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COMPUTED TOMOGRAPHY

Carotid plaque composition by CT angiography in asymptomatic subjects: a head-to-head comparison to ultrasound

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Abstract

Objectives To describe carotid plaque composition by computed tomography angiography (CTA) in asymptomatic subjects and to compare this to carotid plaque assessment by ultrasound, coronary plaques by coronary CTA, and inflammatory biomarkers in plasma.

Methods Middle-aged asymptomatic men, $n = 43$, without known cardiovascular disease and diabetes were included. Plaques in coronary and carotid arteries were evaluated using CTA. Total plaque volumes and plaque composition were assessed by a validated plaque analysis software. The 60% centile cut point was used to divide the population into low or high carotid total plaque volumes. The occurrence of carotid plaques and intima-media thickness (IMT) was estimated by ultrasound.

Results Carotid plaque by ultrasound was undiagnosed in 13 of 28 participants (46%) compared to CTA. Participants having carotid plaques by ultrasound had significantly higher absolute volumes of all CTA-defined carotid plaque subtypes and a higher fraction of calcified plaque. A high carotid total plaque volume was independently associated with age (adjusted odds ratio (OR) 1.41 [95% confidence interval (CI) 1.14–1.74], $p = 0.001$), IMT (adjusted OR 2.26 [95% CI 1.10–4.65], $p = 0.03$), and D-dimer (adjusted OR 8.86 [95% CI 1.26–62.37], $p = 0.03$). All coronary plaque features were significantly higher in participants with a high carotid total plaque volume.

Conclusion The occurrence of carotid plaques in asymptomatic individuals is underestimated by ultrasound compared to plaque assessment by CTA. Carotid plaque composition by CTA is different in individuals with and without carotid plaques by ultrasound.

Key Points

- *The occurrence of carotid plaques by ultrasound was underestimated in 46% of participants who had plaques by carotid CTA.*
- *Participants with carotid plaques by ultrasound had higher volumes of all plaque subtypes and a higher calcified plaque component as determined by carotid CTA compared to participants without carotid plaques by ultrasound.*
- *A high carotid total plaque volume was independently associated with age, intima-media thickness, and D-dimer.*

Keywords Carotid arteries · Computed tomography angiography · Ultrasonography · Plaque · Atherosclerotic

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Abbreviations

Ag	Agatston score
BMI	Body mass index
CAC	Coronary artery calcification
CRP	C-reactive protein
CT	Computed tomography
CTA	Computed tomography angiography
CVD	Cardiovascular disease
HDL	High-density lipoprotein
IMT	Intima-media thickness
IQR	Interquartile range
LDL	Low-density lipoprotein
LD-NCP vol.	Low-density non-calcified plaque volume
MRI	Magnetic resonance imaging
NCP vol.	Non-calcified plaque volume
SD	Standard deviation
TPV	Total plaque volume

Introduction

Carotid atherosclerosis is one of the predominant causes of stroke [1]. Carotid atherosclerosis determined by ultrasound is associated with subsequent risk of vascular events in symptomatic patients [2] and asymptomatic individuals in the general population [3]. Because of its easy setup and since it does not need contrast or radiation, ultrasound has been the preferred screening method for the occurrence of carotid atherosclerosis.

However, ultrasound remains inadequate for a detailed description of carotid plaque composition [4, 5], which recently promoted alternative imaging modalities [6–11]. These studies included both symptomatic and asymptomatic subjects in different clinical settings, using diverse non-invasive imaging modalities [7–10, 12] and histological specimens [3, 11]. The studies emphasize that the relationships between plaque components, plaque surface ulcerations, and intra-plaque hemorrhages in the carotid vasculature are complex, and conflictive findings have been reported accordingly [8, 11].

Coronary plaque characterization by CT angiography (CTA) has demonstrated high reproducibility when compared to intravascular ultrasound [13], is well-described in different patient subsets [14–17], and is associated with adverse cardiac outcomes [18, 19]. Coronary plaque composition has been shown to predict adverse cardiovascular outcomes even in persons without prior coronary artery disease [17]. Carotid plaque composition by CTA and the relation to composition and burden of coronary plaques, and ultrasonic determined carotid vessel wall changes have not previously been examined.

Therefore, this pilot study sought to quantify carotid plaque features by CTA in asymptomatic subjects without known cardiovascular disease (CVD) and to compare these findings with carotid plaque by ultrasound and coronary plaque composition by coronary CTA.

Materials and methods

Design and study population

The present cross-sectional pilot study is based on study participants born in either 1949 or 1959, who were recruited in 2009 and re-invited in 2015 as part of the Danish Risk Score (DanRisk) study [20–23]. The study participants in the current study were male participants from one center, University Hospital of Southern Denmark, Esbjerg. Participants with known CVD and/or diabetes, renal insufficiency (estimated glomerular filtration rate (eGFR) < 45 ml/min or creatinine concentration > 140 micromol/l), body mass index > 35, or atrial fibrillation were excluded. The selection criteria for the current study were based on the carotid ultrasonic findings in 2009. Participants having either a visible carotid plaque and/or intima-media thickness (IMT) in the highest tertile and participants with no visible carotid plaque and IMT in the lowest tertile were included in this study (Fig. 1). Study participants were re-examined in 2015, and the final study population consisted of 43 participants. The current study focuses on the follow-up examinations in 2015 where all examinations and scans were performed.

