.

Clinical Endpoints

The aim of this section is to review evidence linking CRP and fibrinogen as inflammatory markers to clinical cardiovascular outcomes in T2DM. Cardiovascular events that were included in our research are coronary heart disease (CHD: coronary heart disease related death, myocardial infarction, coronary revascularization), peripheral arterial disease (PAD: leg revascularisation, leg amputation, intermittent claudication) and stroke.

Population-based studies

Many large population-based studies have assessed the importance of different cardiovascular risk factors, including inflammation on clinical outcomes. We evaluated these epidemiological studies for evidence of inflammation being a risk factor for the development of cardiovascular events in T2DM (sub)populations. The following studies were included: the Atherosclerosis Risk In Communities study (ARIC), the Framingham heart study, the Munster heart study (PROCAM), the Rotterdam study, the Cardiovascular Health study, the Multinational Monitoring of trends and determinants in Cardiovascular disease study (MONICA) and the Hoorn study. All studies observed a significant positive correlation between CRP levels and incidence of cardiovascular events[55-62]. However, a separate analysis of the diabetic population was made only in the Cardiovascular Health study and the Hoorn study (table 2). Relative risk for the diabetics with CRP >3 mg/L versus those with CRP level <1 mg/L was 1.49 (95%CI 1.02-2.18) whereas in non-diabetics the relative risk was found to be 1.74 (95% CI 1.30-2.32).[57] The Hoorn study provided long-term follow-up data on the effect of cardiovascular risk factors on mortality in a general population (n=2484) in The Netherlands. In a sub-analysis [63] (n=631) it was demonstrated that approximately 43% of the excess cardiovascular mortality in T2DM patients was explained by endothelial dysfunction and low-grade inflammation. The negative impact of endothelial dysfunction on survival was greater in diabetic patients than in non-diabetic patients (hazard ratio for cardiovascular mortality 1.87 [1.43-2.54] in diabetics vs. 1.23 [0.86-1.75] in non-diabetics; p=0.06). However, the contribution of low-grade inflammation to cardiovascular mortality was not significantly different in diabetic and non-diabetic subjects (hazard ratio for cardiovascular mortality 1.43 [1.17-1.77], not mentioned separately for diabetics). These findings suggest that, in the Hoorn study, the presence of endothelial dysfunction (with or without low-grade inflammation) was the main determinant of excess cardiovascular risk in diabetics compared with non-diabetic subjects. The impact of low-grade inflammation alone on cardiovascular mortality, although significant, was not different in diabetic subjects compared to their non-diabetic counterparts.

Table 2 | Summary of studies evaluating the effects of inflammatory markers on surrogate cardiovascular markers in T2DM patients.

Intermediate endpoint studies					
Authors	**n**	**% T2DM**	**Inflammatory marker**	**CVD marker**	**Correlation (coefficient)**
Metcalf PA et al.[39]	12.876	7.2	Fibrinogen	IMT	+ (NR)
Mita T et al.[40]	75	100	CRP	IMT	+ (r=0.484, p<0.0001)
Sander D et al.[69]	3534	25.0	CRP	IMT	+ (B=0.08, p=0.01)
Corrado E et al.[70]	200	50	CRP Fibrinogen	IMT	+ (r=0.591, p<0.0001)
Hedblad B et al.[52]	555	50	CRP	IMT	+ (NR)
Wakabayashi I et al.[45]	97	100	Fibrinogen	PWV	+ (r=0.216, p<0.050)
Nakamura A et al.[46]	82	100	IL-18	IMT PWV	+ (r=0.225, p=0.042) + (r=0.232, p=0.040)
Nystrom T et al.[43]	45	30.8	CRP	FMD	None (r=-0.4, p=NS)
Leionen ES et al.[48]	239	100	CRP	IMT	None (NR)
Sigurdardottir V et al.[49]	271	27.3	CRP	IMT	None (r=0.20, p=NS)
Dullaart RP et al.[50]	169	49.7	CRP TNF-alpha	IMT	None (NR) None (NR)

Most authors report a positive association. In several series no association could be demonstrated. T2DM=type 2 Diabetes Mellitus; CVD=cardiovascular disease; IMT=intima-media thickness; PWV=pulse wave velocity; FMD=flow mediated dilatation; CRP= C-reactive protein; IL=Interleukin; TNF=tumour necrosis factor; NR= not reported

Prospective follow-up studies

In several prospective cohorts as well as cross sectional studies, populations were analyzed in order to determine whether inflammation and its markers played an important role in cardiovascular risk assessment. Only studies specifically addressing T2DM patients and studies with large diabetic subpopulations were included. In a study performed by Matsumoto et al. 350 Japanese T2DM patients were followed for a period of 1-7 years. When patients were subdivided in tertiles according to baseline CRP levels, the relative risk for cardiovascular events in patients in the highest tertile of CRP was 2.00 (95% CI 1.03-3.85) compared to patients in the lowest tertile.[64] High plasma levels of CRP were also associated with an increased risk of incident cardiovascular events among 746 T2DM men in a study performed by Schulze et al. A relative risk of 2.62 (95% CI 1.29-5.32) for cardiovascular events was observed in T2DM patients in the highest quartile of CRP compared to those in the lowest quartile was during a five year follow-up period.[65] Wattanakit et al. followed 1651 diabetic subjects for 10 years using

Table 3 | Summary of studies evaluating the effects of inflammatory markers on cardiovascular events in T2DM patients.

Large population-based studies				
Study (authors)	**n**	**% T2DM**	**Correlation CRP-CVE**	**Correlation assessed in diabetes specifically**
ARIC *(Ballantyne et al.[55])*	12819	21%	+	no
ARIC *(Folsom et al.[58])*	15792	6,8%	+	no
Framingham *(Rost et al.[61])*	1462	9,4%	+	no
PROCAM *(Heinrich et al.[59])*	2116	unclear	+	no
Rotterdam *(Bos et al.[56])*	6340	10,7%	-	no
MONICA *(Koenig et al.[60])*	3435	5,7%	+	no
Cardiovascular Health *(Cushman et al.[57])*	3971	14,4%	+	yes

Clinical cardiovascular end-point studies				
Authors	**n**	**% T2DM**	**Follow-up period**	**RR (95% CI)**
Matsumoto et al.[64]	350	100	1-7 years	2.00 (1.03-3.85)
Schulze et al.[65]	746	100	5 years	2.62 (1.29-5.32)
Schillinger et al.[67]	454	39,9	21 months	2.13 (p=0.007)
Wattanakit et al.[66]	1651	100	8.7 years	+
Jager et al.[68]	610	27,7	5 years	1.4 (0.6-3.5)

In the large-scale epidemiological studies most authors did not specifically report associations in the T2DM sub-population. The studies that assessed diabetics demonstrated significant elevation of relative risk of a cardiovascular event of around twofold in subjects with high inflammatory markers, compared with low levels. *T2DM=type 2 diabetes mellitus; CRP=C-reactive protein; CVE=cardiovascular events; RR=relative risk*

fibrinogen levels as marker for inflammation. Risk for peripheral artery disease was found to be higher in subjects with high fibrinogen levels. The relative risk for cardiovascular events of patients in the highest quartile of fibrinogen compared to those in the lowest was 2.52 (95% CI 1.43-3.24)[66]. Schillinger et al. followed 454 patients, of which 181 had T2DM, for a median of 21 months. HbA1c and CRP were measured at baseline. Levels of CRP correlated positively with incident cardiovascular events. Hazard ratio for subjects in the highest quartile of CRP compared to those in the lowest was 2.13 (95% CI 1.22-3.70). The deleterious effect of CRP on event risk was more evident in subjects with high HbA1c levels [67].

In contrast to these studies, Jager et al. did not observe a significant elevation of relative cardiovascular mortality risk in patients in the highest tertile of CRP compared to those in the lowest (RR= 1.34; 95%CI: 0.41-4.43). The study was

performed in a sample of 169 T2DM subjects selected from a larger population of 610 subjects and followed for 5 years.[68]

Cross-sectional studies

In 202 diabetic patients with or without macrovascular disease mean CRP levels did not significantly differ between the groups (4.2mg/L and 5.5 mg/L respectively). Fibrinogen levels however, were significantly higher in subjects with prevalent macrovascular disease (420 mg/L versus 382 mg/L).[62]

To summarize, several large epidemiological studies showed a positive correlation between levels of inflammation markers and incidence of cardiovascular events. However, whether this can also be found in diabetic subjects was not specifically answered in most of these studies. The one study that did specifically analyse the diabetic population showed that CRP was of added value in the risk assessment in diabetes[57]. All but one follow-up study revealed that higher levels of CRP and fibrinogen were associated with higher risk for cardiovascular events[64-68]. This observation could not be extended to cardiovascular mortality[68]. Based on the current literature it seems that levels of inflammation markers predict incident cardiovascular disease, also in the setting of T2DM.

CONCLUSIONS

At the level of cellular pathogenesis of atherosclerosis, significant differences are seen between diabetic subjects and non-diabetics. Atherosclerotic plaques show higher expression of inflammatory receptors and proteins, marked infiltration of inflammatory cells and larger necrotic-core size in the setting of T2DM. It is likely, but not unequivocally proven, that increased inflammation markers in atherosclerotic plaques are related to systemic inflammation.

Regarding intermediate endpoints there was a positive correlation with inflammation in most but not all studies in T2DM. The impact of inflammation seems to be more outspoken when related to clinical endpoints compared to intermediate endpoints. High inflammation markers are linked with more cardiovascular events, of which most are to be expected to have a negative

influence on life expectancy. This may support the hypothesis that increased cardiovascular risk in diabetes in not only due to increased progression of atherosclerosis (as measured by most intermediate endpoints) but also to more severe atherothrombosis.

Whether levels of CRP and fibrinogen can be seen as causal factors for developing cardiovascular events in type 2 diabetes patients has still to be proven. To date, most studies used them as markers of a state of low grade systemic inflammation. However, based on the current review linking systemic inflammation to the pathophysiology and outcome of atherosclerosis in T2DM, it seems warranted to further unravel the components of the systemic and local inflammatory components in prospective research. Hopefully this will result in new therapeutic strategies to treat the major cause of mortality in patients with T2DM.

