3

The role of atherosclerosis in stroke

3.1 Markers of subclinical vascular disease and stroke; a review

INTRODUCTION

It is estimated that 70% of all strokes are related to vascular disease caused by atherosclerosis. Nowadays, a large arsenal of techniques is available to measure subclinical vascular pathology in various arterial segments. To the extent that subclinical vascular pathology predicts stroke risk, it can be used to identify persons who are at increased risk of stroke and, hence, may benefit most from preventive interventions. In this chapter, we review the relation between markers of subclinical vascular disease and its relation with stroke. If possible, we focus on population-based evidence. Some measures of vascular disease are relatively new and their relation with stroke in the population still has to be explored. In those cases, we discuss results from hospital-based studies. First, we focus on measures of generalized atherosclerosis. Then, we shall discuss some structural and functional properties of vascular disease. Finally, possible expressions of vascular disease as detected by brain MRI will be discussed.

MEASURES OF GENERALIZED ATHEROSCLEROSIS

Ankle-brachial index

The ankle-brachial index is a relatively simple and non-invasive measure, that is used to assess peripheral arterial disease and atherosclerosis.[1,2] The ankle-brachial index is calculated as the ratio of systolic blood pressure measured at the ankle to the systolic blood pressure at the arm. An ankle-brachial index below 0.90 is considered to reflect presence of peripheral arterial disease.[3] Peripheral arterial disease is common in elderly persons without overt symptoms of claudication and the prevalence in subjects aged over 65 years is estimated to be 10%.[4] Several studies have consistently shown that a low ankle-brachial index is a predictor of stroke (table 1).[5-9]

Table 1

Prospective population-based cohort studies on ankle-arm index and risk of stroke.

Study	Study population (Age)	Follow-up	No. of strokes	Relative risk (95% CI) of stroke in low (<0.9) vs high (>0.9) ankle arm index
Edinburgh Artery Study [5]	1592 subjects (55-74 years)	5 years	50	1.9 (1.0-3.5)* 1.9 (1.0-3.4)¶
Cardiovascular Health Study[6]	5714 subjects (65 years or over)	6 years	67	1.4 (0.9-2.2)*§ 1.4 (0.9-2.3)¶§ 1.6 (1.1-2.4)*‡ 1.1 (0.7-1.7)¶‡
Atherosclerosis Risk In Communities Study [7]	14,839 subjects (45-64 years)	7 years	206	5.7 (2.8-11.7)*† 1.4 (0.7-2.6)¶†
Rotterdam Study [8]	6450 subjects (55 years or over)	3.7 years	135	2.3 (1.5-3.3)*
Honolulu Heart Program [9]	2767 men (71-93 years)	3.6 years	91	2.0 (1.1-3.5)¶

* Adjusted for age and gender, ¶ Adjusted for age, gender and cardiovascular risk factors, ‡ In participants without previous cardiovascular disease, † Relative risk in ankle arm index <0.8 vs >1.2, § In participants with previous cardiovascular disease.

It has been suggested that a low ankle-brachial index can be used as a screening tool to identify subjects at high risk for disease, although a high ankle-brachial index does not rule out the presence of atherosclerosis. The Atherosclerosis Risk In Communities Study has shown that the risk associated with a low ankle-arm index diminished after adjustment for cardiovascular risk factors including systolic blood pressure, antihypertensive medication, diabetes, smoking, pack-years smoking, LDL-cholesterol, HDL-cholesterol and prevalent coronary heart disease. Therefore, it is doubtful whether assessment of ankle-arm index has prognostic ability beyond traditional risk factors in the prediction of stroke.

Carotid artery intima-media thickness

Atherosclerosis is accompanied by thickening of the intimal layer of the artery. B-mode ultrasonography allows non-invasive visualization of intima-media thickness. Although no distinction is made between the intimal and medial arterial layer, intima-media thickness has shown to be related with cardiovascular risk factors and with other measures of atherosclerosis. Therefore, an increased intima-media thickness is considered to reflect generalized atherosclerosis.[10] Carotid intima-media thickness can be measured in different carotid arterial segments (common carotid artery, bifurcation or internal carotid artery). Several studies have shown that carotid intima-media thickness is positively related to the risk of stroke [11,12] and cerebral infarction,[13] irrespective of location in the carotid artery.[12] Table 2 gives an overview of these studies. In summary, carotid intima-media thickness is related to all subtypes of stroke and cerebral infarction.

Carotid plaques

Plaques represent more advanced stages of atherosclerosis and are predominantly present in arterial segments with a turbulent blood flow like the carotid bifurcation. Presence of carotid plaques and plaque characteristics like echolucency, ulceration, intraplaque hemorrhage and surface regularity can be assessed through ultrasonography or angiography. Carotid plaques are frequently found in stroke patients and are related to the risk of stroke in the population.[14-17] It is still a matter of debate as to what the underlying mechanism is that relates carotid plaques and stroke. One proposed mechanism is rupture and intraplaque hemorrhage, leading to superimposed emboli. The finding that plaques with characteristics like echolucency, ulceration, intraplaque bleeding and irregularity of the plaque surface are associated with symptoms supports this.[14-19] Further, it was reported that hypoechoic, but not hyperechoic plaques were related to an increased risk of non-cardioembolic ischemic stroke in the population.[17] Another explanation is that carotid plaques simply reflect generalized atherosclerosis. The finding in the Rotterdam Study that total plaque score is related to the risk of stroke, cerebral infarction and lacunar infarction confirms that carotid plaques are markers of generalized atherosclerosis. Table 3 gives an overview of the prospective studies on carotid plaques and stroke.

Table 2

Studies on IMT and risk of stroke.

Study	Study design	Population	Determinant	Unity of IMT	Outcome	Relative risk (95% CI) of stroke per SD increase in CCA-IMT
Cardiovascular Health Study[12]	Population based cohort	4476 subjects aged >65 years	Maximal IMT in CCA and ICA	Per SD increase and in quartiles	Stroke	1.4 (1.3-1.5)*
Atherosclerosis Risk in Communities Study[13]	Population based cohort	14214 subjects aged 45-64 years	Mean IMT in CCA, BIF and ICA	Per SD increase and in tertiles and quintiles	Ischemic stroke, caused by thrombosis or embolism	Women: 1.7 (1.5-2.0)¶ Men: 1.5 (1.3-1.8)¶
Genetique de l'Infarctus Cerebral Study[60]	Hospital-based case-control	470 stroke cases and 463 controls	Mean far wall IMT in CCA	Per SD increase	Cerebral infarction and subtypes	1.8 (1.5-2.2)
Rotterdam Study	Population based cohort	5679 subjects aged >55 years	Mean, maximal IMT and mean far wall IMT in CCA	Per SD increase and in quartiles	Stroke, hemorrhagic stroke and subtypes of infarction	1.5 (1.4-1.6)* 1.4 (1.2-1.6)¶

IMT: Intima-media thickness, CCA: Common carotid artery, BIF: Carotid bifurcation, ICA: Internal carotid artery, * Per SD increase in maximal IMT, ¶ Per SD increase in mean IMT.

Table 3

Prospective studies on carotid plaques and stroke

Study	Study design	Population	Determinant	Outcome	Results§
Cardiovascular Health Study[17]	Population-based cohort	4886 asymptomatic subjects aged >65 years	Echogenicity of dominant plaque in internal carotid artery	Non-cardioembolic ischemic strokes	Hypoechoic plaques increase the risk of stroke in asymptomatic adults (2.5 (10.4-4.5)) *†
Rotterdam Study	Population-based cohort	4217 neurologically asymptomatic subjects aged >55 years	Plaques in six locations in the carotid artery	Stroke and subtypes of cerebral infarction	Plaques increase risk of stroke (2.4 (1.4-4.2)) lacunar (10.8 (1.70-69.7)) and non-lacunar infarction in anterior (3.2 (1.1-2.6)), but not in posterior circulation (0.6 (0.1-4.9)) *†‡
Tromsø Study[18]	Population-based follow-up study	223 subjects with and 215 without carotid stenosis	Echogenicity of plaque	Stroke, cerebral infarction and TIA	Subjects with both stenosis and echolucent plaques have increased risk of stroke compared to subjects without carotid stenosis (2.8 (4.4-37.2)) *
Grønholdt et al.[19]	Hospital-based follow-up study	111 asymptomatic and 135 symptomatic patients with >50% carotid stenosis	Echogenicity of plaque	Ischemic stroke in ipsilateral hemisphere	Echolucent plaques compared to echorich plaques increase risk of stroke in symptomatic (2.9 (1.2-7.0)), but not in asymptomatic subjects (1.0 (0.3-3.3)) ¶

* Adjusted for age and gender, ¶ Adjusted for age, gender and cardiovascular risk factors, † Subjects without plaques as reference, ‡ Relative risk in subjects with 5 to 6 plaques in the carotid arteries, § Figures represent relative risk (95% CI).

Aortic arch atherosclerosis

Atherosclerosis in the aortic arch, and in particular disrupted and protruding plaques as assessed with transesophageal echocardiography is frequently observed in stroke patients.[20] Follow-up studies showed that aortic plaque morphology (ulceration, calcification, hypoechoic plaques, irregularities and mobile thrombus[21,22] and thickness[23]) was related to the risk of recurrent stroke. Several mechanisms have been proposed to explain the relation between aortic plaques and stroke.[24] First, aortic plaques are potential sources of thrombo-emboli. This was confirmed by patient series in which a relation between aortic arch atheroma and cerebral micro-emboli was found.[25] Secondly, obstruction of the origin of the carotid and vertebral artery could lead to hemodynamic obstruction of the cerebral blood flow. And thirdly, aortic plaques are considered to be markers of generalized atherosclerosis. Thus far, the relation between plaques in the aortic arch and stroke has not yet been investigated in a population-based setting.