The study participants filled in a questionnaire concerning medical history, family history regarding vascular events, current medication, and smoking habits. The questionnaire was followed by a standardized personal interview. The physical examination and registrations of participants included height, weight, waist circumference, blood pressure, resting ECG, and a venous blood sample.

Written informed consent was obtained from all individual participants included in the study. All procedures performed in the study were conducted in accordance with the ethical standards of the Regional Scientific Ethics Committee for Southern Denmark and with the ethical guidelines of the 1975 Declaration of Helsinki and its later amendments. The protocol was approved by the Regional Scientific Ethics Committee for Southern Denmark, reference number: S20080140 and S20130169 (45023).

Carotid ultrasound

Ultrasound of the carotid arteries was performed using a Philips iE33 High resolution, B-mode cardiovascular ultrasound system (Philips Medical Systems) with a 11–3-MHz linear array transducer with 288 elements and extended operating frequency range, and IMT was analyzed using a semi-automatic edge-detection software package (Q-LAB, version 8.0 with software plug-in IMT). The proximal, mid, and distal

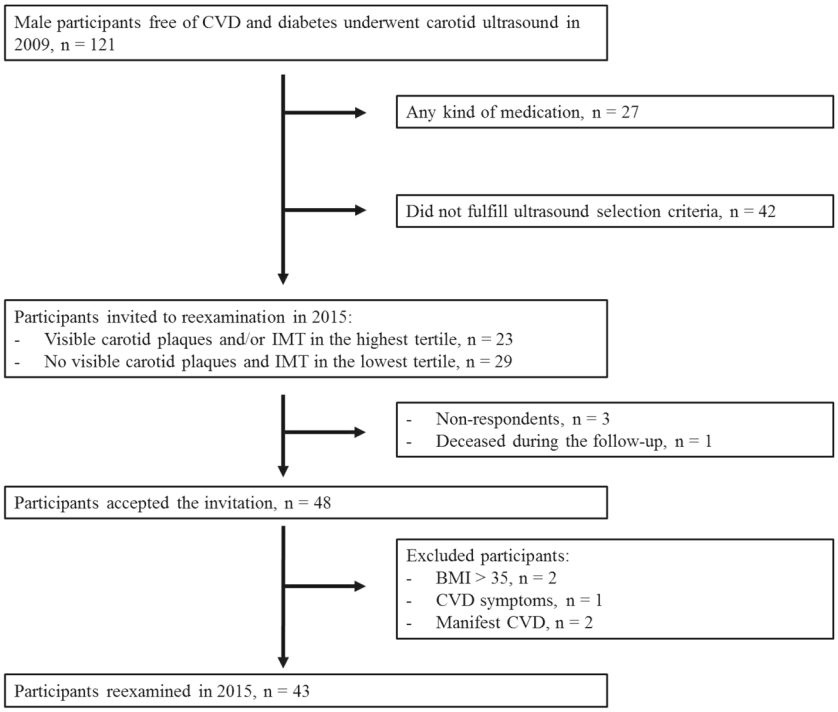


Fig. 1 Flowchart

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

common artery and carotid bifurcation were systematically recorded both horizontally and longitudinally in 0°, 90°, and 180° angles. Intima-media thickness (IMT) was determined at the far wall in each recording during the end-diastole and was based on average of IMT measurements in the common carotid artery and the bifurcation.

Interpretation of the ultrasound scans was performed off-line by the same trained reader blinded to all clinical data. The scan protocol has been described previously [21]. A carotid plaque on ultrasound was defined either as (1) a thickening of the focal wall that was at least 50% greater than the thickening of the surrounding vessel wall or (2) a focal region with an IMT of more than 1.5 mm.

Cardiac CT

Coronary artery calcification (CAC) was expressed as the Agatston score (Ag) [24] and was assessed using a Philips 64-slice scanner (Brilliance 64, Philips Healthcare). Non-contrast scan data were acquired during an inspiratory breath hold. The following technical settings were used: gantry rotation time 400 ms, collimation 64 × 0.625 mm, slice thickness 2.5 mm, 120 kV tube voltage, 220 mA tube current, and prospective gating at 75% of the R-R interval. Median (interquartile range (IQR), range, k-factor) estimated radiation dose was 1.12 mSv (1.01–1.22, 0.54–1.77 mSv, 0.0145).

All CT scans were analyzed by the same experienced cardiologist, who was blinded to all other patient data. The internal validity of calcium scoring was high [22].

Carotid and coronary CTA

Combined carotid and coronary CTA was performed using a Siemens Definition FLASH Dual Source CT scanner. Optiray (Mallinckrodt Pharmaceuticals) was used as the contrast medium. A dynamic test bolus scan was performed at the mid-level of the heart. A retrospective ECG-gated reconstruction acquisition protocol was applied. Technical settings were gantry rotation time 280 ms (temporal resolution of 0.75 ms), collimation 2 × 64 × 0.6 mm, reconstructed slice thickness is 0.6 mm with an increment of 0.3 mm in a matrix of 512 × 512 pixels, 120 kV tube voltage, and 20 mAs tube current with a quality reference mAs of 320 (using CAREDose4D Automatic Exposure Correction). Pitch was automatically adapted to the heart rate. ECG pulsing was set to 100% dose for 65–75% of the R-R interval. For the remaining part of the cardiac cycle, dose was reduced to 4% (MinDose). The CT acquisition was performed in the caudocranial direction, starting 2 cm distally of the apex of the heart to the level of the external auditory canal.