Learning Points

- Current literature indicates that atherosclerotic plaques in the setting of type 2 diabetes mellitus exhibit more inflammatory properties than non-diabetic plaques
- Most published data suggest that elevated inflammatory markers are associated with detrimental outcome of intermediate cardiovascular endpoints in patients with type 2 diabetes mellitus. This association, however, is not unequivocally established
- The association between inflammation and unfavourable cardiovascular outcome in type 2 diabetes patients is most consistent when regarding clinical endpoints

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CHAPTER 7

Vascular Phenotype and Subclinical Inflammation in Diabetic Asian Indians Without Overt Cardiovascular Disease

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ABSTRACT

Although Asian Indian (AI) patients with diabetes mellitus type 2 (DM2) are at high risk for cardiovascular disease (CVD), not all patients develop CVD. The vascular phenotype of AI-DM2 without CVD has not been elucidated and may point to protective features. Using baseline data from a clinical trial we provide an initial description of vascular parameters in AI-DM2 compared to European Caucasian controls (ECs) matched for age and gender. Endpoints of the study were endothelial function, low-grade systemic inflammation (CRP), and carotid intima-media thickness (cIMT).

AIs had longer duration of diabetes, worse glycemic control and more microangiopathy. Both groups demonstrated marked endothelial dysfunction. CRP levels were similar: 1.7 (4.9) mg/L in AIs and 2.8 (3.6) mg/L in ECs. cIMT values were significantly lower in AI-DM2 than EC-DM2 (0.655mm (0.12) vs. 0.711mm (0.15), p=0.03). Multiple regression analysis showed that variability in CRP was mainly determined by waist circumference, not by ethnicity. In contrast, ethnicity was a significantly explanatory variable for cIMT.

Vascular phenotype of AI-DM2 without CVD was characterized by endothelial dysfunction and relatively low levels of CRP, comparable to EC-DM2 controls. In contrast, lower cIMT values were observed in AI-DM2 despite longer duration of diabetes and worse metabolic control. We propose that mechanisms slowing its progression may have atheroprotective potential in AI-DM2.

INTRODUCTION

In the Netherlands a large community from the former Dutch colony of Surinam, originally of Asian Indian (AI) descent, has settled. Epidemiologic data suggest that the excess of type 2 diabetes mellitus (DM2) and cardiovascular disease (CVD) noted in AI populations across the world [1] [2] is also present in AIs in the Netherlands [3] [4]. An important pathogenic factor is the high prevalence of insulin resistance and DM2 in AIs. However, traditional risk factors do not fully explain the excess of CVD [5]. Several other risk factors such as low grade systemic inflammation [6] [7] and endothelial dysfunction [8] [9] have been proposed to contribute to initiation and progression of atherosclerosis in AIs.

Despite the well-established high cardiovascular risk, not all AI-DM2 develop CVD. The vascular phenotype of AI-DM2 without CVD has not been elucidated and may point to protective features regarding the development of CVD. We aimed to provide a first evaluation of vascular parameters in AIs and matched EC controls with DM2 but without CVD.

MATERIALS AND METHODS

Subjects

This study is a substudy of a previously reported randomized clinical trial. The study design and results of which have been described elsewhere[10-12]. Using this database, we were able to identified 48 subjects of AI descent and 48 EC subjects from the same cohort matched for age and gender. There were no differences in demographics between the two groups, both living in an urban area in the Netherlands. The predecessors of the AI population migrated from India to Surinam starting 1873. Most of our study subjects were first generation immigrants in this country and third or fourth generation out of India. Patients were eligible for the study if they had been diagnosed with DM2 for at least 1 year, aged 30-80 years and without CVD. CVD was defined as angina pectoris, clinically manifest coronary artery disease, ECG criteria for a past myocardial infarction, ischemic stroke, peripheral artery bypass surgery, percutaneous transluminal angioplasty, or amputation because of atherosclerotic disease. Patients with marked dyslipidemia (fasting total cholesterol >6.9 mmol/l or triglycerides >6.0

mmol/l) were excluded from the original population, as prior statin therapy was an exclusion criterion in the clinical trial. Eligible patients gave their written informed consent. The study was approved by the hospital's Medical Ethics Committee.

Endpoints

The endpoints of this study were differences in inflammatory markers (serum C-reactive protein (CRP) and fibrinogen levels), endothelial function (as estimated using measurement of flow mediated dilatation (FMD)) and cIMT as a non-invasive measure of atherosclerosis. Furthermore, presence and risk of coronary atherosclerosis was assessed using measurement of silent coronary ischemia (ambulatory ECG) and UKPDS risk scores for CVD.

Clinical Examination

Anthropometric measurements were performed by two observers using standardized methods. Waist circumference (WC) was measured midway between the iliac crest and the lowest costal margin at the end of normal expiration; hip circumference (HC) was measured at the maximal circumference at the level of the femoral trochanters. Blood pressure was measured using a standard sphygmomanometer after a 10 minute resting period in supine position. Hypertension was defined as systolic BP ≥140mmHg and/or diastolic BP ≥90mmHg. The presence or absence of retinopathy was determined from the subject's medical files, wherein reports from ophthalmologists were retrieved.

Laboratory Investigations

Lipid and safety measurements were performed at the Department of Clinical Chemistry and Hematology of the Leyenburg Hospital, according to ISO 15189 standard procedures. Blood samples were collected after an overnight fast. A urine sample was collected for the determination of the albumin over creatinine ratio. Serum or plasma was isolated by centrifugation at 2900 rpm for 5 minutes. Levels of total cholesterol and triglycerides were measured by enzymatic methods on a Synchron LX20-analyzer (Beckman Coulter, Brea, USA). LDL-cholesterol was calculated according to the Friedewald formula[13]. If triglycerides were above 4.5 mmol/l, LDL-cholesterol was measured directly with the use of a reagent kit (Genzyme Diagnostics). HDL-cholesterol levels were determined after dextran

sulfate-magnesium precipitation of apolipoprotein B-containing lipoproteins. Creatinine kinase and alaninaminotransferase were measured by an enzymatic rate method on a Synchron LX20 multichannel chemistry analyzer, according to IFCC-methods. HbA1c was measured by HPLC on a Variant II (BioRad, USA). For the urine sample, a Jaffé rate method was used for the measurement of creatinine on a Synchron LX20-analyzer, while albumin was measured by rate nephelometry. Presence of microalbuminuria was defined as >2.5 gram albunim/mol creatinine for men and >3.5 gram albumin/mol creatinine for women.

The high-sensitivity CRP assay was performed in the Leiden University Medical Center with the Tinaquant CRP (latex) high-sensitive assay from Roche. This particle enhanced immunoturbidimetric assay was carried out on a Roche Module P using serum.

CVD risk scores, aECGs and Metabolic Syndrome criteria

Absolute 10-year risk scores for developing a cardiovascular event were calculated using the UKPDS risk engine version 2.0 [14]. For patients using anti-hypertensive medication the systolic blood pressure was arbitrarily set at 160 mmHg. The aECG registration and analysis were conducted as previously described [15]. Criteria for the presence of the metabolic syndrome (MS) were according to the European Group for the Study of Insulin Resistance modification of the WHO guidelines[16,17]: presence of DM2 (per definition in our population), and 2 or more of the following characteristics: waist circumference ≥ 94cm in males and ≥ 80cm in females; triglycerides > 1.7 mmol/L; HDL-cholesterol < 0.9 mmol/L in males and < 1.0 mmol/L in females; blood pressure ≥140/≥90 mmHg.

Ultrasound protocol

Ultrasound imaging was performed with an Acuson Aspen scanner with a linear array 7.5 MHz probe. For FMD, an optimal longitudinal image of the brachial artery at, or just above the elbow, was established and kept stable using a specially designed fixative. The exact FMD protocol was described earlier [12]. For cIMT, all images were recorded digitally for off line, blinded, analysis by an independent core laboratory, Heartcore, Leiden, the Netherlands as described previously [10]. Briefly, the left and right distal 1.0 cm of the common carotid arteries, near and far walls, was examined longitudinally in the angle resulting in an optimal and maximal cIMT (while avoiding plaques). For each segment, three

R-wave triggered images were stored. Mean cIMT was measured, when possible, over the entire 1 cm of the vessel segment. Mean common cIMT was obtained by averaging the mean IMTs of far and near wall, left and right.

STATISTICAL ANALYSIS

All binary data were analyzed using the Pearson chi-square test. All continuous outcome data were significantly skewed and therefore analyzed using the non-parametric Mann-Whitney test or log transformed (hsCRP, lp(a)) before being analyzed using the student t-test. Values are reported as medians (IQR). P-values <0.05 were considered statistically significant. Correlations were calculated with the Spearman's rank test. To test the impact of correlated parameters on the variability of the outcome variables a stepwise regression analysis was performed.

RESULTS

Patient characteristics are given in table 1. Despite similar age distribution AIs had a significantly longer duration of diabetes (12.4yrs vs. 6.3yrs; $p < 0.001$) and worse glycemic control as shown by higher median HbA1c levels (7.85% vs. 7,20%; p=0.006). Microangiopathy was observed more frequently in AIs, as shown by elevated prevalence of retinopathy (29% vs. 6%; p=0.003) and higher level of microalbuminuria (1.3mg/L vs. 0.6mg/L; p=0.009).

Cardiovascular risk and anthropometry

Smoking was less prevalent in AIs compared to ECs, both at present and ex-smokers. No significant differences were observed in hypertension (defined as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg or the use of antihypertensive medication) and family history of CVD in first degree relatives. Lipid parameters including plasma HDL-C levels were comparable in both ethnic groups. Lp(a) was significantly different between the groups (215.5mg/dL (410) in AIs vs. 95.0mg/dL (316) in ECs (p=0.02)). The UKPDS risk scores for myocardial infarction were found to be 14.9% /10years in AIs vs. 10.5% /10 years in ECs (p=NS).