STRUCTURAL AND FUNCTIONAL PROPERTIES OF SUBCLINICAL VASCULAR DISEASE

Calcifications in the vessel wall

Calcium deposits in the coronary and extra coronary vessels are considered to indicate the extent of atherosclerotic lesions. Therefore, they are putative markers of subclinical vascular disease. Calcifications in the coronary arteries can be highly sensitively visualized by electron-beam tomography. Quantitative measures of coronary calcification are closely related to the amount of atherosclerotic plaques in histopathologic investigation.[26]

Several population-based studies have explored the relation between calcifications and stroke. Iribarren and colleagues investigated the relation between calcification of the aortic arch, detected by X-ray and risk of stroke in a population of 139,849 subjects aged 30 to 89 years.[22] They found that aortic arch calcification increased the risk of ischemic stroke in women (RR 1.46, 95% CI 1.28-1.67), but not significantly in men (RR 1.17, 95% CI 0.97-1.42). The Rotterdam Study investigated the relation between coronary calcification and presence of stroke in 2,013 men and women in whom 34 men and 16 women

had experienced a stroke.[27] Coronary calcifications in the epicardial arteries were assessed on electron-beam tomography and the calcium score was obtained. Participants with a higher calcium score were more likely to have experienced a stroke than subjects in the reference category (RR 5.2 (95% CI 1.5-17.8) in men and 2.4 (95% CI 0.7-7.8) in women). These results indicate that electron-beam tomography is promising for the selection of subjects at high risk for cerebrovascular events. Prospective studies are needed to confirm these findings.

Arterial stiffness

An important risk factor for stroke is hypertension. The prevalence of isolated systolic hypertension increases with age.[28,29] Stiffening of the arterial tree is seen as the main cause of an elevated systolic blood pressure and a decreased diastolic blood pressure, and thus, an elevated pulse pressure in the arterial system. Recently, accurate methods to non-invasively measure arterial stiffness have become available. One of these methods is the measurement of arterial distensibility, i.e. change in arterial diameter due to change in arterial pressure over the cardiac cycle.[30,31] The relation between carotid distensibility and risk of stroke has been scarcely addressed. The Rotterdam Study investigated the relation between carotid distensibility and history of stroke.[32] The study was performed in 3,818 subjects in whom 78 had experienced a previous stroke. Participants in the lowest quartile of carotid distensibility were more than 12 times more likely to have experienced a stroke (OR 12.6, 95% CI 2.7-58.1), compared to the first quartile. This relationship remained significant after adjustment for carotid plaques and ankle to brachial index. The most likely underlying mechanism of this association is an elevation of pulse pressure, induced by increased arterial stiffness. In addition, it has been suggested that the risk of embolism due to rupture of plaques is increased in stiff arteries.[33] Additional prospective studies are needed to confirm the result from this cross-sectional study.

Another measurement of arterial stiffness is the pulse-wave velocity. The pulse-wave velocity is calculated as the ratio between the transit time for the foot of the pulse wave to travel along the arterial tree and the distance of the arterial segment. A higher pulse-wave velocity reflects a stiff artery. The pulse-wave velocity can be measured in the thoraco-abdominal aorta. One case-control study investigated the relation between pulse-wave velocity and stroke in 20

stroke patients and 20 controls without cardiovascular disease.[34] The pulse-wave velocity was significantly increased in stroke patients compared to controls, independent of blood pressure level. These findings, however, were not confirmed by researchers from the Rotterdam Study who failed to find a clear relationship with a history of stroke.[32] More studies are needed to elucidate the relationship between arterial stiffness and stroke.

Indices of cerebral circulation, measured by trans cranial doppler

Trans cranial doppler ultrasonography allows detection of micro-embolic signals and evaluation of cerebral hemodynamics such as cerebral blood flow and vasomotor reactivity. Various hospital-based studies have shown that presence of micro-emboli as assessed by trans- cranial doppler has diagnostic and prognostic value for stroke[35-37], as presence of micro-embolic signals were related to cardio-embolic strokes[38], severe carotid stenosis[39], poorer outcome[37] and a higher recurrence rate of stroke.[36,40] Trans-cranial doppler can also be used to assess cerebral hemodynamic parameters such as the blood flow velocity, pulsatility index and reactivity to CO_2. Cardiovascular risk factors have been shown to be related to these parameters.[41,42] However, the prognostic value of these hemodynamic measures themselves still needs to be investigated.

Consequences of vascular disease

Silent brain infarctions and white matter lesions are frequently observed in elderly subjects. It is estimated that 20% to 33% of healthy elderly have silent brain infarctions[43,44,45] and that, depending on the scoring method, 5 to 90% have white matter lesions.[46] The prevalence of these lesions substantially increases with age.[43,44,47,48,49] The lesions are associated with cardiovascular risk factors[50-52,43] and are considered to reflect small vessel pathology.

Silent brain infarctions as risk factor for stroke

One hospital-based study investigated the relationship between presence of silent brain infarctions and risk of vascular events in stroke patients with non-rheumatic atrial fibrillation.[53] The investigators observed that presence of silent brain infarctions is associated with increased risk of vascular events and stroke, in particular. Kobayashi and colleagues followed 933 healthy subjects aged 30 to 81 years for 1-7 years.[47] They reported that presence of silent brain infarctions was related to a more than 10-fold increased risk of stroke. The Cardiovascular

Health Study followed 3,324 participants aged 65 years or over for 4 years.[45] Presence of silent brain infarctions almost doubled the risk of stroke (RR 1.9, 95% CI 1.2-2.8).

White matter lesions as risk factor for stroke

It has been observed that white matter lesions are related to all kinds of stroke subtypes, but especially lacunar infarctions and deep cerebral hemorrhages.[54] These findings suggest that lacunes and white matter lesions share a common type of vasculopathy located either in the deep perforator vessels, or deep medullary arterioles. Little data exist on the relationship between white matter lesions and risk of future stroke or other cardiovascular events. The studies that have been performed were based on patient series and have shown that presence of white matter lesions in elderly neurological patients is related to an increased risk of cardiovascular death.[55,56] Also, higher recurrence rates of stroke in patients with white matter lesions have been reported.[57-59] These studies, however, were based on selected patient groups and prospective population-based studies are awaited.

SUMMARY

Various measures of subclinical vascular disease are related to stroke and its subtypes. Ankle-arm index, carotid IMT and plaques are markers of generalized atherosclerosis and are related to the risk of stroke in the general population. Plaque characteristics like echodensity and surface regularity may provide additional information. It is still debated whether these measures have prognostic value beyond traditional risk factors. Less established measures are aortic arch atherosclerosis, calcifications in the vessel wall, arterial stiffness and indices of cerebral circulation measured by trans-cranial doppler. They have been shown to be related to stroke, but quantification of this relationship in prospective population-based studies is needed. Finally, consequences of vascular disease like silent brain infarctions have recently been confirmed as risk factor for stroke.

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3.2 Carotid artery intima-media thickness and risk of intracerebral hemorrhage and lacunar infarction

Abstract

Background - The relation between an increased intima-media thickness and all subtypes of stroke is not yet clear. We investigated the relation between intima-media thickness and stroke subtypes.
Methods - The study was performed within the population based Rotterdam Study. At baseline (1990-1993), common carotid intima-media thickness was measured in 5,675 participants, aged 55 years or over, who were free from previous stroke. Follow-up for stroke was complete until January 1, 1999.
The relation between carotid intima-media thickness and risk of stroke and subtypes of cerebral infarction was assessed with Cox proportional hazards regression.
Results - A total of 309 strokes had occurred. Increase per SD in mean intima-media thickness increased the risk of stroke and cerebral infarction by 38% and 32%, respectively. The corresponding risks for lacunar infarction and intracerebral hemorrhage increased by 58% and 54%, respectively.
Conclusions - Intima media thickness is particularly related to lacunar infarction and intracerebral hemorrhage.

INTRODUCTION

Several population-based cohort studies have shown that carotid artery intima-media thickness as measure of generalized atherosclerosis is useful in estimating the risk of stroke.[1-3] There is still unclarity as to whether an increased intima-media thickness is related to stroke subtypes, and in particular lacunar infarction. For lacunar infarction, small vessel disease associated with hypertension and diabetes is considered the main underlying mechanism. However, recently some studies identified lacunes with an embolic cause.[4] One case control study reported that an increased intima-media thickness is related to all subtypes of cerebral infarction,[5] including lacunar infarctions, whereas another study suggested that intima-media thickness has more prognostic value to predict non-lacunar as compared to lacunar infarction.[6] Furthermore, the relation between carotid intima-media thickness and risk of intracerebral hemorrhage has not been investigated. Clarification of the relationship between intima-media thickness and stroke subtypes may give more insight in the pathophysiology of stroke. We investigated the relationship between common carotid intima-media thickness and the risk of subtypes of stroke in a large, population based cohort study of elderly people.

MATERIALS AND METHODS

Population

The study was performed within the framework of the Rotterdam Study, a population-based, single-center cohort study on chronic diseases in the elderly.[7] All inhabitants of Ommoord, a district of Rotterdam, aged 55 years or more were invited. People living in homes for the elderly were included. The participation rate of those invited for the study was 78% and a total of 7,983 subjects participated. The Medical Ethics Committee of Erasmus University Rotterdam approved the study. Written informed consent to retrieve information from treating physicians was obtained from all participants. Baseline measurements were done from 1990 to 1993 and included an interview at home and two visits to the research center for physical examination. From the 7,129 subjects who visited the research center at baseline, 5,854 underwent Duplex ultrasonography of both carotid arteries. Missing ultrasound data were mainly

due to logistic reasons such as restricted availability of ultrasonographers. Among those with ultrasound examination, 179 had experienced a previous stroke. These were excluded from the present study, leaving a cohort of 5,675 participants for the present study.

Carotid artery intima-media thickness (IMT) measurements

During Duplex examination with a 7.5 MHz linear-array transducer (ATL UltraMark IV), the optimal longitudinal images of the near and far wall of the distal common carotid artery were frozen on the R-wave of the electrocardiogram. This was repeated three times for the left and right sided carotid artery. All images were stored on videotape. Presence (yes or no) of carotid plaques was assessed at six locations (right and left sided common carotid artery, bifurcation and internal carotid artery).[8] The total plaque score reflected the number of sites with plaques (range 0-6). Carotid artery intima media thickness measurements were performed off-line with help of specially dedicated computer software.[9] This procedure has been described in detail elsewhere.[10] The readers were blinded to all clinical information. Maximal carotid IMT, mean carotid IMT and mean far wall carotid IMT in the distal 10 mm of the common carotid artery were measured. We defined maximal common carotid IMT as the mean of 3 consecutive measurements of the maximal IMT, measured in four locations (near wall of left common carotid artery, near wall of right common carotid artery, far wall of left common carotid artery and far wall of right common carotid artery). In case of missing values at either location, we used the average of the available measurements. Similarly, we defined mean common carotid IMT as the average of 3 consecutive measurements of mean common carotid IMT, measured in the same four locations. Far wall common carotid IMT was defined as the average of the mean far wall common carotid IMT, measured in left and right sided carotid artery. We assessed common carotid IMT in 95% of those in whom ultrasonography was performed. Common IMT measurements were shown to have good reproducibility.[11,12]

Assessment of stroke and subtypes

During the baseline interview a previous stroke was assessed by asking “Did you ever suffer from a stroke, diagnosed by a physician?”. Medical records of subjects who answered ‘yes’ were checked in order to verify the diagnosis.[13] A history of TIA was also assessed during the baseline interview. All TIAs were

reviewed by a neurologist.[14] Once entered into the Rotterdam Study subjects are continuously monitored for major events through automated linkage of the study database with the files from general practitioners. Information on vital status was obtained at regular intervals from the municipal authorities in Rotterdam. When an event or death has been reported, additional information is obtained by interviewing the general practitioner and scrutinizing information from hospital discharge records in case of admittance or referral.