Two separate datasets were reconstructed. The first range covered the heart and the second range covered the area from the aortic arch to the proximal limit of the scan range. Mean (SD, range, k-factor) estimated radiation dose for the coronary and the carotid scans were 5.82 (1.48, 3.71–10.48, 0.0145) and 2.05 (0.52, 1.30–3.69, 0.0051), respectively. All scans were transferred to a dedicated workstation, Syngo.via (Siemens Healthcare) for off-line analysis.

The carotid and the coronary scans were analyzed by an experienced neuro-radiologist and an experienced CT

cardiologist, respectively. Both were blinded to all the clinical data. The location of lesions in the coronary arteries was reported using a 17-segment model [25], and a stenosis severity > 50% was classified as significant. Coronary scans were rated non-evaluable if three or more segments were non-evaluable. Lesions in the carotid arteries were reported as located in the common carotid artery, the carotid bifurcation, and the internal and external carotid arteries [26], and a stenosis severity > 70% was classified as significant.

Plaque quantification on carotid and coronary CTA

A semi-automated plaque software validated by coronary CTA, Autoplaque © research software, version 2.0. [13, 27], was used to quantify both the carotid and the coronary plaques by the same trained reader, blinded to all other data. The applied software has been used in CTA datasets of various protocols from a variety of commercial CT scanners [15, 28]. The software has not previously been validated on CTA datasets derived from carotid arteries.

For both the carotid and the coronary CTA, window settings were set to standard CTA and modified when necessary. Plaques were identified in multiplanar windows both with cross-sectional and longitudinal views. This was followed by a definition of the proximal and the distal point of the plaque. A circular region of interest for a reference blood pool in the descending aorta was set before proceeding to the center-line editor and subsequently to the automated lesion quantification using predefined scan-specific threshold values. The reader performed edge-detection, automatic visual adjustments of plaque composition, and changing of threshold values of non-calcified plaque volume (NCP vol.) and calcified plaque volume before finalizing the result.

Examples of plaque analyses in the carotid arteries are depicted in Fig. 2. Volumes of NCP (red overlay), calcified plaque (yellow overlay), low-density non-calcified plaque (LD-NCP), and total plaque were registered. The carotid bulb was defined as a visual enlargement of the carotid vasculature before the bifurcation, and the beginning of the common carotid artery was defined as 60 mm centrally from the most distal point of the carotid bulb. The common carotid artery was separated into three segments, distal, mid, and proximal, each with a length of 20 mm. The bifurcation was defined as the exact branching point of the carotid bulb. The internal carotid artery and the external carotid artery were each separated into two segments, distal and mid, each with a length of 20 mm.

In the coronary scans, proximal and distal points of the plaque were set before the automatic plaque quantification, while the arterial lumen was determined by a series of control points in the region of interest in the carotid scans.

The population was separated in individuals having a low or a high carotid total plaque volume based on a previously defined 60% centile cut point [17], giving a threshold of 228.4 mm^3. All coronary segments with a lumen diameter ≥ 2 mm were analyzed in accordance with the Society of Cardiovascular Computed Tomography (SCCT) guideline [29]. In addition, each lesion was classified according to the SYNTAX Score I (synergy between percutaneous coronary intervention with TAXUS™ and cardiac surgery I) [30, 31].

Blood collection and handling

Blood samples were drawn from an antecubital vein into sterile vacuum plastic tubes containing either 0.109 mol/L citrate or no anticoagulants, and the plasma or serum isolated after 20-min centrifugation at 2000*g* at 20 °C were frozen and stored in aliquots at − 80 °C until analysis. Lipids were analyzed using Cholesterol, Direct LDL, Ultra HDL, and Triglycerides kits employing the Architect C16000 analyzer. Kits and analyzer were from Abbott. Concentrations of CRP and fibrinogen were determined on a BN-II nephelometer using antibodies and reagents from Siemens Healthcare Diagnostics GmbH. The protein concentration of D-dimer was determined with the STA-Liatest D-DI kit from Diagnostica Stago. The assay was performed using the STA-R Evolution autoanalyzer from Diagnostica Stago.

Statistics

Continuous variables were expressed as mean ± SD or median and IQR. Dichotomous variables are shown as numbers and percentages. Student's *t* test was used for comparison of normally distributed continuous variables, and the Mann-Whitney rank test for comparison of non-normally distributed continuous variables. Fisher's exact test was performed for comparison of dichotomous variables. The Spearman rank correlation test was used to examine correlation between continuous variables. To examine independent associations, multivariate logistic regression models with stepwise backward analyses using carotid total plaque volume as the outcome variable were generated. The models tested variables that significantly differentiated the groups in the univariate analysis, age, tobacco use, antihypertensive treatment, D-dimer, IMT, and coronary total plaque volume. The intra- and inter-observer analyses were based on 12 carotid ultrasound and CTA scans and Bland-Altman plots, Spearman rank correlation, Wilcoxon signed rank test, and kappa statistics were used to test observer variations. *P* values < 0.05 were considered statistically significant in both the univariate and multivariate analyses. Stata 15.0, StataCorp was used for statistical analyses.