The anthropometric data are summarized in table 2. AIs were significantly

Table 1 | Patient characteristics and Laboratory findings

	Asian Indians (n=48)	European Caucasians (n=48)	p-values
male gender	20 (42%)	20 (42%)	1.0
age (yrs)*	50.7 (8.6)	50.9 (7.6)	0.89
diabetes duration (yrs)*	12.4 (8.2)	6.3 (5.4)	<0.001
HbA1c (%)	7.85 (1.9)	7.20 (1.7)	0.006
retinopathy	14 (29%)	3 (6%)	0.003
microalbunimuria	14 (29%)	8 (17%)	0.15
microalbuminuria† (mg/l)	1.3 (7.7)	0.6 (1.1)	0.009
family history of CVD	16 (33%)	13 (27%)	0.51
hypertension	23 (48%)	19 (40%)	0.41
Smokers	20 (42%)	31 (65%)	0.024
UKPDS (%/10yrs)	14.9 (14.1)	10.5 (13.7)	0.29
creatinine (µmol/l)	80.0 (30)	76.0 (19)	0.32
Clearance (ml/min)	81.2 (34.1)	101.9 (29.7)	<0.001
total cholesterol (mmol/l)	5.2 (1.1)	5.5 (1.1)	0.26
HDL-cholesterol	1.1 (0.4)	1.2 (0.5)	0.26
LDL-cholesterol	3.3 (1.4)	3.5 (1.3)	0.83
triglycerides (mmol/l)	1.6 (1.1)	1.7 (1.2)	0.51
lipoprotein(a)† (mg/dL)	215.5 (410)	95.0 (316)	0.02
fibrinogen (g/L)	3.6 (1.9)	3.2 (1.3)	0.88
hsCRP† (mg/L)	1.7 (4.9)	2.8 (3.6)	0.83
hsCRP≥3.0mg/L	14 (29%)	18 (38%)	0.39

All continuous data are expressed in medians (IQR) and compared using non-parametric test (Mann-Whitney) except:
*Data were normally distributed and expressed in means (sd), compared using student t-test
†Data were compared after log transformation using student t-test

smaller and lighter. EC women had higher values for WC and HC, as well as higher average BMI as compared to AI women. In men no differences were observed regarding these parameters. The MS score was fully comparable in the two groups, and did not change using ethnicity-specific cut-off values as recently proposed by the International Diabetes Federation (data not shown).

Inflammation, endothelial function and vascular parameters

No differences were observed between the groups for low-grade chronic inflammation as assessed by CRP. The median value was 1.7 mg/L (4.9) in AIs vs. 2.8 mg/L (3.6) in ECs (p=0.83). In addition the number of subjects with evidence of low-grade inflammation (defined as CRP levels ≥ 3 mg/L and < 15mg/L) did not significantly differ between the groups and was 14 (29%) in AIs and 18 (38%) in ECs (p=0.39). Median serum levels of fibrinogen were comparable (3.6 g/L (1.9) in AIs and 3.2 g/L (1.3) in ECs; p=0.88).

Endothelial dysfunction was observed in both groups with FMD levels under 2% (table 2) but comparable between AIs and ECs. In both groups 9 subjects (19.1%) had abnormal findings on their aECG suggesting silent ischemia. These 18 subjects had comparable values of IMT (0.730mm (0.18) vs. 0.680mm (0.15) in subjects with normal aECGs, p=0.472). Further analysis of these subjects revealed no ethnic difference for the number of ischemic episodes, the duration of ischemia or the ischemic burden (data not shown).

AIs were found to have significantly lower median cIMT values of 0.655mm (0.12) compared to ECs (0.711mm (0.15); p=0.03). Luminal diameter was not a predetermined endpoint, but was assessed in 66 cases (35 ECs and 31 AIs). In this subset AIs had smaller lumina (7.294mm (1,12) vs 7.770mm (1.05) in ECs; p=0.02).

In a stepwise regression analysis logCRP, IMT, FMD and logLp(a) were entered as dependent variables (table 3). LogLp(a) was included because it has consistently been found to be high in AI patients. Co-variables that were taken into account were: age, race, duration of diabetes, HbA1c, smoking status, WC, LDL- and HDL-cholesterol, triglycerides and systolic BP. FMD was impacted by age only (r^2=0.05; β=-0.01; p=0.003). The strongest determinant of variance in Lp(a) levels was race (r^2=0.11; β=0.37; p=0.003). WC had the greatest impact on variance in CRP levels (r^2=0.24; β=1.24; p=0.001) and race did not contribute significantly. Finally, age and race explained variance in cIMT (r^2=0.13; β=0.004; p=0.005 for age and β=-0.47; p=0.047 for race).

CONCLUSIONS

In this study we observed, for the first time, a low cIMT in AI-DM2 patients without CVD, compared to matched EC counterparts. Low cIMT was present despite longer duration of diabetes and worse glycemic control, the significance of the

Table 2 | Anthropometry and Vascular parameters.

	Asian Indians (n=48)	European Caucasians (n=48)	p-values
height (cm)			
♂	167.5 (12.0)	180.0 (11.0)	<0.001
♀	156.5 (8.0)	164.5 (9.0)	<0.001
weight (kg)			
♂	77.0 (10.0)	87.0 (21.0)	0.003
♀	76.0 (18.0)	96.0 (28.0)	0.001
body mass index (kg/m^2)			
♂	27.1 (5.2)	27.2 (4.9)	0.98
♀	30.8 (7.0)	34.3 (8.9)	0.016
waist circumference (cm)			
♂	97.5 (13.0)	98.0 (15.0)	0.83
♀	100.0 (19.0)	108.5 (20.0)	0.045
hip circumference (cm)			
♂	97.5 (8.0)	102.5 (8.0)	0.42
♀	101.5 (11.0)	109.5 (19.0)	0.001
waist/hip ratio			
♂	1.0 (0.09)	0.99 (0.10)	0.33
♀	0.98 (0.13)	0.99 (0.12)	0.87
metabolic syndrome	33 (69%)	37 (77%)	0.36
MS score*	2.10 (1.1)	2.13 (0.87)	0.92
FMD (%)	1.56 (2.5)	1.88 (2.8)	0.44
cIMT (mm)	0.655 (0.12)	0.711 (0.15)	0.03
abnormal aECG	9/47 (19.1%)	9/47 (19.1%)	1.0

Table 3 | Multiregression analysis

	age	race	waist circumference	model p-value
cIMT (r^2=0.15)	β=0.004 *(p=0.005)*	B=-0.47 *(p=0.047)*	-	0.003
FMD (r^2=0.05)	β=-0.01 *(p=0.045)*	-	-	0.045
log CRP (r^2=0.16)	β=-0.009 *(p=0.136)*	B=0.055 *(p=0.589)*	β=1.24 *(p=0.001)*	0.004
Log Lp(a) (r^2=0.11)	β=0.002 *(p=0.799)*	B=0.371 *(p=0.003)*	β=0.552 *(p=0.137)*	0.035

latter being illustrated by increased measures of microangiopathy in AI-DM2. Longer duration of diabetes and increased prevalence of microalbuminuria are in line with previous publications on AIs in Netherlands, and elsewhere [18] [19]. In addition, the high Lp(a) levels observed in AIs have been previously reported [19]. Thus, the low cIMT is a new and intriguing finding and it was found in a population with very similar characteristics to these earlier reports with one exception: absence of overt CVD, despite presence of DM2 in our study population.

Previous studies have suggested that endothelial function may be more vulnerable in AIs than in ECs and thus contributes to the development of atherosclerosis [20]. In our population of DM2 patients without CVD both ethnic groups exhibited endothelial dysfunction and we could not demonstrate ethnic differences.

CRP is a cardiovascular risk indicator with additional predictive power to the Framingham risk scores [21]. CRP levels were found to be higher in AI migrants compared to native populations in several [7,22] but not all [23] studies. We observed intermediate values of CRP in AI-DM2 and EC-DM2 with medians of respectively 1.7 and 2.8 mg/L. It could be hypothesized that an attenuated individual inflammatory response could be part of a protective phenotype, thus being in line with epidemiologic data relating CRP to CVD. The intermediate CRP levels were observed in AI men and EC men with similar WCs. Using the recent ethnicity specific cut-off values for WC, AI-DM2 patients had a more outspoken abdominal obesity compared to EC-DM2. As several reports in literature link central and overall adiposity to CRP levels in AIs [7,22] [24-27], we expected higher CRP levels in AI-DM2. These relatively low levels of CRP were therefore compatible with the hypothesis of an attenuated inflammatory response in these patients. Further studies should be performed to explore the possible abrogation of inflammation in high-risk subjects without overt CVD.

The most interesting observation was the relatively low cIMT values in AIs, despite longer duration of diabetes and worse glycemic control. This could be a race-related phenomenon, which is in line with the observation of smaller luminal diameters in AIs. To date, IMT studies on predictive power[28,29] have not taken diameter into account. In our subjects a significant variability of IMT for a given diameter was observed (data not shown), indicating the need for further explorative studies. There are no firm data on ethnicity-specific IMT values. We observed a median cIMT of 0.66mm (0.12). Previous IMT studies in AIs have

reported cIMT values of 0.59mm (+/-0.17) in non-diabetics and 0.63mm (+/-0.22) in DM2 patients living in South India [30]; and cIMT values of 0.93 +/- 0.36mm vs. 0.85 +/- 0.21 mm in diabetics with and without retinopathy, respectively have been reported in same population [31]. Based on our and other studies we calculated that future prospective comparative studies in different ethnic groups would require a sample size of at least 115 AIs vs 115 ECs to detect a 0.05mm difference in cIMT with a power of 0.80 and a two-sided significance of 0.05

The low cIMT observed may also have been due to pathophysiologic differences between AI-DM2 and EC-DM2 leading to slower progression of cIMT. Pathophysiologic changes directly related to diabetes seem unlikely as DM2 was milder in EC than in AI in this study. Thus, low cIMT is for instance not readily explained by decreased glycosylation of the extracellular matrix. Other candidate pathophysiologic mechanisms influencing cIMT progression in AI-DM2 could be endothelium dependent, i.e. intrinsic or environmental acquired resistance against oxidative stress. In this regard the lower smoking rates in AI-DM2 may be of relevance. Mechanisms could also be endothelium-independent and more related to the pathophysiology of the intima. An attenuated intimal inflammatory response, in line with the intermediate levels of low-grade inflammation observed, would be such a mechanism.

In summary, the data presented provide a first description of vascular parameters in AI-DM2 from Surinam *without* CVD. In these patients, we observed ethnicity-defined, significantly lower cIMT than EC-DM2, despite presence of a number of robust cardiovascular risk factors. Following this interesting observation, reported for the first time, we propose that atheroprotective mechanisms are in play, slowing progression of cIMT and CVD. This and other similar AI cohorts should be intensivly researched to unravel he protectiv factor(s).