A neurologist (P.J.K.) reviewed information on all possible strokes. A stroke was classified as definite if the diagnosis was based on typical clinical symptoms and neuro-imaging excluded other diagnoses. A stroke was considered probable in case typical clinical symptoms were present but neuro-imaging was not performed. For fatal strokes, other causes of death, especially cardiac should have been excluded. A stroke was classified as possible if clinical symptoms were less typical and neuro-imaging was not performed, or if a cardiac cause of death could not be excluded in case of a fatal stroke. We used only definite and probable strokes in the analyses. Subclassification in hemorrhagic or ischemic stroke was based on neuro-imaging, which was available for 67.5% of all cases. Cerebral infarctions were classified as lacunar if consciousness and higher cerebral function were unaffected and symptoms matched one of the typical lacunar syndromes. CT or MRI usually showed a small (<1.5 cm) infarction in the territories supplied by the perforating branches of major cerebral arteries. For the present study, follow up for stroke was complete for all participants until January 1, 1999.

Medical history and risk factors

A computerized questionnaire was used to obtain information on current health status and medical history at baseline. History of myocardial infarction, coronary bypass surgery (CABG), coronary angioplasty (PTCA), stroke and TIA was obtained through direct questioning. All reported myocardial infarctions were verified by using medical records. History of intermittent claudication and angina pectoris was assessed through the Rose questionnaire.[15] Smoking was assessed during the interview and subjects were classified as current, former or never smoker. At the research center, non-fasting blood samples were taken and serum total cholesterol and high-density lipoprotein (HDL) cholesterol was measured using an automated enzymatic procedure. Sitting blood pressure was measured on the right upper arm with a random-zero

sphygmomanometer. In the analyses we used the average of two measurements, measured at one occasion. Hypertension was defined as a systolic blood pressure of 140 mmHg or over, or a diastolic blood pressure of 90 mmHg or over, or current use of antihypertensive drugs for the indication of hypertension.[16] Diabetes mellitus was defined as use of oral blood glucose lowering drugs or insulin or random or a post-load serum glucose level higher than 11.0 mmol/l. Atrial fibrillation was assessed by electrocardiogram. A history of cardiovascular disease was coded if participants had a history of TIA, myocardial infarction, angina pectoris, intermittent claudication, atrial fibrillation, coronary bypass surgery and/or coronary angioplasty.

Data analysis

The association between common carotid IMT and risk of stroke was assessed through Cox proportional hazards regression. We calculated rate ratios (RR) of stroke per standard deviation increase in mean IMT, maximal IMT and mean far wall IMT. We adjusted for age and gender and additionally for cardiovascular risk factors, including systolic and diastolic blood pressure, diabetes, smoking, total and HDL-cholesterol. Subsequently, analyses were repeated after exclusion of subjects with previous cardiovascular disease. Results are presented as rate ratios with corresponding 95% confidence intervals.

Results

A total of 309 strokes occurred during a mean follow-up time of 6.2 years (35,164 person years). Baseline characteristics of the study population are given in table 1. Subtyping revealed 167 cerebral infarctions, 25 intracerebral hemorrhages, 2 subarachnoid hemorrhages and 115 unspecified strokes. The infarction was lacunar in 43 cases and non-lacunar in 107 cases. In 17 cases, the subtype could not be determined.

Carotid IMT, stroke and stroke subtypes

Increase in mean common carotid IMT increased the risk of stroke and cerebral infarction by respectively 38% and 32% per SD (table 2). We found similar relationships for mean far wall land maximal intima-media thickness. The relationships remained after adjustment for cardiovascular risk factors, or restriction to participants without previous cardiovascular disease (table 2).

Table 1

Baseline characteristics of the study population.

	Cohort (n=5675)	Stroke (n=309)
Age	69.3 (9.0)	74.9 (8.5)
Gender (% female)	60.4	57.0
Systolic blood pressure (mm Hg)	139.1 (22.3)	150.2 (23.4)
Diastolic blood pressure (mm Hg)	73.4 (11.7)	74.8 (13.2)
Total-cholesterol (mmol/L)	6.6 (1.2)	6.5 (1.2)
HDL-cholesterol (mmol/L)	1.4 (0.4)	1.3 (0.4)
Smoking (% current)	22.9	23.8
Diabetes (%)	10.1	19.1
Previous cardiovascular disease (%)	23.2	40.3
Mean IMT far wall common carotid artery (mm)	0.78 (0.19)	0.89 (0.27)
Mean IMT common carotid artery (mm)	0.80 (0.16)	0.89 (0.19)
Maximal IMT common carotid artery (mm)	1.03 (0.22)	1.18 (0.31)
Total plaque score	1.44 (1.62)	2.12 (1.83)

Values represent means (SD) or percentages.

Overall, the risk of lacunar infarction significantly increased by almost 60% per SD increase in mean intima-media thickness (table 2). The risk estimate for lacunar infarction attenuated to a 38% increase after adjustment for cardiovascular risk factors, but remained significantly increased. When we restricted the analysis to persons without previous cardiovascular disease, the higher risks of lacunar infarction associated with increased mean intima-media thickness lost statistical significance, but remained significant, however, with maximal IMT. In persons with a low plaque score (0 to 1), the relative risk of lacunar infarction per SD increase in mean IMT was 2.39 (95% CI 0.83-6.82). Increased mean intima-media thickness increased the risk of non-lacunar infarction, although not statistically significantly. We observed no significant differences between the risk of lacunar versus non-lacunar infarction for the different measures of IMT.

Table 2

Rate ratio (95% CI) of stroke and subtypes per standard deviation increase in intima-media thickness of the common carotid artery (cIMT).

	Stroke n=309	Intracerebral hemorrhage n=25	Cerebral infarction n=167	Non-lacunar infarction n=107	Lacunar infarction n=43
All subjects*					
Max cIMT	1.43 (1.32-1.55)	1.42 (1.07-1.89)	1.45 (1.30-1.62)	1.33 (1.14-1.55)	1.67 (1.37-2.02)
Mean cIMT	1.38 (1.25-1.52)	1.54 (1.13-2.12)	1.32 (1.15-1.53)	1.19 (0.99-1.44)	1.58 (1.23-2.03)
Mean far wall cIMT	1.34 (1.25-1.43)	1.36 (1.10-1.69)	1.31 (1.19-1.45)	1.22 (1.05-142)	1.47 (1.25-1.72)
All subjects ý					
Max cIMT	1.37 (1.25-1.49)	1.33 (0.97-1.82)	1.39 (1.23-1.56)	1.29 (1.10-1.52)	1.50 (1.21-1.86)
Mean cIMT	1.29 (1.16-1.44)	1.42 (1.00-2.00)	1.25 (1.07-1.45)	1.14 (0.93-1.40)	1.38 (1.06-1.82)
Mean far wall cIMT	1.29 (1.19-1.39)	1.30 (1.01-1.66)	1.27 (1.14-1.43)	1.19 (1.00-1.40)	1.39 (1.15-1.67)
Subjects without CVD*					
Max cIMT	1.47 (1.32-1.63)	1.48 (1.08-2.05)	1.44 (1.22-1.69)	1.43 (1.17-1.75)	1.50 (1.21-1.86)
Mean cIMT	1.40 (1.23-1.60)	1.58 (1.12-2.23)	1.34 (1.10-1.63)	1.29 (1.00-1.65)	1.32 (0.92-1.93)
Mean far wall cIMT	1.38 (1.27-1.51)	1.30 (0.96-1.77)	1.32 (1.13-1.53)	1.29 (1.06-1.57)	1.28 (0.92-1.78)

* adjusted for age and gender.

ý adjusted for age, gender, smoking, diastolic blood pressure, systolic blood pressure, total cholesterol, HDL-cholesterol, diabetes.

CVD: Cardiovascular disease.

Mean intima media thickening per SD was associated with a 54% increased risk of intracerebral hemorrhage (RR 1.54 (95% CI 1.13-2.12)) (table 2). After adjustment for cardiovascular risk factors or restriction to persons without previous cardiovascular disease, the corresponding risks of intracerebral hemorrhage were 1.42 (95% CI 1.00-2.00) and 1.63 (95% CI 1.17-2.27), respectively. In participants without hypertension (n=3,693), increase in mean intima-media thickness elevated the risk of hemorrhagic stroke 2-fold (RR 1.98 (95% CI 1.23-3.19) per SD of mean IMT).

DISCUSSION

Our data show that increased carotid intima-media thickness particularly increases the risk of intracerebral hemorrhage and lacunar infarction, irrespective of cardiovascular risk factors.

Before we interpret our results, some methodological issued need to be considered. We may have misclassified some strokes. However, since classification of strokes was done blinded to information on carotid IMT, any misclassification would have led to an underestimation of the risks. We restricted the analysis to CT or MRI confirmed strokes to reduce misclassification.

A few other studies have reported a relationship between carotid IMT and risk of stroke.[1-3,5] However, none of these studies investigated intracerebral hemorrhages and only two hospital-based case control studies reported on subtypes of cerebral infarction.[5,6] In one study, a relation between mean far wall IMT and risk of all subtypes of cerebral infarction was found. The odds ratio for lacunar infarction per SD increase in IMT was 1.74, which is in line with the risk that we found. In contrast, another hospital based case control study of 292 strokes recently reported higher IMT values in non-lacunar versus lacunar infarctions.[6] The authors suggested that IMT may have predictive value with respect to non-lacunar versus lacunar infarction. Our study does not support that suggestion.