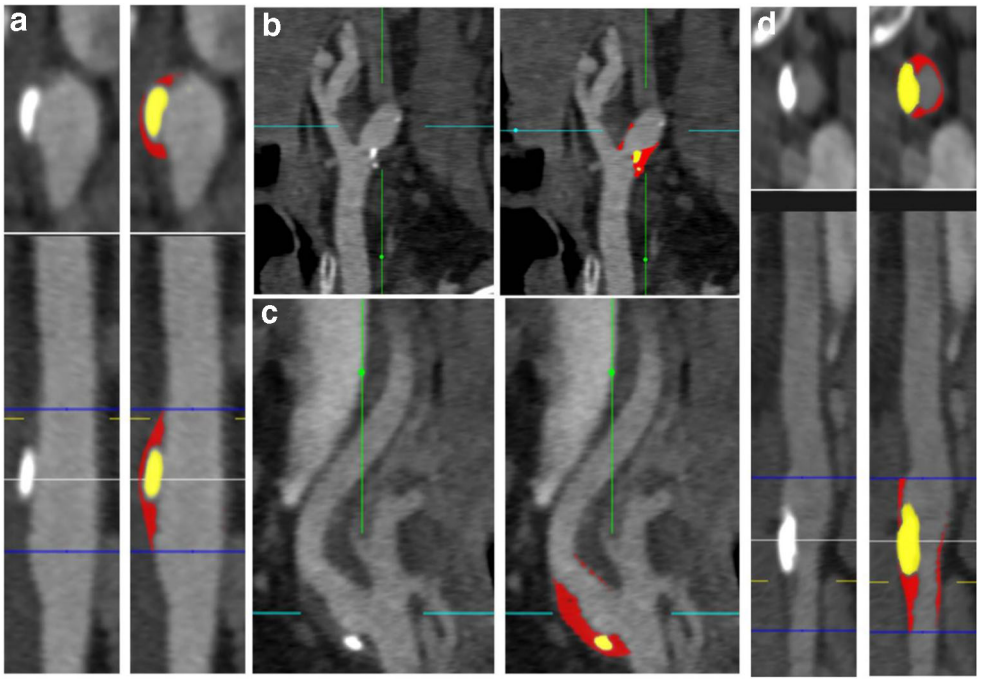


Fig. 2 Screenshots from the semi-automated plaque quantification in the carotid arteries. Red overlay indicates non-calcified plaques (NCP); yellow overlay indicates calcified plaques. **a** Stretched and horizontal views with mixed plaque in the right carotid bifurcation. **b** Longitudinal view with mixed plaque in the left carotid bifurcation. **c** Mixed plaque in the right internal carotid artery. **d** Stretched and horizontal views with mixed plaque in the left external carotid artery

Results

Basic characteristics and correlation analyses

Baseline characteristics, inflammatory biomarkers, carotid ultrasound findings, and coronary plaque features according to carotid total plaque volume by CTA are shown in Table 1. Age, tobacco use, antihypertensive treatment, D-dimer, IMT, prevalence of carotid plaque by ultrasound, and coronary plaque characteristics significantly differed between groups.

One participant had a significant coronary stenosis, while no significant stenosis in the carotid arteries was found. There were no differences in CT acquisition characteristics between participants with or without carotid plaque by ultrasound.

D-dimer, IMT, the SYNTAX score I, Ag, and coronary total plaque volume were significantly correlated with each of the carotid plaque composition variables, Table 2. IMT correlated with the SYNTAX score I, and coronary NCP, calcified plaque, and total plaque volumes, while Ag and carotid total plaque volume correlated with the SYNTAX score I and all the coronary plaque composition variables, Table 4 (supplementary results). D-dimer did not correlate with any of the coronary plaque composition variables.

Reproducibility analyses

The observer analyses of the ultrasound examinations on carotid plaque occurrence revealed good observer concordances (intra-observer analysis: $r = 0.63$, $p < 0.01$, κ value of 0.63; inter-observer analyses: $r = 0.71$, $p < 0.01$, κ value of 0.67). The observer analyses on carotid total plaque volume by CTA revealed good observer concordances (intra-observer analyses: $r = 0.97$, $p < 0.001$, κ value = 0.79; inter-observer analyses: $r = 0.95$, $p < 0.0001$, κ value = 0.79) (Fig. 3).

Head-to-head comparison between carotid ultrasound and CTA

Participants with carotid plaques by ultrasound had a different carotid plaque composition both in absolute (Table 3) and relative (Fig. 4) terms, compared to participants with no carotid plaques by ultrasound. The coronary plaque composition was similar in participants with and without carotid plaque by ultrasound, Table 5 (supplementary results).

Carotid plaques were found in 28 participants determined by CTA of whom 13 (46%) had no carotid plaques by ultrasound. Screenshot examples of the carotid CTA and ultrasound are shown in Fig. 5. Median (interquartile range) total carotid plaque volume by CTA in the latter 13 participants was 238.7 mm^3 (109.8–402.3 mm^3) compared to 317.7 mm^3 (141.6–548.9 mm^3) in participants with carotid plaques by both CTA and ultrasound ($n = 16$), $p = 0.34$. Plaques that were undiagnosed by ultrasound were located in the internal/external carotid arteries in five participants, in the carotid bifurcation in two participants and in both the carotid bifurcation and the internal/external carotid arteries in six participants.