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CHAPTER 8

Can and should carotid ultrasound be used in cardiovascular risk assessment? – the internist's perspective

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ABSTRACT

Cardiovascular risk management is a major and challenging task for internists. Risk scores using algorithms based on traditional risk factors are helpful in identifying patients in whom intensive prevention strategies are warranted or can be withheld. However there remains a need for more accurate screening tools to allow clinicians to individualize the primary prevention programs to their patients.

Approximately 40-80% of apparently healthy, asymptomatic subjects exhibit increased thickness of the lamina intima-media of the carotid artery or have atherosclerotic carotid plaques. These abnormalities can be measured safely and at low cost by ultrasound. Subclinical carotid lesions are strongly associated with generalized atherosclerotic burden and the risk of future cardiovascular events. Although many cardiovascular risk management guidelines recommend the use of these parameters incorporation in clinical practice is still not commonplace.

Based on the current literature it seems that in high risk patients there is no additional value of the measurements because even in absence of carotid lesions these patients should receive an intensive risk reduction regime. In the large low-intermediate risk group however carotid ultrasound findings seem to carry subtle but possibly clinically relevant information about cardiovascular risk profile. The effect of treatment decisions based on carotid ultrasound parameters has not been studied and they should only be made in conjunction with all other cardiovascular risk factors. Sequential measurements to monitor progression and evaluate treatment response on an individual basis are not sufficiently reproducible and are therefore not recommended.

INTRODUCTION

Case example

During your outpatient clinic you are visited by a 55 year old male. He has been under your care for cardiovascular risk management for the last 3 years. You are treating him for hypertension, with a thiazide diuretic and an ACE inhibitor; his blood pressure is relatively well regulated with values around 140/85 mmHg. His BMI is 32 kg/m^2 with predominantly visceral adiposity. Fasting blood glucose levels are slightly elevated but HbA1c is normal and stable. His LDL-C is 3.2 mmol/L with an HDL of 0.9 mmol/L, fasting triglycerides are 2.2 mmol/L. He has no specific complaints, leads a sedentary lifestyle which makes it difficult for you to determine whether he has angina on exertion. You have advised him to stop smoking and two months ago he quit after 35 packyears.

Your Framingham risk calculator tells you that he is currently at intermediate risk for suffering a heart attack in the next 10 years (15.3%). This does not warrant more aggressive management of his cardiovascular risk factors. However your clinical intuition gives you an uneasy feeling about your patient partly because had he not quit smoking his risk score would be significantly higher (28.4%) and the impending diabetes is not taken into account in the prediction model. All your efforts during his visits are aimed at determining whether the obviously present risk factors have led to the development of atherosclerosis. If so, you feel it is justifiable to aim for secondary prevention targets for blood pressure and lipids and are considering adding a statin and aspirin to his treatment regimen.

Could ultrasound examination of the carotid arteries of your patient help you decide? Although many guidelines on cardiovascular disease prevention recommend using cIMT and carotid plaque detection in risk assessment strategies its implementation in clinical practice is still not commonplace. This may partly be explained by conflicting data on the additional value of IMT above risk assessment tools such as the Framingham (40) PROCAM (41) and SCORE (42) algorithms among others.

The current review will summarize the pathophysiological and epidemiological basis for the use of IMT measurement and carotid plaque detection as possible predictors of future cardiovascular events. Technical and methodological considerations important for the interpretation of results will be addressed first.

Three main issues considering clinical applicability in individual patients will be discussed:

- Is the presence of subclinical carotid atherosclerosis representative of generalized and particularly coronary atherosclerosis?
- Does the presence of subclinical carotid atherosclerosis increase the risk of suffering a cardiovascular event?
- Can progression of subclinical carotid atherosclerosis be used to monitor the efficacy of cardiovascular risk management in individual patients?

The review will conclude with recommendations on implementation of carotid ultrasound in individual risk stratification.

Technical and methodological considerations

B-mode ultrasound imaging is able to visualize the intima-media complex of large arteries. The thickness of these vascular structures can be measured offline. Several autopsy studies have validated these measurements and found them to be highly accurate when compared with histological findings in the same arterial segment (43) (14). Early atherosclerotic changes in these arteries (smooth muscle cell proliferation, fatty streaks and non-stenotic plaques) can be detected by thickening of these vascular structures (figure 1). Carotid ultrasound is able to visualize morphological changes in the arterial wall, before the advanced stages of atherosclerosis are reached. It is therefore possible to identify vascular damage in patients before they develop clinical signs and symptoms.

In a substantial percentage of asymptomatic adults this form of subclinical atherosclerosis is present. Salonen et. al. observed lesions in 80% of male subjects in the general population by the age of 60 years. (44) Carotid ultrasound is a non-invasive, safe and inexpensive imaging modality. However, there is as of yet no standardized international imaging protocol dictating how to perform IMT measurements and plaque detection. In general, all current ultrasound devices provide sufficient resolution to assure accurate measurement, figure 2 shows a representative example.

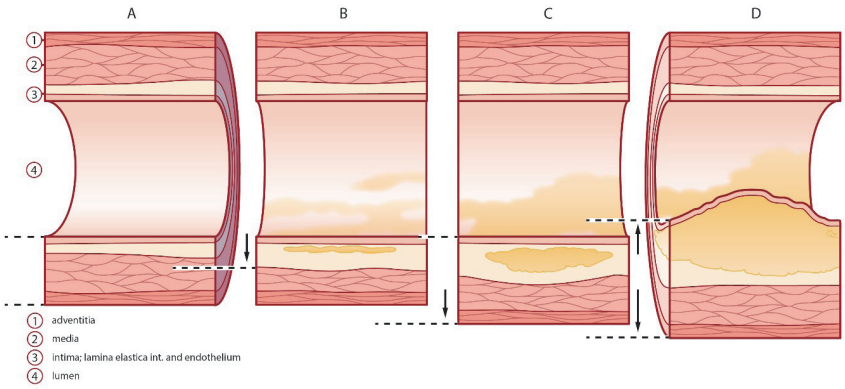

Figure 1 | Schematic representation of the progression of arterial atherosclerosis over a period of decades.

A) Normal arterial architecture as seen in healthy young subjects. B) Formation of fatty streaks in de arterial wall can be seen in post-mortem microscopy as early as in adolescence and represents a physiological vascular response to injury. Although these arterial changes can be a precursor of manifest atherosclerosis, absence of or adequate management of vascular risk factors can keep progression in check. Current imaging techniques are unable to objectify or quantify these subtle changes. C) With advancing age and under the influence of vascular risk factors the fatty streaks can progress to overt intimal thickening. In this stage of the atherosclerotic process the artery responds by an outward remodelling. The artery hereby preserves its lumen diameter and flow. Imaging studies with angiography and Doppler duplex will therefore not detect this early stage of atherosclerosis. Ultrasound measurement of intima media thickness however is sensitive enough to identify these lesions. D) Finally the atherosclerotic plaque starts encroaching into the lumen causing flow changes and stenosis which lead to tissue dysfunctions downstream. In this stage all imaging modalities are useful in diagnosing and evaluating the lesions, however a window for early intervention has passed.

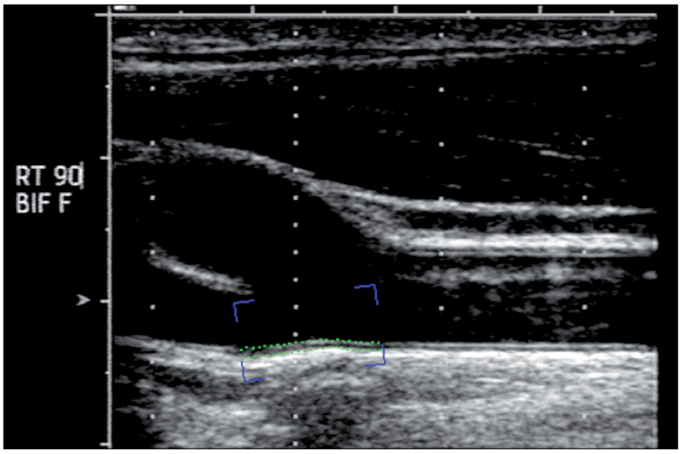

Figure 2 | Representative example of a longitudinal, cross-sectional carotid ultrasound image of a 62-year old male patient (a). The blue box shows an example of a region of interest for computer aided edge detection (green dotted lines). The distance between the green lines represents the IMT.

Ultrasound investigations are prone to operator variability, but several studies show excellent inter- and intra-observer reproducibility in the case of IMT measurement (45) (46) (47). The most frequently used location for the measurement is the most cranial one centimeter of the common carotid artery, but the carotid bifurcation and the internal carotid artery are other possible sites for measurement. Further differences in protocols arise because most authors calculate the mean IMT over one centimeter whereas others use the maximum value. As atherosclerosis is an asymmetrical process the angle at which the artery is approached by the operator can vastly influence the end results. Some authors try to limit the variance this causes by measuring IMT at several angles and using the mean value. Unilateral versus the average of bilateral measurements is another factor limiting uniformity. Due to these and other factors it remains unclear how to define normal IMT values. In healthy young adults IMT will be approximately 0.5mm slowly increasing with age. It is unclear where the threshold for higher cardiovascular risk lies and if this threshold is comparable in different populations (diabetics vs. non-diabetics; men vs. women; CKD vs. non-CKD; ethnic differences etc.). cIMT values exceeding 0.9mm are thought to imply increased cardiovascular risk. Atherosclerotic plaque is defined as a focal structure that encroaches into the arterial lumen of at least 0.5 mm or 50% of the surrounding cIMT value or demonstrates a thickness >1.5 mm.

Association with atherosclerotic burden

Increased IMT or the presence of plaque in the carotid artery has been directly correlated to coronary artery and overall atherosclerotic burden in several autopsy studies (48) (49). Iwakiri et al. recently published findings in 111 autopsy studies in which they found a positive association between cIMT and coronary intima media thickening ($R=0.31$, $p>0.001$). Although the correlation is statistically significant the strength of the association is modest. Abnormal cIMT in these subjects was also linked to the presence of a necrotic core in the atherosclerotic lesions. Fifty percent of subjects in the lowest tertile of cIMT demonstrated vulnerable plaques at various vascular beds. This increased to 80% in subjects with increased IMT. The cutoff point for the lowest tertile was <1,091mm. A cIMT value of <0.9mm is generally seen as the clinical threshold for higher cardiovascular risk. It would be of interest to know whether subjects

under this clinical cut-off point had less plaque burden or histologically less vulnerable plaques but these data were not published. The findings of this autopsy study support the hypothesis that carotid atherosclerosis represents more generalized vasculopathy but also suggest that the absence of carotid atherosclerosis does not necessarily exclude coronary pathology.