A possible explanation for the relation between increased IMT and lacunar infarction is that IMT also reflects the thickness of the medial arterial layer, and possibly thickening of the medial layer in small arteries, which is known to precede small vessel disease. Another possible explanation is that micro-emboli from atherosclerotic lesions cause lacunar infarctions.[4] We explored this by assessing the relation between IMT and lacunar infarction in

persons with a low number of carotid plaques. The risk was still elevated, but not statistically significant, probably due to lack of statistical power. This finding is in favor of the hypothesis that carotid IMT is related to small vessel disease.

Our study shows that an increased IMT is related to a higher risk of intracerebral hemorrhage, even in persons without hypertension or previous cardiovascular disease. It is likely that an increased IMT reflects vulnerability of intracranial vessels for rupture.

In summary, apart from being a risk factor for stroke, an increased IMT is particularly related to an increased risk of intracerebral hemorrhage and lacunar infarction, independent of cardiovascular risk factors.

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3.3 Carotid plaques increase the risk of stroke and subtypes of cerebral infarction in asymptomatic elderly

Abstract

Background - Few studies have quantified the relation between carotid plaques and stroke in asymptomatic subjects and limited data exist on the importance of location of plaques, or the association with subtypes of cerebral infarction. We investigated the relationship between carotid plaques, measured at different locations, and risk of stroke and subtypes of cerebral infarction in a population based study.

Methods and results - The study was based on the Rotterdam Study and included 4,217 neurologically asymptomatic subjects aged 55 years or over. Presence of carotid plaques at six locations in the carotid arteries was assessed at baseline. Severity was categorized according to the number of affected sites. After a mean follow up of 5.2 years 160 strokes had occurred. Data were analyzed using Cox proportional hazards regression. Plaques increased the risk of stroke and cerebral infarction approximately 1.5-fold, irrespective of plaque location. Severe carotid plaques increased the risk of non-lacunar infarction in anterior (RR 3.2 (95% CI 1.1-9.7)), but not in posterior circulation (RR 0.6 (95% CI 0.1-4.9)). A more than 10-fold increased risk of lacunar infarction was found in subjects with severe plaques (RR 10.8 (95% CI 1.7-69.7)). No clear difference in risk estimates was seen between ipsilateral and contralateral infarction.

Conclusions - Carotid plaques increase the risk of stroke and cerebral infarction, irrespective of their location. Plaques increase the risk of infarctions in the anterior, but not in the posterior circulation. It is likely that carotid plaques in neurologically asymptomatic subjects are both markers of generalized atherosclerosis and sources of thrombo-emboli.

Introduction

Carotid plaques have frequently been found in subjects who suffered from a stroke.[1-3] Few studies have quantified the association between carotid plaques and risk of subsequent stroke in asymptomatic subjects.[4,5] Also, limited information is available on the relationship with subtypes of cerebral infarction,[4,5] as well as on the impact of location of the carotid plaque in relation to the risk of stroke.[5,6] One could hypothesize that because of a more turbulent blood flow, bifurcation and internal carotid artery plaques carry a higher risk than plaques in the common carotid artery.[5,7] It is also still controversial whether carotid plaques merely reflect generalized atherosclerosis or are directly causally related to subsequent stroke by release of thrombo-emboli.[5,8,9] We investigated the association between asymptomatic plaques, measured at six locations in the carotid arteries, and the risk of stroke and subtypes of cerebral infarction in a population-based cohort of elderly persons.

Methods

Population

The study is part of the Rotterdam Study, a population-based cohort study on chronic and disabling diseases in the elderly. All inhabitants of Ommoord, a suburb of Rotterdam, aged 55 years or more were invited. People living in homes for the elderly were included. Participation rate of those invited for the study was 78% and in total 7,983 subjects participated.[10] The Medical Ethics Committee of Erasmus University Rotterdam approved the study. Written informed consent to retrieve information from treating physicians was obtained from all participants. Baseline measurements were obtained from 1990 to 1993 and consisted of an interview at home and two visits to the research center for physical examination. From the 7,129 subjects who visited the research center, 419 had experienced a previous stroke or TIA. Participants with a previous stroke or TIA were excluded from the present study.

Assessment of carotid plaques

At baseline 5,494 participants who were free from previous stroke or TIA underwent B-mode ultrasonography of both carotid arteries with a 7.5-MHZ

linear array transducer (ATL Ultra-Mark IV) to assess presence of plaques in the common carotid artery, bifurcation and internal carotid artery.[11] Missing ultrasound data were mainly due to restricted availability of ultrasonographers. We defined plaques as focal widenings of the vessel wall of more than 50% relative to adjacent segments, with protrusion into the lumen, composed of calcified or non-calcified components (figure 1).

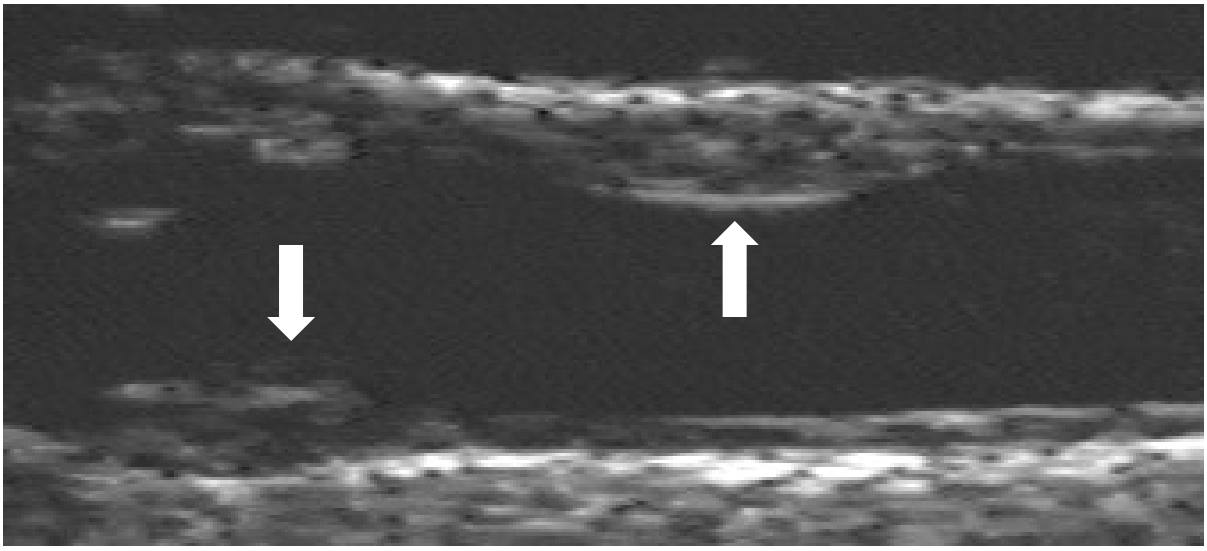

Figure 1
B-mode image of the carotid artery with plaques in the common carotid artery (right arrow) and bifurcation (left arrow).

The protrusion was evaluated by eyeballing judgement, without measuring the thickness of the lesions and of the adjacent structure. Plaques were assessed in 5,276 randomly selected neurologically asymptomatic participants. The assessment was performed off-line in 1,471 participants and on-line in the remainder. Ultrasonographers who did the plaque-assessment were blinded for clinical information. The total plaque score reflected the total number of sites with plaques and ranged from 0 to 6 (left and right-sided common carotid artery, bifurcation and internal carotid artery).

A total of 4,217 participants had information on plaques at six locations. Participants with a missing plaque score at one or more locations on average had a 1.9 mm Hg (95%CI 0.5-3.3) lower diastolic blood pressure, a 2.0 mm Hg (95% CI 1.3-2.8) lower systolic blood pressure and a 0.5 kg/m^2 (95% CI 0.2-0.7) higher body mass index, compared with those with information on plaques at six locations. The groups did not differ according to other risk factors.

A reproducibility study of the on line plaque assessment showed kappa's of 0.66 for the left, 0.68 for the right carotid artery and 0.67 for either side, indicating moderate agreement. Corresponding figures for the off-line reproducibility were 0.59, 0.65 and 0.60, respectively.[11] In a subgroup of 954 participants from the Rotterdam Study, assessment of stenosis in the right internal carotid artery was performed on-line with help of 5-MHz pulsed Doppler.[12] Interpretation of velocity profiles was done according to standard criteria.[13] The prevalence of clinically relevant stenosis was low (stenosis >50% 1.4%; stenosis >80% 0.3%).

Assessment of stroke and subtypes

During the baseline interview a previous stroke was assessed by asking "did you ever suffer from a stroke, diagnosed by a physician?". Medical records of subjects who answered 'yes' were checked in order to verify the diagnosis.[14] A history of TIA was also assessed during the baseline interview. All TIAs were reviewed by a neurologist.[15] Once subjects enter the Rotterdam Study they are continuously monitored for major events through automated linkage of the study database with the files from general practitioners. Information on vital status is obtained at regular intervals from the municipal authorities in Rotterdam. When an event or death has been reported, additional information is obtained by interviewing the general practitioner and scrutinizing information from hospital discharge records in case of admittance or referral.

A neurologist (P.J.K.) reviewed information on all possible strokes. A stroke was classified as definite if the diagnosis was based on typical clinical symptoms and neuro-imaging excluded other diagnoses. A stroke was considered probable in case typical clinical symptoms were present but neuro-imaging was not performed. For fatal strokes, other causes of death, especially cardiac should have been excluded. A stroke was classified as possible if clinical symptoms were less typical and neuro-imaging was not performed, or if a cardiac cause of death could not be excluded in case of a fatal stroke. We only used definite and probable strokes in the analyses.

Subclassification in hemorrhagic or ischemic stroke was based on neuro-imaging, which was available for 67.5% of all cases. Cerebral infarctions were lacunar if consciousness and higher cerebral function was maintained in the setting of one of the typical lacunar syndromes. CT or MRI usually showed a small (<1.5 cm) infarction in the territories supplied by the perforating branches

of major cerebral arteries. We further classified infarctions as located in anterior or posterior circulation. For the present study, follow up for stroke was complete for all participants until January 1, 1998.