Coronary plaques were found in 25 participants, of whom 20 (80%) had carotid plaques by CTA, while 12 (48%) had carotid plaque by ultrasound.

Table 1 Baseline characteristics, inflammatory biomarkers, carotid ultrasound, and coronary plaque features according to carotid total plaque volume by computed tomography angiography

Carotid total plaque volume			
$n = 43$	Low, $n = 26$	High, $n = 17$	p value
Baseline characteristics			
Born			
1949	4	13	
1959	22	4	< 0.001
Tobacco use			
Never	15 (58)	3 (18)	
Prior/current	11 (42)	14 (82)	< 0.05
Systolic blood pressure, mmHg	138 (19)	137 (14)	0.87
Diastolic blood pressure, mmHg	81 (9)	81 (8)	0.97
Body mass index, kg/m^2	26.9 (2.4)	27.8 (4.2)	0.38
Total cholesterol, mmol/l	5.27 (1.01)	5.49 (0.73)	0.46
LDL cholesterol, mmol/l	3.23 (0.98)	3.51 (0.67)	0.32
HDL cholesterol, mmol/l	1.32 (0.30)	1.31 (0.35)	0.97
Triglycerides, mmol/l	1.88 (1.25)	1.75 (0.70)	0.72
Antihypertensive treatment	0 (0)	3 (18)	0.06
Lipid-lowering treatment	3 (12)	1 (6)	0.97
Antithrombotic treatment	0 (0)	0 (0)	NA
Anticoagulative treatment	1 (4)	0 (0)	0.40
Biomarkers			
C-reactive protein, mg/l	0.89 (0.87)	1.10 (1.30)	0.53
Fibrinogen, μmol/l	8.92 (1.39)	8.92 (1.53)	0.98
D-dimer, mg/l	0.31 (0.21)	0.38 (0.13)	< 0.05
Carotid ultrasound features			
Intima-media thickness, mm	0.75 (0.12)	0.87 (0.13)	< 0.01
Carotid plaques by ultrasound			
No	20	7	
Yes	6	10	< 0.05
Coronary plaque characteristics			
SYNTAX score I	0 (0–15)	16 (9–34)	< 0.05
Agatston score, U	0 (0–111)	15 (4–228)	< 0.05
NCP volume, mm^3	0 (0–150.1)	205.1 (78.8–302.0)	< 0.05
Calcified plaque volume, mm^3	0 (0–26.2)	6.3 (0.1–82.2)	< 0.05
LD-NCP volume, mm^3	0 (0–11.1)	23.3 (5.9–39.0)	< 0.01
Total plaque volume, mm^3	0 (0–218.7)	210.5 (85.1–335.7)	< 0.05
Number of affected coronary segments			
0 segment	15	3	
1–2 segments	4	5	
3–4 segments	2	5	
≥ 5 segments	5	4	< 0.05

Values are presented as mean (SD), median (IQR), or n (%). Low carotid total plaque volume: carotid total plaque volume < 60% centile cut point; high carotid total plaque volume: carotid total plaque volume ≥ 60% centile cut point

LDL low-density lipoprotein, *HDL* high-density lipoprotein, *NCP volume* non-calcified plaque volume, *LD-NCP volume* low-density non-calcified plaque volume

Multivariate logistic regression revealed that age (adjusted odds ratio (OR) 1.41 [95% confidence interval (CI) 1.14–1.74], $p = 0.001$), IMT (adjusted OR 2.26 [95% CI 1.10–4.65], $p = 0.03$), and D-dimer (adjusted OR 8.86 [95% CI 1.26–62.37], $p = 0.03$) were independently associated with high carotid plaque volume determined by CTA.

Table 2 Correlation (*r*) between carotid plaque characteristics by computed tomography angiography (CTA) and D-dimer, intima-media thickness, SYNTAX Score I, Agatston score, and coronary total plaque volume by CTA

	Carotid NCP volume, mm^3	Carotid calcified plaque volume, mm^3	Carotid LD-NCP volume, mm^3	Carotid total plaque volume, mm^3
D-dimer, mg/l	0.34	0.41	0.32	0.37
Intima-media thickness, mm	0.57	0.62	0.53	0.59
SYNTAX score I	0.43	0.48	0.32	0.45
Agatston score, U	0.51	0.55	0.43	0.53
Coronary total plaque volume, mm^3	0.44	0.54	0.38	0.47

Values are presented as Spearman's correlation coefficient *r*. All $p < 0.05$

NCP non-calcified plaque, *LD-NCP* low-density non-calcified plaque

Discussion

This pilot study in asymptomatic middle-aged men indicated that the presence and burden of carotid plaques by ultrasound compared to CTA are underestimated. Participants with and without carotid plaques by ultrasound had different plaque composition by CTA both in absolute and relative terms. In addition, this study demonstrated that carotid plaque composition by CTA was associated with age, IMT, and D-dimer plasma concentration.