The association has also been studied in vivo. Increased cIMT and/or carotid plaque predicted coronary pathology in quantitative coronary angiography studies (50) (21), intravascular ultrasound examination (20), coronary calcification scores (51) and myocardial scintigraphy studies (22). The strength of the correlations are yet again modest at best (R=0.23-0.44) but all the findings were statistically significant and consistent. Most authors conclude that cIMT measurement and plaque detection may be useful as a non-invasive tool to approximate the presence of coronary artery disease. Whether asymptomatic patients with abnormal carotid ultrasound findings therefore require a more intensive CVD risk management strategies remains unclear.

Epidemiological evidence

Table 1 lists the population-based studies examining the association of cIMT with cardiovascular risk. It can be concluded that all current prospective data confirm the correlation between abnormal carotid ultrasound findings and elevated risk for future cardio- or cerebrovascular events. After correction for traditional risk factors the strength of association attenuates but cIMT and carotid plaque presence appear to be independent predictors. The strength of the association varies between a 20% and a 5-fold risk increase depending on the outcome parameter studied (MI, stroke and/or death), carotid scanning protocol segment used (internal carotid, external carotid, bifurcation, inclusion of plaque) and the level of statistical correction.

Whether this justifies its use in a routine cardiovascular screening setting is dependent on the additional predictive power of carotid ultrasound above the current standard risk prediction models. Analyses of the Framingham offspring and MESA studies as well as a recent meta-analysis have addressed this issue. In 2011 Polak et al. published data from the Framingham offspring study. A subset of 2965 members of this cohort underwent cIMT measurements. During the 7.2 years of follow-up 296 participants suffered a cardiovascular event. The same

Table 1 | Summary of population-based studies on the association between carotid ultrasound abnormalities and cardiovascular risk.

Author	n	Follow-up	Carotid parameter	Outcome parameter	Correction for traditional risk factors	HR [95% CI;p-value]]
Salonen et al 1991 (52)	1288	1m-2.5y	Bilateral CCA & BIF	Coronary artery event	none	6.71 [1.33-33.91;p<0.01] stenotic plaque 4.51 [1.51-11.47;p<0.01] minor plaque 2.17 [0.70-6.76;p=NS] increased cIMT
Belcaro et al 1996 (53)	2322	6y	Bilateral CCA, BIF, ICA	Cardiovascular event or death	none	*No formal HR; incident event distribution:* 0% events with normal ultrasound 5.5 % with increased cIMT (p<0.05) 18.4% with minor plaque (p<0.025) 42% with stenotic plaque (p<0.025)
Chambless et al 1997 (54)	14054	10.2y	Bilateral CCA, BIF, ICA	Cardiovascular events or death	Age, gender	5.07 [3.08-8.36;p<0.01] MI in women 8.54 [3.52-20.74;p<0.01] stroke in women 1.87 [1.28-2.69];p<0.01] MI in men 3.62 [1.45-9.15;p<0.01] stroke in men
Bots et al 1997 (55)	7983	2.7y	CCA	Cardiovascular events or death	Age, gender, BMI, smoking BP, lipids, diabetes, prior cardiovascular event	1.38[1.21-1.58;p<0.01] MI 2.23 [1.48-3.36;p<0.01] stroke
O'Leary et al 1999 (56)	5858	6.2y	CCA, ICA	MI, stroke	Age, gender, BP, presence of atrial fibrillation, smoking, diabetes	3.15[2.19-4.52;p<0.01] MI or stroke
Kitamura et al 2004 (57)	1289	4.5y	CCA, BIF, ICA	stroke	Age, BP, BMI	4.8 [1.9-12.0;p<0.01] stroke
Rosvall et al 2005 (58) (59)	5163	7y	CCA	MI, stroke	Age, gender, physical activity, smoking, BP, diabetes, lipids, waist circumference	1.23 [1.07-1.41;p<0.01] MI 1.21 [1.02-1.44;p<0.01] stroke
Lorentz et al 2006 (60)	5056	4.2y	CCA, BIF, ICA	MI, stroke	Age, gender, BMI, BP, lipids, smoking, diabetes	1.85 [1.09-3.15;P<0.01] combined stroke, MI or death
Polak et al 2011 (61)	2965	7.2y	CCA, BIF, ICA	Cardiovascular events or death	Age, gender, BP, lipids, smoking	1.21 [1.13-1.29;p<0.01] per 1SD increase in cIMT 1.92 [1.49-2.47;p<0.01] plaque
Polak et al 2013 (62)	6562	7.8y	CCA, ICA	Cardiovascular events or death	Age, gender, BP, lipids, smoking	1.45 [1.20-1.76;p<0.01] minor plaque 1.65 [1.34-2.03;p<0.01] stenotic plaque 1.33 [1.18-1.49;p<0.01] increased cIMT

Abbreviations: HR: Hazard ratio; CCA: common carotid artery; BIF: bifurcation; ICA: internal carotid artery; BP: blood pressure

authors recently reported their findings in het MESA-study population. A total of 6562 subjects were followed for an average of 7.8 years. A base prediction model was able to predict 74,3% of all CVD events [95% CI: 72.4-76.2], 72.9% of coronary events [95% CI: 70.5-75.2] and 77.4% of cerebrovascular events [95% CI: 73.9-80.9]. Presence of subclinical carotid atherosclerosis (plaque or increased cIMT) was significantly and independently associated with future events with hazard ratios ranging from 1.21 [95% CI: 1.13-1.30] for maximum cIMT in the internal carotid artery to 1.65 [95% CI: 1.34-2.03] for stenotic plaque. The predictive power of a model containing ultrasound parameters was slightly better than the base model. The increase in C-statistic regarding all cardiovascular events was marginal (0.46%-0.65%) but statistically significant. Looking at cerebrovascular events alone, the addition of carotid ultrasound findings did not significantly impact the performance of the baseline model. For coronary events cIMT and plaque presence appeared to be more relevant in this population. Including these parameters improved predictive ability of the base model by 0.89%-1.31%. Perhaps a more clinically relevant parameter is the net reclassification improvement (NRI) (63). This statistic quantifies in how many subjects the risk classification (low, intermediate, high) was rightly changed by taking cIMT and plaque presence into account. For coronary artery events the NRI was statistically significant for all ultrasound parameters and strongest for maximum IMT in the internal carotid artery (7%) and presence of stenotic plaque (5%).

Den Ruijter et al. combined the data from 14 large population based cohorts resulting in a database of 45.828 individuals without know cardiovascular disease (64). During a median follow-up of 11 years 4007 first time myocardial infarctions or strokes occurred. A model based on the Framingham Risk Score performed reasonably well in predicting the clinical events (75.7% [95% CI: 74.9-76.4]). Adding common carotid IMT to this model did not significantly improve performance of the model (75.9% [95% CI: 75.2-76.6]). In the entire cohort more than 90% of subjects stayed in the same risk category after adding cIMT values. Therefore the net reclassification improvement was marginal (0.8% [95% CI 0.1%-1.6%). The added value of cIMT improved slightly when subjects at intermediate risk alone were analyzed. In this subset the net reclassification improvement was approximately 4%. In patients with diabetes there was no effect on the area under the ROC or net reclassification improvement observable. The authors conclude that although cIMT improves risk prediction, especially in intermediate

risk patients, the additional value is too marginal to warrant its use.

Carotid atherosclerosis progression and cardiovascular risk

Several randomized intervention studies have shown that pharmacological interventions (statins, niacin, anti-hypertensive drugs, etc.) can slow cIMT progression when compared to a placebo control group. The suggestion in these trials is that limiting progression may lead to fewer future cardiovascular events. To clinicians it may therefore seem that sequential measurements of IMT to monitor progression can serve as a tool in evaluating the efficacy of individual cardiovascular risk management strategies. However there are no clear long-term data supporting this assumption. Several studies have looked at carotid atherosclerosis progression and its association with future events.

Results from the Multiethnic Study of Atherosclerosis suggested that cIMT progression is associated with risk of myocardial infarction and stroke. A recent meta-analysis evaluated this correlation on a larger scale. Pooled data from 10 cohort studies provided results from 36.984 primary prevention patients with 257.067 person-years of follow up. The robust, independent and significant correlation between cIMT and subsequent clinical end-point is confirmed in this analysis. By contrast a consistent null result is found for cIMT progression. Overall hazard ratios were found to be approaching 1.0 in unadjusted models as well as after adjustment for cardiovascular risk factors and baseline cIMT.

In a meta-analysis the relationship between changes in cIMT and the occurrence of major vascular events was analyzed (65). Data from 41 intervention studies were included. Most studies examined the effect of statins on cIMT progression but the dataset also included trials with antihypertensive drugs, lifestyle interventions, estradiol and anti-oxidants. The main finding was that progression or regression of cIMT was not correlated with more or fewer events in these trials. This does not detract from the beneficial effect of the interventions in the trials but demonstrates that cIMT changes do not accurately reflect these effects.

The recently published results from the IMPROVE study confirm the lack of predictive value of standard cIMT progression parameters for future events (66). A cohort of 3482 subjects with three or more vascular risk factors was included in the study. A novel parameter, the fastest progression of maximum IMT, was postulated by the authors. There was a significant and independent association

between this new value and the occurrence of CV events during a mean follow-up time of 21.5 months

Wannarong et al. compared changes in carotid IMT, plaque area and plaque volume in 349 subjects as predictors of myocardial infarction, stroke, transient ischemic attacks and death (67) . During the median follow-up time of 3.17 years only progression of plaque volume independently predicted future events.