Medical history and risk factors

At baseline, information on current health status, medication use and medical history including previous myocardial infarction, coronary bypass surgery and coronary angioplasty was obtained using a computerized questionnaire. All reported myocardial infarctions were verified by using medical records. History of intermittent claudication and angina pectoris was assessed through the Rose questionnaire.[16] Participants were classified as current, former or never smoker. Non-fasting blood samples were taken and serum total cholesterol and high-density lipoprotein (HDL) cholesterol was measured using an automated enzymatic procedure. Sitting blood pressure was measured twice on the right arm with a random-zero sphygmomanometer. We used the average of the two measurements in the analyses. Diabetes mellitus was defined as use of oral blood glucose lowering drugs or insulin or random or post-load serum glucose level higher than 11.0 mmol/L. Atrial fibrillation was assessed by an electrocardiogram. A history of cardiovascular disease was coded if participants had a history of myocardial infarction, angina pectoris, intermittent claudication, atrial fibrillation, coronary bypass surgery and/or coronary angioplasty.

Data analysis

The relation between carotid plaques and the risk of stroke was assessed with Cox proportional hazards regression. We tested for linearity of the plaque score by comparing the log likelihood of models including plaque score as categorical and continuous variable by means of a X^2 test. We assessed the relation between total plaque score and risk of stroke and cerebral infarction. Participants without any plaques were taken as the reference. We also examined the presence of one or more plaques at different locations (left and/or right sided common carotid artery, bifurcation and internal carotid artery) in relation to the risk of stroke and cerebral infarction.

In order to distinguish between generalized atherosclerosis and thrombo-embolism we assessed whether the association between left and right-sided plaque score (0-3) and risk of infarction was different for ipsilateral and contralateral infarction. Further, we determined the relation between carotid

plaques and subtypes of infarction (non-lacunar infarction in the anterior and posterior circulation and lacunar infarction). All analyses were adjusted for age and gender and additionally for cardiovascular risk factors (blood pressure, diabetes mellitus, smoking, HDL- and total-cholesterol).

We performed additional analyses adjusting for statin use, stratified for aspirin use and excluding subjects with a history of cardiovascular disease. Results are presented as rate ratios with corresponding 95% confidence intervals.

Results

Table 1 shows the baseline characteristics of the study population. During 21,967 person years of follow-up (mean follow-up 5.2 years) 160 definite or probable strokes occurred.

Table 1

Baseline characteristics of the study population.

	Study population (n=4,217)	Stroke (n=160)
Age	68.8 (838)	74.3 (8.9)
Gender (% female)	60.1	53.8
Systolic blood pressure (mm Hg)	139.4 (22.4)	149.0 (23.8)
Diastolic blood pressure (mm Hg)	73.9 (11.7)	75.1 (13.5)
Total-cholesterol (mmol/L)	6.6 (1.2)	6.5 (1.3)
HDL-cholesterol (mmol/L)	1.4 (0.4)	1.3 (0.4)
Diabetes (%)	9.9	21.3
Previous myocardial infarction (%)	11.3	16.9
Smoking (% current smokers)	23.2	24.1
Atrial fibrillation (%)	2.4	5.6
Cardiovascular disease (%)	20.1	28.1
Any carotid plaques (%)	60.0	74.4
Common carotid artery plaque(s) (%)	15.1	25.0
Bifurcation plaque(s) (%)	53.2	70.6
Internal carotid artery plaque(s) (%)	34.5	44.4
Left carotid artery plaque(s) (%)	49.3	64.4
Right carotid artery plaque(s) (%)	48.1	64.4
Total plaque score (range 0-6)	1.5 (1.7)	2.3 (2.0)

Values represent means (SD), or percentages.

Subtyping revealed 85 cerebral infarctions and 12 intracerebral hemorrhages. A total of 63 strokes could not be subtyped because neuro-imaging was lacking or due to limited information. The infarction was lacunar in 17 cases (24%). A total of 52 infarctions were non-lacunar, 34 of which were located in the anterior and 18 in the posterior circulation. We could not determine the exact type in 16 infarctions. The number of plaques in the left and right carotid artery were significantly correlated (Spearman correlation coefficient 0.63).

Carotid plaques and risk of stroke and cerebral infarction

Testing for linearity showed that continuous analysis of total plaque score was justified. The risk of stroke and cerebral infarction gradually increased with increasing total plaque score (RR's per plaque increase 1.15 (95% CI 1.05-1.26) for stroke and 1.17 (95% CI 1.03-1.33) for cerebral infarction). The results were largely similar when we adjusted for cardiovascular risk factors (RR 1.13 (95% CI 1.03-1.24) and 1.12 (95% CI 0.99-1.29), respectively) or restricted the analyses to persons without previous cardiovascular disease (RR 1.15 (95% CI 1.03-1.29) and 1.22 (95% CI 1.04-1.42), respectively). The risk of stroke in subjects with severe plaques (score 5 to 6) was 2.4 fold increased (RR 2.44 (95% CI 1.42-4.20)) and the risk of cerebral infarction almost tripled (RR 2.70 (95% CI 1.27-5.77)), compared to subjects without plaques (table 2).

When we looked at the risk associated with plaques at the different locations we saw no indication that plaques at locations with a turbulent blood flow, like the carotid bifurcation and internal carotid artery, carried a higher risk than plaques in the common carotid artery (table 3).

Increase in total plaque score remained statistically significantly related to the risk of stroke and cerebral infarction when we put total plaque score and plaque location in one model (RR 1.48 (95% CI 1.14-1.93) and 1.52 (95% CI 1.04-2.20), respectively). However, it should be noted that there is a large overlap between plaques at the different locations, which limits the interpretation of the results.

Carotid plaques in relation to hemisphere and subtype of infarction

Table 3 shows that carotid plaques were not consistently related to higher risks of infarction in the ipsi vs. contralateral hemisphere. Most cases occurred in participants with plaques in both carotid arteries. The risk of infarction in the left hemisphere significantly increased 3-fold in participants with plaques only in the

left and in both carotid arteries (RR 3.23 (95% CI 1.04-10.06) and 2.98 (95% CI 1.15-7.75), respectively). The risk of infarction in the right hemisphere was increased when plaques were present in both carotid arteries, although not statistically significantly.

Severe plaques (5-6 plaques) increased the risk of non-lacunar infarction in the anterior circulation more than 3-fold (RR 3.24 (95% CI 1.08-9.72)) (table 4). The risk of anterior circulation infarction was very similar for plaques at the different locations (common carotid artery, bifurcation or internal carotid artery). However, the risk increases for anterior circulation infarction were not statistically significant. Neither total plaque score nor plaques at different segments of the carotid artery showed a significant relationship with the risk of non-lacunar infarction in the posterior circulation. Increase in total plaque score gradually increased risk of lacunar infarction. Participants with 3-4 plaques had a more than five times increased risk and participants with 5-6 plaques had a more than ten-fold increased risk of lacunar infarction (age and gender adjusted RR 10.84 (95% CI 1.70-69.67)). With adjustment for systolic and diastolic blood pressure and diabetes the relative risk associated with severe plaques decreased to 7.86 (95% CI 1.20-51.72). Further adjustment was not possible because of paucity of data.

Medication use

A total of respectively 372 (8%) and 92 (2%) participants reported current use of aspirin and statins. The number of statin users was too low to perform stratified analysis. Additional adjustment for statin use did not change the results. We found that carotid plaques increased the risk of cerebral infarction in non-aspirin users (RR 1.24 (95% CI 1.08-1.42) per plaque increase). Plaques did not increase the risk in aspirin-users (RR 0.82 (95% CI 0.57-1.16)). Corresponding relative risks in participants without previous cardiovascular disease were 1.27 (95% CI 1.08-1.50) and 0.92 (95% CI 0.61-1.38), respectively.

Table 2

Rate ratio of stroke and cerebral infarction in relation to carotid plaques, adjusted for age and gender.

Plaque		Stroke		Cerebral infarction	
	Subjects at risk	No. of events	Rate ratio (95% CI)	No. of events	Rate ratio (95% CI)
No plaque (reference)	1685	41	1.00 (reference)	24	1.00 (reference)
Total plaque score					
1-2 plaques	1459	55	1.14 (0.74-41.74)	30	1.22 (0.69-2.17)
3-4 plaques	795	38	1.27 (0.79-2.04)	18	1.38 (0.72-2.65)
5-6 plaques	278	26	2.44 (1.42-4.20)	13	2.70 (1.27-5.77)
Any plaque	2532	119	1.31 (0.90-1.91)	61	1.40 (0.84-2.34)
Plaques at different locations *					
≥1 plaque in left or right CCA†	638	40	1.58 (0.97-2.59)	17	1.45 (0.72-2.94)
≥1 plaque in left or right BIF‡	2242	113	1.42 (0.97-2.09)	59	1.52 (0.90-2.55)
≥1 plaque in left or right ICA§	1452	71	1.25 (0.82-1.91)	37	1.37 (0.78-2.42)

* Groups with plaques at different locations are not mutually exclusive.

† CCA: Common carotid artery.

‡ BIF: Bifurcation.

§ ICA: Internal carotid artery.

Table 3

Rate ratio of ipsi and contralateral infarction in relation to the side of carotid plaque, adjusted for age and gender.

Plaque		Infarction in right hemisphere		Infarction in left hemisphere	
	Subjects at risk	No. of events	Rate ratio (95% CI)	No. of events	Rate ratio (95% CI)
Right carotid artery*					
0 plaques	2190	8	1.00 (reference)	12	1.00 (reference)
1 plaque	1085	7	1.58 (0.56-4.42)	12	1.83 (0.81-4.12)
2 plaques	687	9	3.10 (1.16-8.26)	8	1.92 (0.76-4.81)
3 plaques	255	3	2.80 (0.72-11.00)	2	1.40 (0.30-6.44)
Any plaques	2027	19	2.23 (0.95-5.23)	22	1.81 (0.87-3.75)
Left carotid artery*					
0 plaques	2137	9	1.00 (reference)	10	1.00 (reference)
1 plaque	1147	6	1.12 (0.39-3.17)	11	1.93 (0.81-4.89)
2 plaques	683	7	2.18 (0.79-6.03)	9	2.75 (1.09-6.93)
3 plaques	250	5	4.05 (1.28-12.83)	4	3.49 (1.04-11.68)
Any plaque	2080	18	1.77 (0.78-4.04)	24	2.33 (1.09-4.98)
No plaques	1685	7	1.00 (reference)	6	1.00 (reference)
Plaque(s) only in left CA	505	1	0.45 (0.05-3.64)	6	3.23 (1.04-10.06)
Plaque(s) only in right CA	452	2	0.98 (0.20-4.76)	4	2.30 (0.64-8.21)
Plaque(s) in both carotid arteries	1575	17	2.20 (0.88-5.51)	18	2.98 (1.15-7.75)

* Number of locations where plaques were observed (range 0-3).