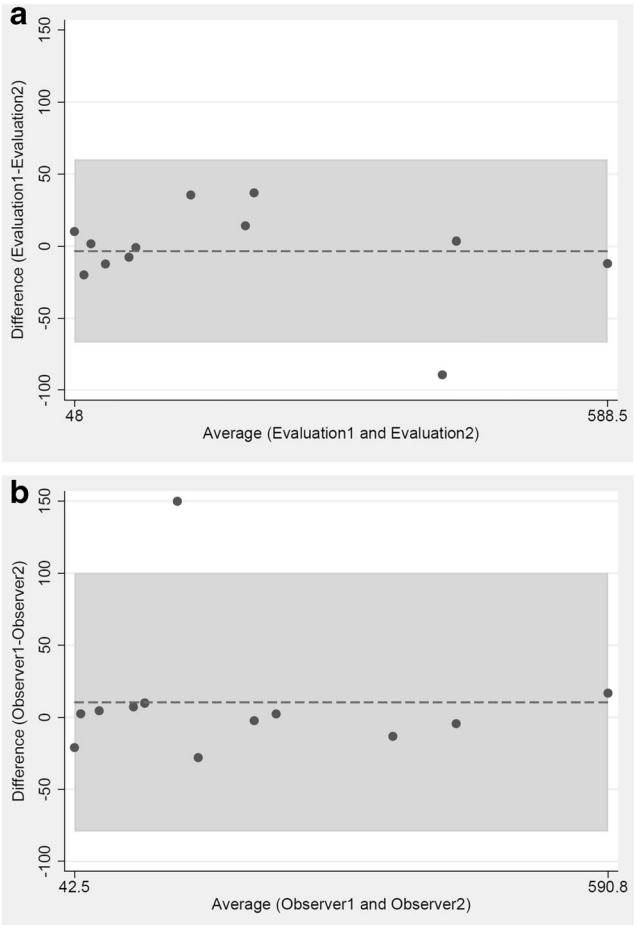


Fig. 3 Bland-Altmann plot of **a** intra-observer and **b** inter-observer variation of total plaque volume by carotid computed tomography angiography (CTA)

Carotid atherosclerosis is assessed by ultrasonic determined IMT for predicting CVD, in particular cerebrovascular disease [2, 6, 32, 33]. Although assessment of carotid atherosclerosis in the internal carotid artery improves prediction of CVD better than IMT in the common carotid artery [34], several studies do not include evaluation of IMT in the internal carotid artery due to poor accessibility when using ultrasound [35]. Moreover, scan protocols may vary in terms of visualized segments, scan angles used, definition of carotid plaque, and definitions of cut points for abnormal IMT [34], and a substantial observer variability has been found [34, 35]. The present study reveals that the presence of carotid plaques is underestimated by ultrasound, as 46% of plaques diagnosed by CTA went undiagnosed by ultrasound, and 62% of these were actually located in the carotid bifurcation. Previously, it has been demonstrated that ultrasound is highly accurate in detecting the presence of large calcified plaques [36]. Accordingly, in this study, participants with ultrasound-verified carotid plaques had a significantly larger fraction of calcified plaques compared to participants without carotid plaques by ultrasound. However, the fraction of non-calcified plaques constituted the largest proportion of total plaque volume both in participants with and without carotid plaques by ultrasound. In this relation, the non-calcified part of plaques has been associated with carotid ulcerations in vulnerable plaques [10], while multiple calcifications have been associated with intra-plaque hemorrhage [37]. Moreover, studies have documented a discrepancy and a poor correlation between carotid ultrasound features and CTA regarding plaque ulcerations and stenosis grading [38–40] in symptomatic patients. Although the current study demonstrates an independent association between carotid total plaque volume by CTA and IMT by ultrasound [41], the study also emphasizes the discrepancy between carotid ultrasound and CTA in asymptomatic subjects.

The plaque analysis software used in the present study has previously been used to demonstrate the value of coronary

Table 3 Carotid plaque characteristics by computed tomography angiography according to findings by carotid ultrasound

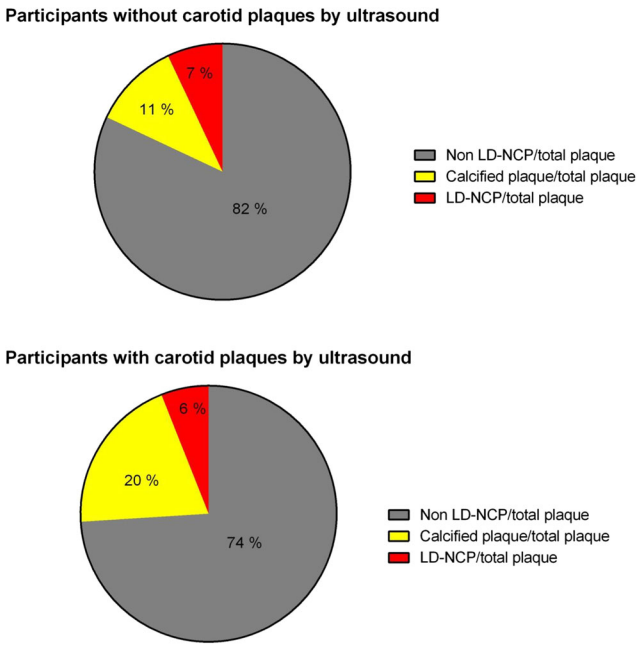
	÷ Carotid plaques by ultrasound, $n = 27$	+ Carotid plaques by ultrasound, $n = 16$	p value
Carotid plaque characteristics			
NCP volume, mm^3	0 (0–227.1)	226.9 (62.8–358.6)	< 0.01
Calcified plaque volume, mm^3	0 (0–12.4)	39.7 (7.4–82.4)	< 0.01
LD-NCP volume, mm^3	0 (0–11.0)	9.1 (2.6–17.7)	< 0.05
Total plaque volume, mm^3	0 (0–238.7)	302.4 (86.0–471.5)	< 0.01
Number of affected carotid segments on CTA			
0 segment	14 (52)	1 (6)	
1–2 segments	11 (41)	10 (63)	
3–4 segments	2 (7)	5 (31)	
≥ 5 segments	0 (0)	0 (0)	< 0.05