DISCUSSION

It remains a challenge for clinician involved in primary prevention of cardiovascular disease to correctly identify patients at elevated risk. Assessment tools using models based on traditional risk factors have aided clinical decision-making tremendously. However, especially for patients at intermediate risk the need for more accurate risk markers persists. It is specifically this group of patients in which under- or overtreatment may occur. The vast majority of published data on the correlation of carotid atherosclerosis and coronary atherosclerosis demonstrates a close association. Carotid ultrasound provides a safe, patient-friendly and affordable means to objectify and quantify carotid atherosclerosis. In 1996 Belcaro et al. estimated the cost of one carotid-femoral IMT examination to be around 12 euros or approximately 15 US dollars, including training, equipment and staff expenses. (53) Long-term follow up studies with large sample sizes consistently confirm that elevated IMT and the presence of carotid plaques lead to higher risk of suffering coronary and cerebrovascular events. When adjusted for traditional cardiovascular risk factors the additional risk appears to be approximately 20-30%, suggesting that cIMT is an independent risk factor. Unadjusted odds ratios far exceed these figures.

Herein lies a problem in the interpretation of the studies. Is the aim of carotid ultrasound to provide the clinician with another cardiovascular risk factor to take into account in treatment decisions or can it be seen as the sum result of all traditional and non-traditional risk factors in a specific patient? There are strong arguments for the latter. Over the last decades cIMT has been shown to independently correlate to a plethora of non-traditional risk factors for atherosclerosis, including lp(a), homocysteine levels, lymphocyte/neutrophil ratios, inflammatory markers, circulating endothelial progenitor cells, markers

of endothelial function, etc. Moreover it has been suggested that traditional risk factors are not the major contributors to cIMT variance. It would seem useful for clinicians to obtain a general idea about the atherosclerotic state of the patient before making treatment decisions. For most of the abovementioned non-traditional risk factors however there is no known or proven treatment. Finding subclinical atherosclerosis in the carotid arteries in non-smoking patients without diabetes, hypertension or hyperlipidemia therefore presents the clinician with a dilemma. It does not seem prudent to look for non-traditional risk factors if it has no treatment consequences. If there are treatable risk factors present it is still unclear whether intensifying prevention interventions, e.g. setting lower LDL-cholesterol, blood pressure and HbA1C targets or adding aspirin, will lead to better outcomes. Conversely there is no evidence that absence of carotid atherosclerosis justifies withholding or delaying treatment of traditional risk factors.

The meta-analysis of the largest dataset examining these issues concludes that cIMT measurement improves risk assessment to such a limited extent that it is not a useful tool. On a population level this conclusion is accurate. However on an individual patient level the discussion is more subtle. In approximately 5% of patients at intermediate cardiovascular risk the addition of cIMT would have rightly reclassified them as high risk patients. Plaque detection and cIMT measurements in the carotid bifurcation and the internal carotid artery were not included in this analysis. Adding these parameters is likely to have positively affected the contribution of carotid ultrasound to the prediction model as they were the strongest predictors of future coronary artery events in the MESA-study. Although the group of patients who may benefit from carotid ultrasound measurements will remain small the consequences are potentially profound. Initiating early risk reduction strategies could prevent or delay morbidity and mortality due to progression of atherosclerotic burden.

Evaluating the response to an implemented risk management strategy could greatly aide clinicians in decisions about intensifying treatment. Monitoring cIMT progression would be a relatively simple and low-cost method to this end. However the current data suggest that progression of cIMT is not correlated with risk of cardiovascular events. A possible explanation for this finding is that one-time cIMT measurements are highly reproducible but the progression of cIMT is prone to inter- and intra-observer variability. This is due to the fact

that cIMT varies between 0.5mm and 1.2mm whereas yearly cIMT progression is in the range of 0.001-0.030mm. Sequential measurements are therefore much more susceptible to changes in the exact location of the reading (angle of the transducer, position of the subjects head etc.). Although advances in ultrasound equipment and scanning protocols are increasingly minimizing these variations it appears that cIMT progression is too unreliable to use as a marker to individualize risk management strategies. The most recent studies suggest that focal changes in carotid atherosclerosis (increasing plaque volume and fastest progression of maximum IMT) may prove helpful in monitoring treatments. Both these longitudinal parameters were found to independently predict future events, however further studies confirming these initial findings are needed. In addition, these novel parameters are not routinely measured and validation and standardization are required before they can be utilized in clinical practice.

FUTURE PERSPECTIVES

To fully elucidate the value of carotid ultrasound a four-armed management study is needed in which subjects with and without carotid atherosclerosis are randomized for intensive versus standard risk prevention strategies based on the ultrasound findings. Promising work is being done in the area of plaque characterization with ultrasound. Detailed description was beyond the scope of this review but by utilizing gray scaling techniques it is possible to identify lipid-rich, vulnerable carotid plaques. (68) These appear to be even more strongly associated with future vascular events, particularly ischemic strokes. (25) (69) (70) Finally, newer ultrasound equipment can perform real-time quantification of cIMT instead of the time consuming off-line procedure employed at the moment. These portable ultrasound machines make quick office-based measurement of cIMT and plaque detection available without the need for a dedicated vascular laboratory or radiology department. Growing experience with and exposure to carotid ultrasound will likely help clinicians properly implement and interpret it in clinical practice.

Recommendations & Learning points

- In patients at high cardiovascular risk there is no additional value of carotid ultrasound
- In patients at low or intermediate risk carotid ultrasound can aide clinicians by giving an indication of overall atherosclerotic burden; presence of carotid plaque or increased cIMT should prompt reclassification of the patient to a higher risk category
- Sequential measurement of cIMT to monitor progression are unreliable and the predictive value of longitudinal changes in cIMT is uncertain; it cannot be used to individualize risk management strategies
- Bilateral examination of the common carotid artery, the carotid bulb and the internal carotid artery from several angles provide the optimum information on cIMT and plaque presence
- The effect of treatment decisions based on carotid ultrasound parameters has not been studied and they should only be made in conjunction with all other cardiovascular risk factors

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CHAPTER 9

Summary

Carotid artery imaging has played a crucial and lasting role in cardiovascular research in the last decades. By proving to be a reliable and valid intermediate cardiovascular end-point it has facilitated clinical trials with shorter follow-up time and limited sample sizes, thereby aiding in the evaluation and development of pharmacological therapies. In this thesis the main focus has been on two other uses for carotid imaging technology, namely as a new parameter in 1) cardiovascular risk assessment in individual patients and 2) pathophysiological studies on atherosclerosis. Two imaging modalities were utilized for measuring carotid vessel wall geometry: high resolution ultrasound plaque detection and quantification of intima-media thickness and magnetic resonance imaging of total vessel wall thickness and area. Ultrasound-based measurements are at a stage of development that may allow its transition from research parameter to clinical tool. This thesis addresses several critical issues surrounding such a transition to clinical practice. Magnetic resonance imaging is a relatively new method for carotid artery assessment. Its potential role in clinical practice and in pathophysiological studies on vascular pathology is not yet clearly defined. This thesis has provided an initial recommendation on the use of magnetic resonance imaging in a clinical setting. Furthermore, a novel parameter is put forth, derived from a combination of ultrasound and magnetic resonance imaging for future studies on the role of the lamina adventitia in the atherosclerotic process.

In chapter 1 a general outline is provided on the current uses of carotid artery imaging and a several key technical issues are addressed.

In chapter 2 the results of a study on the practical feasibility of carotid ultrasound measurement by clinicians are discussed. The study included 112 patients recruited from the vascular outpatient department. Two trained physicians performed ultrasound scans during their normal clinical outpatient routine. The results were compared to the findings of an experienced vascular sonographer in a specialized vascular laboratory, as the gold standard for carotid ultrasound. The results of the study demonstrate that physicians, after a short training program are able to incorporate ultrasound scans into their regular clinical routine, with an average scan time of 7.3 minutes. A high level of accuracy was achieved by the physicians regarding carotid plaque detection but not for measurement of intima-media thickness. Based on these findings it can be suggested that physicians

could carry out office-based carotid plaque detection in their vascular patients without the aid of radiologists of laboratory technicians. Quantification of intima-media thickness should be done in a vascular laboratory or using specialized equipment. Current guidelines recommend some form of testing for subclinical atherosclerosis in the work-up for cardiovascular risk assessment, be it ankle-brachial index, CT-based coronary calcification scores, pulse wave analysis or carotid ultrasound. Increasingly physicians are utilizing (hand-held) ultrasound in clinical practice for point of care testing. Based on our study results and the recommendations in the guidelines we would advise physicians to screen for carotid plaque in office, especially in patients at intermediate cardiovascular risk. If abnormalities are found, intensifying risk management strategies can be considered. It should be noted that there is as of now no evidence that imaging-based management of cardiovascular risk improves outcome. Informing patients carefully about this fact, but also of the fact that they may be at slightly elevated risk will hopefully lead to a more shared and balanced decision.

In chapter 3 the effect of incorporating carotid ultrasound parameters in clinical decision making is explored further. Carotid ultrasound may not be readily available in a primary care setting. In the Netherlands initial cardiovascular risk management is done by general practitioners. It can therefore be assumed that patients referred to a specialized vascular care unit will already be at an elevated risk because the initial management was deemed to be insufficient by the general practitioner. In a high risk population performing carotid ultrasound testing is unnecessary because their risk profile already warrants intensive management. We therefore looked at the distribution of carotid abnormalities in relation to calculated risk in specifically this referred population.
The carotid parameters measured in the 112 patients from the vascular outpatient department were related to traditional cardiovascular risk factors and calculated risk scores. Intima-media thickness and carotid plaque presence were related to, but only partly explained by traditional cardiovascular risk factors. A high prevalence of subclinical carotid atherosclerosis was demonstrated in patients not treated with statins and with low calculated cardiovascular risk scores. Adding the carotid plaque presence to the clinical decision process would result in a 22% increase in patients classified as higher risk. When including both plaque presence and increased intima-media thickness to the decision process 36% of subjects

would be reclassified. These results suggest that including carotid ultrasound parameters in clinical practice may aide in identifying high-risk patients at an early stage. Seen in combination with the results discussed in chapter 2 it can be argued that especially carotid plaque detection may be an essential tool in cardiovascular risk assessment and should be broadly implemented in clinical practice. Reclassifying patients with carotid plaque to a higher risk category is now also recommended by several guidelines. Implementing standard carotid scanning in vascular outpatients treated in a primary prevention setting will, according to our findings, lead to identifying a large number of subjects with low calculated risk who nonetheless have subclinical atherosclerosis. For these patients it will be a shared decision with their physician whether to step up risk reduction efforts.