Table 4

Rate ratio of subtypes of cerebral infarction in relation to carotid plaques, adjusted for age and gender.

Plaque		Non-lacunar infarction				Lacunar infarction	
		Anterior circulation		Posterior circulation			
	Subjects at risk	No. of events	Rate ratio (95% CI)	No. of events	Rate ratio (95% CI)	No. of events	Rate ratio (95% CI)
No plaque	1685	9	1.00 (reference)	9	1.00 (reference)	2	1.00 (reference)
Total plaque score							
1-2 plaques	1459	12	0.97 (0.38-2.48)	5	0.64 (0.21-1.97)	6	3.50 (0.70-17.56)
3-4 plaques	795	5	0.91 (0.30-2.83)	3	0.60 (0.15-2.36)	6	5.54 (1.03-29.77)
5-6 plaques	278	8	3.24 (1.08-9.72)	1	0.59 (0.07-4.88)	3	10.84 (1.70-69.67)
Any plaque	2532	25	1.16 (0.51-2.63)	9	0.62 (0.23-1.66)	15	4.64 (1.03-20.96)
Plaques at different locations*							
≥1 plaque in left or right CCA†	638	8	1.27 (0.42-3.83)	2	0.51 (0.10-2.58)	4	5.69 (0.93-34.73)
≥1 plaque in left or right BIF‡	2242	23	1.27 (0.56-2.91)	9	0.70 (0.26-1.89)	15	5.35 (1.18-24.20)
≥1 plaque in left or right ICA§	1452	17	1.32 (0.54-3.21)	6	0.62 (0.21-1.88)	9	4.62 (0.93-23.00)

* Groups with plaques at different locations are not mutually exclusive.

† CCA: Common carotid artery.

‡ BIF: Bifurcation.

§ ICA: Internal carotid artery.

DISCUSSION

The present study shows a dose-dependent relation between carotid plaques and the risk of stroke and cerebral infarction, irrespective of location of plaques. The risk estimates were highest for lacunar infarctions. Carotid plaques increase the risk of infarction in the anterior but not in the posterior circulation.

A few methodological issues need to be addressed. First, apart from slightly lower blood pressure levels and higher body mass index in those with compared to those without missing plaque values we found no differences in cardiovascular risk factors. Therefore, we think that restriction to participants with complete plaque score did not lead to a selection bias. The reproducibility of plaques showed moderate agreement and some misclassification may have occurred. We may also have misclassified some strokes, especially when information was limited. However, since plaque assessment was performed blinded for case status and vice versa, misclassification, if any, was non-differential, leading to an underestimation of the true associations.[17] For stroke subtypes we restricted ourselves to CT or MRI-confirmed infarctions to reduce possible misclassification. In clinical practice, cerebral infarctions are often classified according to presumed etiology.[18] Using such a classification in etiologic research would introduce a circular argument. For example, carotid atherosclerosis would by definition be related to large vessel strokes. Therefore, we deliberately classified cerebral infarctions according to size and location.

The present study was performed in neurologically asymptomatic subjects in whom carotid stenosis was rare. Most studies on carotid plaques and stroke were performed in patients with carotid stenosis or in subjects who suffered from a stroke or TIA.[1-3,19] The Cardiovascular Health Study is the only other population-based cohort study that has investigated carotid plaques in relation to subsequent stroke in asymptomatic subjects.[4] In that study, 4,886 participants were followed for 3.3 years and 175 strokes occurred. The study focused on characteristics of the most prominent plaque in the bifurcation or internal carotid artery and concluded that hypoechogenic, but not hyperechogenic plaques increased the risk of ischemic stroke. We add to those findings in that we show a dose-dependent relation between number of sites affected with plaques and risk of stroke, but no differences in risk associated with plaques located at different sites in the carotid arteries.

An important question is whether carotid plaques are related to stroke as markers of generalized atherosclerosis or as sources of thrombo-emboli. Observations in favor of the generalized atherosclerosis hypothesis include first that the risk of cerebral infarction does not depend on plaque location. However, the considerable overlap of plaques at different locations limits the potential to decisively assess this. Secondly, and in line with findings in a few other studies,[8,20] we found no differences between risk of infarction in the ipsi and the contralateral hemisphere in relation to the side of the carotid plaque, as we would have expected if plaques were primarily sources of emboli. Thirdly, plaques were related to lacunar infarction. Since only a minority of lacunar infarctions are caused by thrombo-emboli,[21] this suggests that carotid plaques are associated with lipohyalinosis or intracranial atherosclerosis on small vessel level. There are two findings that argue in favor of thrombo-emboli. First, we observed that carotid plaques increase the risk of anterior, but not of posterior circulation infarction. The anterior cerebral circulation mostly is supplied by the carotid arteries whereas the posterior circulation is not. Emboli from the carotid arteries will most likely go to the anterior circulation. Secondly, carotid plaques do not increase the risk of stroke in aspirin users. Underlying this may be that aspirin stabilizes plaques resulting in less thrombo-emboli. Since the effects of aspirin are manifold and confounding by indication may play a role, this should not be over-interpreted.

We conclude that carotid plaques are associated with increased risk of stroke, irrespective of their location. It is likely that carotid plaques in neurologically asymptomatic subjects are both markers of generalized atherosclerosis and sources of thrombo-emboli.

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3.4 Stroke is associated with coronary calcification as detected by electron beam computed tomography

Abstract

Background - Coronary calcification detected by electron-beam computed tomography (EBCT) measures the atherosclerotic plaque burden and predicts coronary events. Since atherosclerosis is a generalized process, coronary calcification may also be associated with manifest atherosclerotic diseases at other sites of the vascular tree. We examined whether coronary calcification is related to the presence of cerebrovascular events.
Methods - From 1998 to 2000, participants from the population-based Rotterdam Study were invited for EBCT scanning to detect coronary calcification. Calcifications were quantified according to Agatston's method. Calcium scores were available for 1,874 subjects with a mean age (standard deviation) of 71 (5.6) years. Stroke was present in 50 subjects at the time of scanning.
Results - A graded association was found between the calcium score and the presence of stroke. In men, the age-adjusted odds ratios for stroke were 3.4 (95% confidence interval 0.9-12.7) in subjects in the calcium score category 101-500, and 5.1 (1.5-17.4) in subjects with a calcium score above 500, compared to subjects in the lowest calcium score category (0–100). In women, corresponding odds ratios were 1.3 (0.4-4.6) and 2.4 (0.7-7.9), respectively. Additional adjustment for cardiovascular risk factors did not materially alter the risk estimates.
Conclusions - In this large population-based study, a strong and graded association was found between coronary calcification and stroke. The results suggest that coronary calcification, detected by EBCT, may be used to identify subjects at high risk of stroke.

INTRODUCTION

A number of studies have shown that non-invasive measures of atherosclerosis, like intima-media thickness of the carotid artery and ankle to brachial blood pressure index, predict cerebrovascular events.[1-8] Coronary calcification, detected by electron-beam computed tomography (EBCT), is a rather new measurement that is closely related to the amount of coronary atherosclerotic plaque in histopathologic investigations.[9] Several studies have shown that coronary calcification predicts coronary events.[10-14] There is a close relation between calcification of the coronary arteries and the extracoronary plaque burden.[15,16] This finding suggests that coronary calcification may also be associated with manifest atherosclerotic diseases at other sites of the vascular tree, such as stroke. No study has yet examined whether coronary calcification is related to cerebrovascular events. To approach this issue, we studied the association of coronary calcification, detected by EBCT, and the presence of stroke in 1,874 elderly men and women participating in the population-based Rotterdam Study.

METHODS

Study population

The Rotterdam Study is a population-based cohort study that aims at assessing the occurrence, risk factors and prognosis of major chronic diseases.[17] In the baseline examination phase (1990-1993), 7,983 subjects aged 55 and over participated. Data were collected during a home interview and at two visits to the study center. From 1997–1999, subjects were invited to participate in the third examination phase. Between the baseline and the third examination phase, 1992 subjects had died, 35 were lost to follow-up, and 55 were not contacted because they were living in nursing homes outside the study area, leaving 5,901 subjects who were invited. Of the invited subjects, 4,730 (80%) participated, of which 4,148 completed the third examination. For EBCT scanning, non-institutionalized participants of 85 years and younger were eligible (n=3,371). From 2,263 (67%) of these participants EBCT scans were obtained. For 389 participants calcium scores were not available due to archiving problems. Thus, calcium scores of 1,874 participants were available for analysis. The Medical

Ethics Committee of the Erasmus University approved the study. All participants gave informed consent.

Measurement of coronary calcifications

We assessed coronary calcifications in the epicardial coronary arteries detected on EBCT scans. Imaging was performed with a C-150 Imatron scanner (Imatron, South San Francisco, California). From the level of the root of the aorta through the heart, 38 images were obtained with 100 ms scan time and 3 mm slice thickness. Images were acquired at 80% of the R-R interval, using electrocardiogram (ECG) triggering. We quantified coronary calcifications with AccuImage software (AccuImage Diagnostics Corporation, South San Francisco, California) that displays all pixels with a density of over 130 Hounsfield units (HU). A calcification was defined as a minimum of two adjacent pixels (area=0.65 mm^2) with a density over 130 HU using a four-connected algorithm to determine the adjacency. We placed a region of interest around each high-density lesion in the epicardial coronary arteries. The peak density in HU and the area in mm^2 of the individual coronary calcifications were calculated. A calcium score was obtained by multiplying each area of interest with a factor indicating peak density within the individual area, as proposed by Agatston et al.[18] We summated the scores for individual calcifications, resulting in a calcium score for the entire epicardial coronary system.