Values are presented as median (IQR) or n (%)

NCP vol. non-calcified plaque volume, *LD-NCP vol.* low-density non-calcified plaque volume

plaque composition in predicting adverse cardiac outcomes [17]. Thus, the finding in this study, that asymptomatic participants with carotid plaques by CTA have a higher prevalence of coronary plaques by CTA, higher measures of the SYNTAX score I and coronary plaque volumes, acknowledging the systematic nature of the atherosclerotic process, may be of clinical relevance [42, 43]. In addition, we have shown that IMT was significantly correlated with the SYNTAX score I and most of the coronary plaque composition characteristics.

Magnetic resonance imaging (MRI) is another imaging modality used as a confirmatory test to visualize carotid plaques,



LD-NCP: low-density non-calcified plaque volume.

Fig. 4 Distribution of carotid plaque components as a percentage of carotid total plaque volume. Upper panel: participants without carotid plaques by ultrasound. Lower panel: participants with carotid plaques by ultrasound

and contrast-enhanced MRI in particular is able to detect the fibrous cap of the plaque, intra-plaque hemorrhage, and plaque ulcerations [6]. A lower spatial resolution compared with CTA and a varying accessibility of MRI in the clinical setting have been reported [44]. However, novel MRI approaches have shown promising results with respect to the sharpness in carotid plaque visualization [44, 45]. A sub-study of the Rotterdam study [8] implemented both CTA and MRI and reported increased intra-plaque hemorrhage and decreased lipid core with increasing calcification. Contrary to the findings in the Rotterdam study, the LD-NCP proportion representing the lipid core was not significantly different between participants with or without carotid plaque on ultrasound in the current study. Discrepancies between findings in the Rotterdam study and the present study may be explained by a difference in the applied plaque analysis software, inclusion of 12% of diabetic patients in the Rotterdam sub-study, as asymptomatic diabetic patients already at an early stage have more prevalent vulnerable plaque features [16], and the limited sample size in the current study. Although participants in the present study were highly selected, inclusion criteria were chosen to create diversity in the occurrence and composition of carotid plaques. D-dimer is elevated both in asymptomatic subjects with high IMT [46], and in patients with unstable carotid plaques [47], and the number of plaques in the carotid vasculature seems to be associated with D-dimer [48]. In the current study, D-dimer was independently associated with carotid total plaque volume by CTA, presumably as an indicator of increased fibrin metabolism in these participants.

Assessments of flow dynamics in the carotid arteries using Doppler ultrasound have been challenging [44, 49]. Fractional flow reserve (FFR) CT derived from coronary CTA (FFR_{CT}) has recently emerged as a non-invasive modality for assessment of the hemodynamic significance of coronary stenosis in symptomatic patients [50, 51]. Whether CT-derived functional estimates can be applied for evaluation of carotid arteries