The abovementioned considerations are based on many years of experience with and sound population-based research on the predictive potential of carotid ultrasound. Much less is known about other carotid imaging modalities like MRI. The technique provides circumferential images of the artery and is therefore possibly more suited for imaging an asymmetrical process like atherosclerosis than ultrasound is. Digital reconstructions of the vessel geometry make it possible to not only measure wall thickness, but also the volume. It is also a highly reproducible imaging modality. However, MRI scans do not measure the same part of the arterial wall as ultrasound because MRI quantifies the total wall thickness including the lamina adventitia. Conclusions drawn from ultrasound-based IMT studies should not be extrapolated to MRI thickness. In chapter 4 this is illustrated. Ultrasound and MRI scans of the same carotid segment are compared. Although the correlation between MRI and US is very good we observed that MRI measurements were systematically higher. This observation could be explained if indeed the difference between IMT and total vessel thickness represents adventitial thickness. The exact role that this vascular layer and the vasa vasorum therein plays in the atherosclerotic process is subject to ardent research, but is seems clear that the density of the vasa vasorum increases with progressive atherosclerosis. Whether this is a contributing pathological process or a compensatory physiological response is not fully elucidated. The difference between IMT and total wall thickness was more pronounced in the thicker vessels in our study. This would be in line with the hypothesis of concurrent

intimal, medial as well as adventitial thickening. In chapter 5 we explored the possibility of quantifying adventitial thickness by combining ultrasound and MRI measurements in the same vessel. Although our findings are preliminary this may be a step towards facilitating pathophysiological studies on the role of vasa vasorum angiogenesis in vivo. There are data suggesting that it is also possible to visualize and reliably quantify adventitial thickness using ultrasound alone. As of now there is no consensus on the optimal imaging modality for adventitia measurements. Our findings suggest that the combined ultrasound-MRI approach is worth exploring and developing further.

The data reported in chapter 6 address the issue of reference values for IMT in different ethnic groups. Much of the population-based data on the association between IMT and cardiovascular risk is generated in Western Europe and the United States. Pooled data from these studies have shown that IMT increases with age and risk factors and that an IMT value of >0.9mm is associated with higher risk of future cardiovascular events. Guidelines recommend physicians to consider treatment of intensification in patients above this threshold. However, the vast majority of subjects in these large studies was of Caucasian ethnicity. Subjects of Asian Indian descent are susceptible to cardiovascular disease, partly because they develop metabolic syndrome at an earlier age. We studied a cross-section of Asian Indian diabetic patients assumed to be a high cardiovascular risk. When comparing them to age and gender matched Caucasians the risk profile of the Asian Indians was indeed unfavorable, with worse glucose regulation, longer duration of diabetes, more microvascular complications and more pronounced dyslipidemia. IMT values however were found to be significantly lower in the Asian Indians. Although this is a very preliminary finding it suggests that the clinically relevant cut-off values for IMT in Asian Indians may be lower than in Caucasians.

In conclusion, the data reported in this thesis supports the use of carotid plaque detection in routine clinical practice by showing that clinicians themselves can perform the ultrasounds examinations reliably. The data further demonstrate that subclinical atherosclerosis is highly prevalent in a vascular referral center in the Netherlands, also in subjects with low cardiovascular risk scores. Estimating cIMT in office-based ultrasounds is not advised, this requires a specialized

vascular laboratory. Interpreting carotid ultrasound parameters should be done very carefully, taking other factors including ethnicity into account. Carotid MRI is a promising emerging imaging modality. Based on the data in this thesis it is not recommended to extrapolate cIMT data on cardiovascular risk to MRI findings as the two techniques measure different structures. Herein also lies the strength of MRI. Combining MRI-based and ultrasound-based measurements may allow for in vivo studies on the role of the adventitial tissue in the atherosclerotic process.

CHAPTER 10

Nederlandse samenvatting

Beeldvormend onderzoek van de arteria carotis heeft in de afgelopen decades een cruciale en blijvende rol gespeeld in klinisch cardiovasculair wetenschappelijk onderzoek. Echografische parameters zijn een valide intermediair eindpunt gebleken voor cardiovasculaire gebeurtenissen. Hierdoor is het mogelijk geworden om klinische studies te verrichten in kleinere patiëntenpopulaties en met kortere vervolgtijden. Hierdoor is het mogelijk geweest om meer efficient farmacotherapeutische interventies te evalueren en ontwikkelen. In dit proefschrift heeft de nadruk gelegen op twee alternatieve manieren om beeldvormende technieken van de arteria carotis in te zetten, namelijk: 1) voor het bepalen van het cardiovasculaire risicoprofiel van individuele patiënten en 2) voor het faciliteren van pathofysiologische studies naar het atherosclerotische proces in vivo. Hierbij is gebruik gemaakt van twee verschillende beeldvormende modaliteiten: hoog-resolutie echografie voor plaque-detectie en kwantificering van de intima-media dikte alsmede MRI bepalingen van vaatwanddikte en het totale vaatwandoppervlak. Met name de echografische methodiek verkeert momenteel in een fase van ontwikkeling waarbij het denkbaar wordt om deze toe te passen in de klinisch praktijk. In dit proefschrift worden kritieke aspecten geadresseerd die voor een dergelijke transitie van onderzoek naar kliniek van belang zijn. MRI technieken om de arteria carotis te karakteriseren zijn relatief nieuw. De potentiele toegevoegde waarde hiervan voor pathofysiologische studies is nog niet geheel opgehelderd. Dit proefschrift bevat enkele initiële aanbevelingen over het gebruik van carotis-MRI in de kliniek. Tot slot wordt in dit proefschrift een voorstel gedaan om de combinatie van echografie en MRI in te zetten om een in vivo indruk te krijgen over de dikte van de lamina adventitia van de arteria carotis. Deze nieuwe parameter zou verder onderzoek naar de rol van deze vaatstructuur in het atherosclerotische proces kunnen faciliteren.

Hoofdstuk een bevat een algemene introductie van de twee beeldvormende technieken. Een aantal belangrijke epidemiologische en technische aspecten is hierin uiteen gezet.

Hoofdstuk twee beschrijft de resultaten van een studie naar de praktische toepasbaarheid van de echografische techniek door clinici in de spreekkamer. In de studie zijn 112 patiënten van de vasculaire polikliniek geïncludeerd. Twee internisten hebben na een beknopte training de echografie uitgevoerd tijdens reguliere poliklinische consulten. De resultaten zijn vergeleken met bevindingen van een ervaren vasculair echografist in een gespecialiseerd vaatlaboratorium.

De laatstgenoemde meting is gebruikt als goudstandaard. Uit de studie kwam allereerst naar voren dat het praktisch gezien mogelijk is om de echografie te incorporeren in de poliklinische routine. Gemiddeld kostte het de internisten 7.3 minuten om de volledige scan uit te voeren. Met deze meting waren de internisten goed in staat om vast te stellen of er een atherosclerotische plaque aanwezig was. Het accuraat schatten van de dikte van de intima-media echter bleek niet mogelijk. Op basis van deze bevindingen kan worden voorgesteld dat internisten bij geselecteerde patiënten met behulp van echografie in de spreekkamer plaque detectie kunnen uitvoeren. Het kwantificeren van de intima-media dikte zou moeten plaatsvinden in een gespecialiseerd vaatlaboratorium met behulp van specifieke apparatuur. In de huidige richtlijnen voor cardiovasculair risico reductie wordt geadviseerd om een inventarisatie te doen van subklinische atherosclerose. Hiervoor worden parameters als enkel-arm index, coronair calcificatie score met behulp van computer tomografie, pulse wave analyse met tonometrie of carotis-echografie aanbevolen. De afgelopen jaren maken clinici in toenemende mate gebruik van (hand-held) echografie voor andere indicaties. Deze ontwikkeling kan het navolgen van deze richtlijnen vergemakkelijken. De resultaten beschreven in hoofdstuk twee suggereren namelijk dat internisten in staat zijn zelf te screenen voor aanwezigheid van atherosclerotische plaques in de arteria carotis. Als bij individuele patiënten atherosclerotische veranderingen zichtbaar zijn kan worden overwogen de risico verlagende strategie te intensiveren. Hierbij moet de kanttekening worden gemaakt dat er geen goede gegevens zijn die bewijzen dat het incorporeren van beeldvorming in de klinische inventarisatie leidt tot minder cardiovasculaire ziekte op de lange termijn. Het is van belang om dit duidelijk met patiënten te bespreken zodat er een weloverwogen gedeelde beslissing kan worden genomen over het verdere preventieve beleid.

In hoofdstuk drie wordt de vraag over het incorporeren van echografisch onderzoek in de klinische besluitvorming verder geëxploreerd. De meettechniek is momenteel in Nederland niet breed beschikbaar voor eerstelijns zorgverleners. In het Nederlandse zorgsysteem is veel van de preventieve cardiovasculaire zorg ondergebracht in de eerste lijn. Als patiënten worden verwezen naar de tweede of derde lijn voor cardiovasculaire ziekte-preventie kan worden aangenomen dat er een additioneel probleem speelt waardoor het risico a priori verhoogd is. De toegevoegde waarde van carotis-echografie bij patienten met een hoog

risico op andere gronden is discutabel omdat deze reeds een indicatie hebben voor een intensief beleid omtrent risico-reductie. Het al dan niet vaststellen van subklinische atherosclerose heeft derhalve minder gevolgen voor de uiteindelijke behandeling. De in hoofdstuk 3 beschreven studie is gericht geweest op het inventariseren van de aanwezigheid van atherosclerotische veranderingen in de arteria carotis in specifiek deze verwezen patiëntenpopulatie. Intima-media dikte en de aanwezigheid van atherosclerotische plaque is in 112 patiënten bepaald en vervolgens gerelateerd aan traditionele cardiovasculaire risicofactoren en berekende risico-scores. Zoals verwacht waren de afwijkende bevindingen bij echografie geassocieerd met risicoscores. Echter de prevalentie van subklinische atherosclerose in de arteria carotis was ook opvallend hoog in de subpopulatie met een lage berekende risicoscore. Het toevoegen van de aanwezigheid van plaque in de carotiden in de klinische besluitvorming zou hebben geleid tot reclassificering van 22% van de patiënten in een hogere risico-categorie. Dit percentage zou oplopen naar 36% als ook een verdikte intima-media werd meegewogen. Deze resultaten suggereren dat carotis echografie wellicht helpt in het identificeren van patiënten die atherosclerose ontwikkelen ondanks een gunstig traditioneel risico-profiel. In combinatie met de in hoofdstuk twee beschreven resultaten is het derhalve denkbaar dat met name plaque detectie potentieel geschikt is om te implementeren in de routine klinische praktijk van een vasculaire polikliniek, alwaar de techniek veelal relatief makkelijk voor handen is. De studie toont aan dat hierdoor patiënten zullen worden geïdentificeerd met een lage berekende risicoscore en desondanks subklinische atherosclerose. Voor deze patiënten zal het een gedeelde beslissing worden met hun zorgverlener of dit zou moeten leiden meer onderzoek naar minder traditionele risicofactoren en/of meer intensieve preventieve maatregelen.