Diagnosis of stroke

The definition of the presence of stroke was a history of stroke at baseline (1990-1993) or a stroke after baseline but prior to electron-beam CT scanning (1998-2000). A history of stroke at baseline was determined on the basis of the question, "Did you ever suffer from a stroke, diagnosed by a physician?". Signs and symptoms had to last more than 24 hours. The number of strokes that had led to a hospital admission and that had occurred without hospitalization was determined, and age at the time of the stroke was obtained. Of the subjects that responded with "yes", the general practitioner (GP) was asked for supplementary medical information, including a detailed history, information on neuro-imaging, and copies of hospital discharge records. Stroke diagnosis was based on all available medical information. In case of no hospitalization, mention of a "cerebrovascular accident" in the GP records was required to confirm the self-reported information. When possible, information on signs and symptoms was

used in the final classification. In case of hospitalization, the diagnosis of a neurologist was used. Events were evaluated by a neurologist and classified into definite, probable or no stroke.[3] After the baseline examination, GPs in the research area, covering 85% of the cohort, reported all possible cases of stroke to the Rotterdam research center. Information from GPs with practices outside the research area (15% of the cohort) was obtained through checking the participant's GP file and by interviewing the GP annually. When an event was reported, additional information including the date of the possible stroke was obtained by interviewing the GP and scrutinizing information from hospital discharge records and/or neuro-imaging in case of admission or referral. Events were classified after all available information was considered. A neurologist classified the events as definite, probable, possible, and no stroke.[2] Events were coded according to the International Classification of Diseases, 10th version.[19] The present analysis was restricted to definite and probable events.

Measurement of cardiovascular risk factors

Information on smoking was obtained during the home interview. We categorized subjects as current, past or never smokers. Anthropometric measures were obtained during the visit at the research center. Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. We used the mean of two consecutive measurements in the analyses. After an overnight of fasting, blood was obtained at the research center by venapuncture with minimal stasis. Serum total cholesterol was determined by an enzymatic procedure. High-density lipoprotein (HDL) was measured similarly after precipitation of the non-HDL fraction.[20] Glucose was determined enzymatically by the Hexokinase method.

Statistical analysis

Since the distribution of calcium scores was skewed, we report medians and ranges. Three absolute calcium score categories were considered, based on cut-points that were chosen before examining the association with presence of stroke: 0–100, 101–500, and above 500. Age-adjusted odds ratios (OR) with 95% confidence interval (CI) for the presence of stroke were calculated per calcium score category using logistic regression analysis, for men and women separately. Calcium score category 0-100 was used as reference category. Analyses for calcium score categories were repeated with additional adjustment

for cardiovascular risk factors (smoking, systolic and diastolic blood pressure, total cholesterol, HDL cholesterol, fasting glucose). Logistic regression analysis was also performed to calculate age-adjusted ORs (95% CI) for the presence of ischemic strokes (the most prevalent subtype of strokes in our cases) per calcium score category by sex. Because of small number of cases, it was not possible to analyze the association between the calcium score and other subtypes of stroke. SPSS 9.0 for Windows (SPSS Inc., Chicago, Illinois) was used for data analysis.

Results

Table 1 describes the characteristics of the 1,874 study participants. The mean age (± SD) of the study population was 71 years (5.6 years), and 47% were men. The median calcium score was higher for men than for women: 313 (interquartile range 64–969) and 56 (interquartile range 5–261), respectively.

Table 1

Characteristics of the study population.

Variable	Men (n=877)	Women (n=997)
Age (years)	71 ± 5.6	71 ± 5.8
Smokers (%)		
Current	17	15
Past	73	39
SBP (mmHg)	144 ± 21	143 ± 21
DBP (mmHg)	77 ± 11	75 ± 11
Total cholesterol (mmol/l)	5.6 ± 0.9	6.0 ± 0.9
HDL cholesterol (mmol/l)	1.3 ± 0.3	1.5 ± 0.4
Serum glucose (mmol/l)	6.0 ± 1.6	5.8 ± 1.3
Calcium score*	313 (64 – 969)	56 (5 – 261)
Log calcium score	5.3 ± 2.1	3.7 ± 2.3
History of stroke (%)	3.9	1.6
History of myocardial infarction (%)	17.6	6.0

Categorical variables are expressed as percentage. Values of continuous variables are expressed as mean ± standard deviation.

* Value of the calcium score is expressed as median (inter-quartile range) because of its skewed distribution.

SBP: Systolic blood pressure, DBP: Diastolic blood pressure.

The mean of the logarithmically transformed calcium scores (± SD) was 5.3 (2.1) for men and 3.7 (2.3) for women. Thirty-four men (3.9%) and 16 women (1.6%) had experienced a stroke before EBCT scanning. The mean interval between the stroke and scanning (± SD) was 8.8 years (7.8). Comparison of characteristics of subjects who completed the third examination with and without EBCT scanning demonstrated no significant differences in levels of cardiovascular risk factors except for the percentage of men (38% versus 47%) (data not shown). In logistic regression analysis, a graded association was found between the amount of coronary calcification and the presence of stroke (table 2).

Table 2

Risk of stroke in calcium score categories for men and women.

	n	events	Model 1* OR (95% CI)	Model 2† OR (95% CI)
Men				
Calcium score:				
0 – 100	274	3	1.0 (reference)	1.0 (reference)
101 – 500	252	10	3.4 (0.9 – 12.7)	3.2 (0.9 – 11.9)
> 500	351	21	5.1 (1.5 – 17.4)	4.8 (1.4 – 16.6)
Women				
Calcium score:				
0 – 100	579	7	1.0 (reference)	1.0 (reference)
101 – 500	247	4	1.3 (0.4 – 4.6)	1.4 (0.4 – 5.2)
> 500	171	5	2.4 (0.7 – 7.9)	2.5 (0.6 – 9.7)

OR: Odds ratio; CI: Confidence interval; n: number of subjects

* Model 1: adjusted for age

† Model 2: adjusted for age, smoking, systolic and diastolic blood pressure, total cholesterol, HDL cholesterol, and serum glucose

The number of subjects in model 2 is somewhat lower than for model 1, due to missing values for cardiovascular risk factors

In men, age-adjusted odds ratios for the presence of stroke increased from 3.4 (95% CI 0.9–12.7) in the calcium score category 101–500, to 5.1 (1.5–17.4) in those with a calcium score above 500, when compared to subjects in the reference category (calcium score of 0-100). The corresponding age-adjusted odds ratios in women were 1.3 (0.4–4.6) and 2.4 (0.7–7.9), respectively. The results did not materially alter after additional adjustment for cardiovascular risk

factors (model 2). In men, 62% of the strokes occurred in the calcium score category above 500, while in women this percentage was 31.

Of the 50 strokes, 34 (68%) were ischemic. Age-adjusted odds ratios for the presence of ischemic stroke were 1.7 (0.4-7.2) for men in the calcium score category 101-500, and 3.4 (0.9-12.1) for men in the category above 500, compared to the reference calcium score category. In women, the corresponding odds ratios were 0.9 (0.2-4.6) and 3.1 (0.8-11.5), respectively.

Discussion

The amount of coronary calcification showed a graded association with the presence of stroke in a general population of elderly subjects. Men in the highest calcium score category (above 500) were five times more likely to have experienced a stroke compared to men in the lowest calcium score category (up to 100). In women with a calcium score above 500, stroke had occurred 2.4 times more often than in women with a calcium score up to 100.

Various aspects of the study need to be addressed. First, scans were obtained from 2,263 subjects (67%) participating in the Rotterdam study. Comparison of characteristics of subjects who completed the third examination with and without EBCT scanning showed a lower percentage of men in the group without EBCT scan, but otherwise small, mostly non-significant differences. Subjects living in institutions were not invited for EBCT scanning and thus not included in this comparison. Furthermore, subjects with severe disability, possible resulting from a cerebrovascular event, may not have attended the third examination and thus were not invited for scanning. If reasons for non-participation are related to the amount of coronary calcification, this may have limited the range of calcium scores in our study. Second, calcium scores were not available for all subjects who were scanned, due to archiving problems, which occurred randomly and will not have affected the results. Third, various minimal number of pixels have been used for distinguishing true foci of calcium from noise. The threshold we used for detection of coronary calcifications was two consecutive pixels. Some studies have used higher thresholds to reduce the contribution of noise. However, in a subgroup of subjects, we found a very high correlation coefficient (r=0.99) between calcium scores, obtained using a threshold of two pixels and a threshold of four pixels.

Fourth, due to the fact that the association of coronary calcification with stroke was evaluated in a cross-sectional study design, only survivors of a

cerebrovascular event are included as cases. It is uncertain whether we would have found the same risk estimates for fatal events. Moreover, survivors of stroke are more likely to have had less severe types of stroke like lacunar infarctions and partial infarctions. This may have limited the range of stroke severity among the cases. Since there was an interval between the occurrence of stroke and scanning of 8.8 years on average, subjects may have been classified into a different calcium score category than the classification would have been if coronary calcification had been measured at the time of the cerebrovascular event. Furthermore, the cerebrovascular event in the past may have initiated medication use to reduce cardiovascular risk. This could have diminished the contrast in coronary calcification between subjects with and without stroke, resulting in underestimation of the risk estimates. After adjustment for cardiovascular risk factors, risk estimates did not materially change. This may in part be due to changes in risk factors in subjects after the cerebrovascular event, leading to misclassification of risk factors. Finally, most of the cerebrovascular events in our cohort were ischemic strokes (68%). Subtype analyses suggest that associations of coronary calcification with ischemic stroke are similar to those found for all strokes. Because of small numbers of events, we could not study the association between coronary calcification and other types of stroke.

Non-invasive measures of atherosclerosis have been shown to predict cerebrovascular events. Studies investigating the relation of carotid intima-media thickness with stroke, have found relative risks ranging between 3.4 and 8.5 for highest versus lowest quintile or category of intima-media thickness.[2] A low ankle brachial pressure index also indicates an increased risk of stroke, but relative risks of stroke associated with these measures were lower.[6] A positive association with risk of stroke has also been reported for carotid stenosis.[5,21,22] Since we investigated the association between coronary calcification and stroke in a cross-sectional design, the risk estimates cannot be directly compared with the results from these studies. However, in our study coronary calcification was strongly related to the presence of stroke. Prospective studies may find relative risks of coronary calcification for cerebrovascular events comparable to or higher than other non-invasive measures of atherosclerosis. If coronary calcification detected by EBCT is going to be used for selection of asymptomatic subjects at high risk of coronary events, it will be important to realize that the high-risk subjects may also have an increased risk of stroke.

In conclusion, we observed a strong and graded association between the amount of coronary calcification and stroke in both men and women. This is the first study on the association between coronary calcification detected by electron-beam CT and stroke. Although prospective data need to confirm our findings, this population-based cross-sectional study suggests that the amount of coronary calcification may identify subjects at high risk of cerebrovascular events.