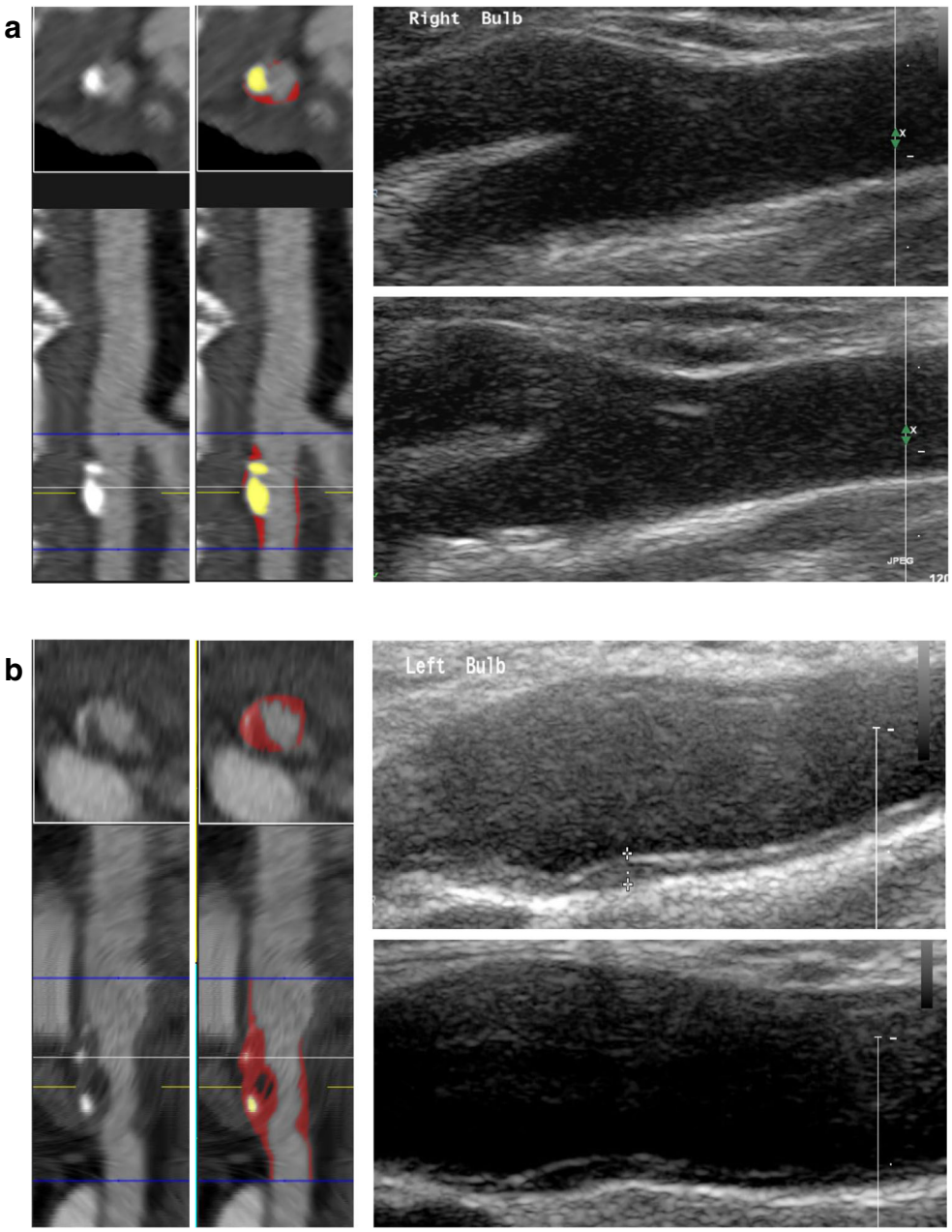


Fig. 5 Examples illustrating cases, where carotid plaques were only diagnosed by computed tomography angiography (CTA). Left panel showing carotid CTA, right panel carotid ultrasound. **a** Mixed plaque in the right internal carotid artery. **b** Mixed plaque in the left carotid bifurcation and in the internal carotid artery. Red overlay indicates non-calcified plaque (NCP) and yellow overlay indicates calcified plaque

remains to be settled in future studies. Current guidelines do not recommend screening of the carotid arteries by CTA in asymptomatic subjects in the general population [52]. Whether the results of this study can be applied for general large-scale screening of carotid plaques by CTA with contemporary CTA acquisition protocols [53] needs further exploration. Owing to the prognostic capabilities of coronary CTA in symptomatic patients [54], CTA using prospective gating and FLASH mode with even lower radiation exposure [53] may be promising in the carotid arteries. In this relation, our study showed lower neck radiation dose compared to the coronary radiation dose.

The single-center study design along with the small sample size hampers any subgroups analyses and limits the conclusions drawn by the present study. The study population consisted solely of men; thus, the extrapolation to a generalized population is not possible. Due to the novel and mechanistic approach of the current study in quantification of carotid plaque composition, further research in a larger population with inclusion of women is needed.

Conclusion

This study indicates that the occurrence of carotid plaques is underestimated by ultrasound compared to plaque assessment by CTA. Carotid plaque composition determined by CTA is

significantly different in individuals with and without carotid plaques by ultrasound. The results need confirmation in larger studies including both sexes.

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Compliance with ethical standards

Guarantor The scientific guarantor of this publication is Dr. Niels Peter R. Sand, PhD, Department of Cardiology, University Hospital of Southern Denmark, Esbjerg, and Department of Regional Health Research, University of Southern Denmark, Denmark.

Conflict of interest The authors of this manuscript declare relationships with the following companies: Dr. Bjarne L. Nørgaard has received research grant from Siemens, Edwards Lifesciences and Heartflow. Dr. Damini Dey is a patent-holder of Autoplaque research software and receives royalties from Cedars-Sinai Medical Center, Los Angeles, CA, USA. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Statistics and biometry Ramshanker Ramanathan and Damini Dey performed the statistical analyses. Statistician Pia V. Larsen, Department of Public Health, University of Southern Denmark, Odense, provided statistical advice.

Informed consent Written informed consent was obtained from all individual participants included in the study. All procedures performed in the study were conducted in accordance with the ethical standards of the Regional Scientific Ethics Committee for Southern Denmark and with the ethical guidelines of the 1975 Declaration of Helsinki and its later amendments.

Ethical approval The study protocol was approved by the Regional Scientific Ethics Committee for Southern Denmark, reference number: S20080140 and S20130169 (45023).

Study subjects or cohorts overlap Some study subjects or cohorts have been previously reported in the following papers: [1–9]. The study subjects recruited in the current study were part of the Danish Risk Score Study (the DanRisk study) conducted in 2009/2010 with follow-up examinations performed in 2015. The DanRisk study was a multicenter study involving four regional centers (Odense, Esbjerg, Vejle, and Svendborg).

The current study is based on follow-up examinations on asymptomatic male study subjects from one study center, Esbjerg, who were free of cardiovascular disease (CVD), diabetes, and any medication at baseline. The current study differs from the other studies by examining the carotid plaque composition by computed tomography angiography (CTA) and by comparing these findings with carotid plaque by ultrasound. In addition, this is the first study from DanRisk focusing on CTA.

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Methodology
- prospective
- cross-sectional study/observational
- performed at one institution

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