De bovengenoemde overwegingen zijn gebaseerd op vele jaren intensief en grootschalig onderzoek naar de potentiele voorspellende waarde van echografie voor cardiovasculaire ziekte. De rol van andere beeldvormende modaliteiten zoals carotis-MRI in klinische besluitvorming is minder uitvoerig onderzocht. De techniek levert een circumferentieel beeld op van de arteria carotis en is derhalve mogelijk meer geschikt voor het kwantificeren van een asymmetrisch proces als atherosclerose dan echografie. Door middel van digitale reconstructie is het tevens mogelijk om niet alleen de dikte maar ook het totale volume van de vaatwand te kwantificeren. Tot slot is een hoge reproduceerbaarheid van de

techniek aangetoond. Echter een belangrijke kanttekening voor de interpretatie van de uitkomsten is dat MRI niet dezelfde vaatstructuren meet als echografie. De wanddikte die gevonden wordt met MRI scans bevat niet alleen het intima-media complex maar waarschijnlijk ook de lamina adventitia. Het is derhalve niet mogelijk om de epidemiologische data van echografische studies direct te extrapoleren naar deze MRI parameters. Dit wordt geillustreerd in hoofdstuk vier. Hier wordt een studie beschreven waarbij echografische bevindingen zijn vergeleken met MRI parameters in eenzelfde segment van de arteria carotis. Hoewel een goede correlatie wordt gezien tussen de twee verschillende modaliteiten wordt aangetoond dat de vaatwanddikte met MRI systematisch hoger is dan met echografie. Dit verschil kan worden verklaard door inclusie van de lamina adventitia met MRI scans naast de intima-media dikte waartoe echografie zich beperkt. Het is nog niet geheel opgehelderd wat de rol van deze buitenste vaatstructuur, waarin de vasa vasorum zich bevinden, is in de pathofyiologie van atherosclerose. De densiteit van de vasa vasorum neemt toe met progressieve atherosclerose. Ook in de studie beschreven in hoofdstuk vier viel op dat het verschil tussen de MRI meting en de echografische meting meer uitgesproken was in de verdikte slagaders. Er wordt momenteel veel onderzoek gedaan naar de vraag of verbreding van de lamina adventitia deel is van het pathologische proces of een compensatoire fysiologische reactie.

In hoofdstuk vijf wordt een voorstel gedaan om de combinatie van carotis MRI en echografie in te zetten om adventitia-dikte te kunnen schatten door de van de totale vaatwanddikte (MRI) de intima-media dikte (echografie) af te trekken. Hoewel deze nieuwe techniek vooralnog niet gevalideerd is zou het een stap kunnen zijn richting het faciliteren van in vivo studies naar vasa vasorum angiogenese. De afgelopen jaren komen er steeds meer data die suggereren dat het met alleen hoog resolutie echografie ook mogelijk is om de lamina adventita te visualiseren en betrouwbaar te kwantificeren. Er is echter nog geen consensus over de optimale methodiek om adventitia-metingen te verrichten. De resultaten die in hoofdstuk vijf zijn beschreven suggereren dat de gecombineerde MRI-echografie benadering het verder exploreren waard is.

In hoofdstuk zeven wordt aandacht besteed aan mogelijke verschillen in referentiewaarden voor intima-media dikte in verschillende ethnische groepen. De overgrote meerderheid van de belangrijke populatie-studies is verricht in West-Europa en de Verenigde Staten. Meta-analyse van deze gegevens heeft

aangetoond dat intima-media dikte toeneemt met de leefttijd en door de aanwezigheid van cardiovasculaire risicofactoren. De huidige consensus is dat het cardiovasculaire risico van patienten significant toeneemt als de dikte 0.9mm overschrijdt. In veel richtlijnen wordt aanbevolen om bij deze grenswaarde te overwegen of het preventieve beleid geintensiveerd zou moeten worden. Het gros van de patienten in de studies waarop dit gebaseerd is waren van caucasische afkomst. Patienten van Hindustaans Surinaamse afkomst hebben een predispositie voor het ontwikkelen van hart- en vaatziekten, deels omdat ze op jongere leeftijd het metabole syndroom ontwikkelen. In de studie die in hoofdstuk zeven is beschreven is gekeken naar groep Hindustaans-Surinaamse patienten met diabetes mellitus en hiermee een verondersteld hoog cardiovasculair risico. Deze groep is vergeleken met caucasische diabetes patienten met vergelijkbare leeftijd en geslacht. Het risicoprofiel bij de Hindustaanse patienten bleek inderdaad ongustig met slechtere glucoseregulatie, langer bestaande diabetes, meer microvasculaire complicaties en ernstigere dyslipidemie. Desondanks werd in de Hindustaanse groep een significant lager waarde van intima-media dikte vastgesteld. Hoewel in deze studie niet is gekeken hoe het ziektebeloop in de verschillende groepen is geweest zou deze opvallende observatie een eerste suggestie kunnen zijn dat de drempelwaarde van intima-media dikte voor verhoogd cardiovasculair risico in deze ethnische groep wellicht lager zou kunnen liggen.

In hoofdstuk acht wordt een samenvatting van de literatuur gepresenteerd met aanbevelingen voor het gebruik van carotis echografie in de klinische praktijk.

Concluderend ondersteunen de data in dit proefschrift het gebruikt van atherosclerotische plaque detectie door te laten zien dat clinici deze metingen op een betrouwbare manier zelf kunnen doen in de spreekkamer en dat het mogelijk is dit te incorporeren in een dagelijkse poliklinische routine. Tevens wordt vastgesteld dat er een hoge prevalentie van subklinische atherosclerose van de arteria carotis is bij patiënten van een vasculaire polikliniek, ook in de groep met een lage berekende risico-score. Schattingen van intima-media dikte door clinici in de spreekkamer zijn te onbetrouwbaar, hiervoor wordt verwijzing naar een gespecialiseerd vaatlaboratorium geadviseerd. De uitkomsten van beeldvormende onderzoeken van de arteria carotis dienen voorzichtig en zorgvuldig te worden geinterpreteerd. Hierbij moeten alle risicofactoren inclusief ethnische afkomst worden meegewogen. De bekende associaties

van echografisch bepaalde intima-media dikte met verhoogd cardiovasculiar risico kunnen niet direct worden geëxtrapoleerd naar afwijkende bevindingen bij MRI onderzoek. De twee beeldvormingstechnieken meten verschillende vaatwandstructuren. Van dit verschil kan mogelijk gebruik worden gemaakt door de twee modaliteiten te combineren om een indruk te krijgen van de lamina adventitia. Verdere ontwikkeling en validering van de methodiek beschreven in dit proefschift zou toekomstige studies naar de rol van vasa vasorum angiogenese in vivo mogelijk kunnen maken.

10

CURRICULUM VITAE

Arghya Ray was born on 17th march 1977 in Eindhoven, the Netherlands. In 1995 he graduated VWO from the Lorentz Lyceum and started studying medicine at the Catholic University of Leuven in Belgium. In 1996 he continued his studies at the Leiden University where he graduated medical school in 2003. Prior to starting his specialization he contributed to the execution of an international clinical trail on the effects op Torcetrapib on carotid intima media thickenss. Exposure to carotid ultrasound during this period led to the conception of the research described in this thesis under supervision and guidance of Prof.dr. M.V. Huisman (promotor), Prof.dr. T.J.Rabelink (promotor) and Dr. J.T.Tamsma (co-promotor).

Arghya was granted a residency in Internal Medicine by Prof.dr. A.E.Meinders and concluded it in 2014 including a fellowship in nephrology under Prof.dr. H de Fijter & Prof.dr. T.J. Rabelink. Three years of this specialization were spent at the HAGA hospital (formerly Leyenbrug & Rode Kruis) in the Hague under guidance of Dr R.M. Valentijn, Dr R.H.Kauffmann & Dr.M.O. van Aken.

Arghya is currently working as an internist-nephrologist at the Leiden University Medical Center. He is married to Poonam and has two children, Ayodhya & Avani.

Arghya Ray werd op 17 maart 1977 geboren in Eindhoven. In 1995 slaagde hij voor zijn VWO aan het Lorentz Lyceum en startte zijn studie geneeskunde aan de Katholieke Universiteit Leuven, Belgie. In 1996 zette hij de studie voort aan de Universiteit Leiden alwaar hij in 2003 afstudeerde. Alvorens te starten met de vervolgopleiding werkte hij mee de uitvoering van een internationale klinische studie naar de effecten van Torcetrapib op carotis intima-media dikte. De ervaringen met carotis-echografie gedurende deze periode hebben geleid tot het formuleren van het onderzoek dat is beschreven in dit proefschrift onder supervisie en begeleiding van Prof. dr. M.V.Huisman (promotor), Prof. dr. T.J. Rabelink (promotor) & Dr. J.T.Tamsma (co-promotor).

Arghya werd in 2008 toegelaten tot de opleiding Interne Geneeskunde door Prof. dr. A.E.Meinders en rondde deze af in 2014 met een differentiatie in de nefrologie onder Prof.dr. H. de Fijter & Prof.dr. T.J.Rabelink. Drie jaar van deze

opleiding genoot hij in het HAGA ziekenhuis (voorheen Leyenburg & Rode Kruis) onder opleiders Dr R.M. Valentijn, Dr R.H.Kauffmann en Dr.M.O. van Aken.

Arghya is werkzaam als internist-nefroloog in het Leids Universitair Medisch Centrum. Hij is getrouwd met Poonam en heeft twee kinderen Ayodhya & Avani.