Carotid imaging in cardiovascular risk assessment

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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.

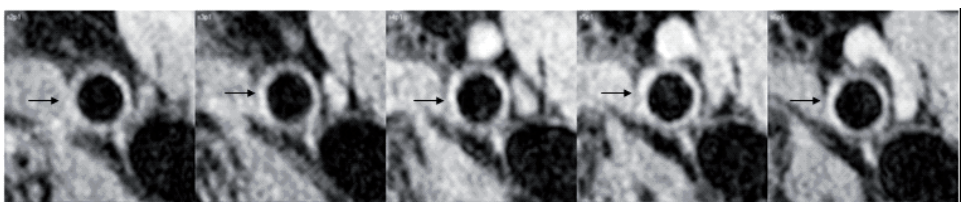

Figure 1 | Representative example of 3-Tesla MRI image of the carotid artery of a 62-year old male subject. Black arrows indicate the carotid artery. Five contiguous transverse slices of 2mm, covering the most distal 1cm of the common carotid artery.

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.

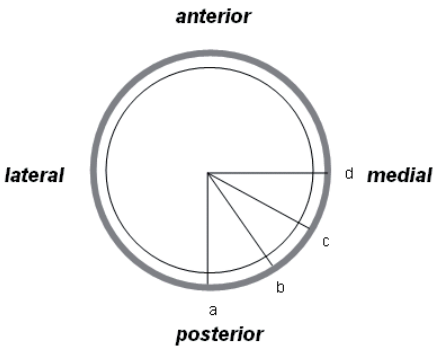

Figure 2 | Schematic illustration of the left common carotid artery. The inner black circle represents the lumen-intima border. The white area of the vessel wall indicates the intima-media complex, which is quantified by high-resolution ultrasound. The outer grey circle represents the lamina adventitia. 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).

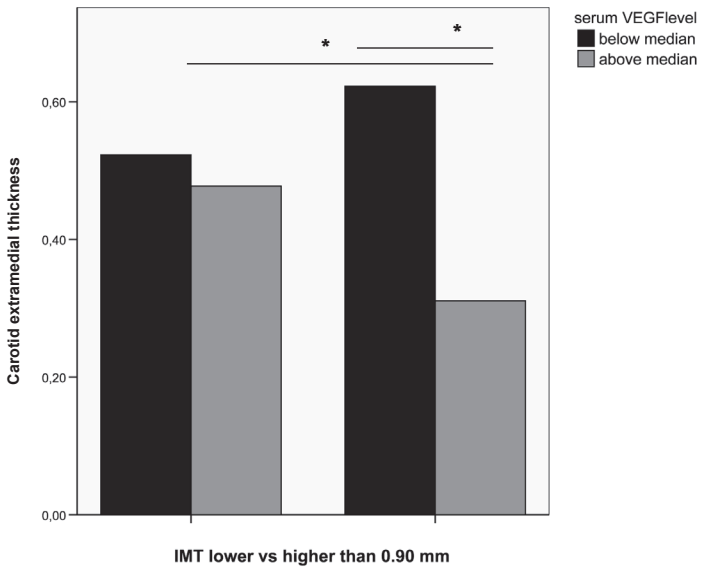

Figure 3 | cEMT values were significantly lower in the patients with elevated IMT levels and VEGF levels above the median (* $p<0.05$)

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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