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3.5 Arterial stiffness and risk of stroke

Abstract

Background - Functional and structural arterial abnormalities have been associated with the risk of cardiovascular disease. In a large population-based study, the association of arterial stiffness with prevalent cardiovascular disease was evaluated and its strength was compared with that of non-invasively measured atherosclerosis.

Methods and Results - The study included 3,818 elderly participants of the third examination phase of the Rotterdam Study. Ninety-five subjects had a myocardial infarction and seventy-eight subjects had a history of stroke since baseline examination. Arterial stiffness was determined at the third examination by carotid-femoral pulse wave velocity as measure of aortic stiffness and by common carotid distensibility as measure of carotid stiffness. Measures of atherosclerosis were the ankle-brachial pressure index and plaques in the carotid artery. Analyses were performed using logistic regression analyses, adjusted for age, sex, mean arterial pressure, and heart rate. Subjects with severe aortic stiffness had four-times more often a previous myocardial infarction then the reference category (odds ratio, 95% confidence interval: 4.0, 1.8-9.2). Aortic stiffness was not clearly associated with stroke. Subjects with severe carotid stiffness had two-times more often a previous myocardial infarction (2.3, 1.1-5.1) and twelve-times more often a previous stroke (12.6, 2.7-58.1) then the reference categories. The strength of the association of arterial stiffness with cardiovascular disease was comparable with the strength of the associations of the ankle-brachial pressure index and plaques in the carotid artery with cardiovascular disease.

Conclusion - The results of this study show that arterial stiffness is related to cardiovascular disease in a general population of elderly subjects**.**

Introduction

Measures of arterial stiffness have been found to be related to coronary artery disease in cross-sectional studies among various populations[1-3] and to cardiovascular and all-cause death in three longitudinal studies, two among subjects with end-stage renal disease[4,5] and one among subjects with essential hypertension.[6] The relation of arterial stiffness with stroke has been scarcely addressed. One small cross-sectional study reported increased aortic stiffness in subjects with stroke.[7] Previous studies were all performed in small groups or in selected subjects. The aim of the present study was to determine the association of arterial stiffness with history of myocardial infarction and stroke in a large general population of elderly subjects. We compared the strength of the association between arterial stiffness and prevalent cardiovascular disease with the strength of the association between non-invasively measured atherosclerosis and prevalent cardiovascular disease. As measures of arterial stiffness we used carotid-femoral pulse wave velocity (PWV) and common carotid arterial (CCA) distensibility. Measures of atherosclerosis were the ankle-brachial pressure index (ABPI) and plaques in the carotid artery.

Methods

Study population

The Rotterdam Study is a population-based cohort study that aims at assessing the occurrence of and risk factors for chronic diseases in the elderly. The rationale and design of the Rotterdam study have been described in detail elsewhere.[8] Shortly, 7,983 subjects aged 55 years or over were included in the first (baseline) examination phase that took place between 1990–1993. The Medical Ethics Committee of the Erasmus Medical Center approved the study and written informed consent was obtained from all participants. From 1997 until 1999 the third examination phase took place for which 5,901 subjects of the original cohort were eligible of which 4,148 subjects attended the physical examinations. Information on PWV or CCA distensibility or both was available at the third examination for 3,818 subjects. A total of 339 subjects were excluded because they had a myocardial infarction or stroke before baseline but not between baseline and the third examination phase. Of the remaining 3,474

subjects, information on PWV, distensibility, the ABPI, and carotid plaques was available for 3,175, 2,825, 3,302, and 3,285 subjects, respectively. Missing information was almost entirely due to logistic reasons.

Arterial Stiffness and Atherosclerosis

Arterial stiffness was measured by two different methods e.g., the carotid-femoral PWV as measure of aortic stiffness and the CCA distensibility coefficient (DC) as measure of CCA stiffness. Both measures were obtained with the subject in supine position on the same day in the same room after five minutes of rest. The order of measurements was fixed with first measurement of PWV and approximately ten minutes later measurement of the DC. Before measurement of PWV, blood pressure was taken twice with a conventional sphygmomanometer. PWV was assessed using an automatic device (Complior, Colson, Garges-lès-Gonesse Cx, France)[9] which measured the time delay between the rapid upstroke of the feet of simultaneously recorded pulse waves. The distance traveled by the pulse wave was measured over the surface of the body with a tape measure. PWV was calculated as the ratio between the distance traveled by the pulse wave and the foot-to-foot time delay. The average of at least 10 successive measurements, to cover a complete respiratory cycle, was used in the analyses. The DC was assessed by measuring vessel wall motion of the right CCA, with a Duplex scanner (Ultramark IV, ATL, Bothell, Washington, USA) connected to a vessel wall movement detector system.[10] The end-diastolic diameter (D) and the change in diameter during systole (ΔD) were computed as the mean of four cardiac cycles of three successive recordings. Blood pressure was measured twice with a Dinamap automatic blood pressure recorder. Pulse pressure (ΔP) was defined as systolic blood pressure (SBP) minus diastolic blood pressure (DBP). The DC was calculated according to the following equation[11]: $DC = (2\Delta D/D)/\Delta P$. Mean arterial pressure (MAP) was defined as DBP + 1/3*ΔP from blood pressure readings during both arterial stiffness measurements. A reproducibility study in 47 subjects showed an intra-class correlation coefficient of 0.80 for both PWV and the DC.

Atherosclerosis was non-invasively assessed by the ABPI and by the presence of carotid artery plaques. Ankle SBP was measured at the left and right posterior tibial artery using an 8 MHz continuous wave doppler probe (Huntleigh 500 D, Huntleigh Technology, Bedfordshire, UK) and a random zero sphygmomanometer with the subject in supine position.[12] The ratio of SBP at

the ankle to SBP at the right arm was measured for both ankles and the lowest ABPI in either leg was used.[13] For assessment of carotid plaques, ultrasonography of both left and right common and internal carotid arteries and carotid artery bifurcation was performed with a 7.5 MHz linear-array transducer (Ultramark IV, ATL, Bothell, Washington, USA). Plaques were defined as a focal widening of the wall with protrusion into the lumen composed of either only calcified deposits or a combination of calcified and non-calcified material. A total carotid plaque score was obtained by summation of presence of plaques at the three locations of far and near wall of the left and right carotid artery. A reproducibility study showed kappa's of 0.66 for the left, 0.68 for the right and 0.67 for either side.

Vascular Disease and Vascular Risk factors

Presence of vascular disease was defined as a first or recurrent myocardial infarction or a first or recurrent stroke that had occurred between baseline examination and the third examination phase (mean duration of follow-up: 6.6 years (range: 5.3 - 10.2 years)). Events were reported by general practitioners by means of a computerized system or collected on yearly visits to their office. In addition, discharge reports and letters of medical specialists were obtained for hospitalized patients. Two independent physicians coded the events according to the ICD 10. In case of disagreement, a medical expert in the field gave the final coding. Of the 78 strokes included, 64 strokes were cerebral infarctions, 3 strokes were primary intracerebral hemorrhages, and 11 strokes were unspecified because no neuro-imaging was available to determine the sub-type.

Information on smoking was obtained using a computerized questionnaire. Height and weight were measured and body mass index (weight/height^2) was calculated. Fasting blood samples were used to determine serum total cholesterol and high-density lipoprotein (HDL) cholesterol by an automatic enzymatic procedure and serum glucose by an enzymatic Hexokinase method (all Boehringer Mannheim, Mannheim, Germany). Information on the use of cardiovascular medication (diuretics, α-blockers, β-blockers, calcium antagonist, ACE-inhibitors, peripheral vasodilators, or lipid lowering drugs) was collected from the pharmacy.

Statistical Analysis

Characteristics of subjects with and without myocardial infarction or stroke were tested for differences between groups after adjustment for age using analyses of covariance for continuous variables and logistic regression analyses for dichotomous variables. The interrelationship between both measures of arterial stiffness and both measures of atherosclerosis was calculated using Pearson's rank correlation. The odds ratios for having a previous myocardial infarction or stroke per quartile of arterial stiffness or atherosclerosis were calculated using logistic regression analyses adjusted for age, sex, MAP and heart rate. For this purpose, we re-coded the carotid plaque score in four categories in such a way that each category comprised approximately 25% of the subjects. Increasing quartiles of both PWV and carotid plaques represent increasing severity, while increasing quartiles of both the DC and ABPI represent decreasing severity. Accordingly, the lowest or the highest quartile was chosen as reference category. Analyses were repeated with additional adjustment for several cardiovascular risk factors (total cholesterol, HDL-cholesterol, glucose, body mass index, and smoking) and after adjustment for the use of cardiovascular medication. Next, we examined whether the associations of both arterial stiffness and atherosclerosis with previous myocardial infarction and stroke were independent of each other by additional adjustment for the other. All analyses were performed using the SPSS 9.0 statistical package for Windows 98 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Characteristics of the study population are presented in table 1. Subjects without a previous myocardial infarction or stroke were younger, less often male, had fewer vascular risk factors and less often atherosclerotic disease as compared to subjects with a previous myocardial infarction or stroke. Subjects with a previous myocardial infarction had a lower SBP, DBP, and heart rate as compared to subjects without a previous myocardial infarction or stroke. The pearson's rank correlation between PWV and the DC was –0.40 ($p<0.001$). The pearson's rank correlation between carotid plaques and the ABPI was –0.17 ($p<0.001$).

PWV and carotid plaques had the strongest association with previous myocardial infarction (Table 2, Model 1). Subjects in the highest quartile of both

Table 1

Characteristics of the study population.

Characteristic	Subjects without myocardial infarction or stroke	Subjects with myocardial infarction	Subjects with stroke
Number	3310	95	78
Age (years)	72 (6.8)	74 (6.7)*	76(7.6)‡
Men (%)	39	67†	48§,◊
Systolic blood pressure (mmHg)	143 (21)	138 (21)†	146 (20)◊
Diastolic blood pressure (mmHg)	76 (11)	70 (11)†	75 (12)◊
Pulse pressure (mmHg)	67 (17)	66 (18)	73 (19)◊
Heart rate (bpm)	74 (12)	66 (12)†	71 (13)◊
Smoking			
Current(%)	15.8	17.9	13.0
Past(%)	48.4	60.0†	57.1
Body mass index (kg/m2)	26.8 (4.0)	26.1 (4.1)	26.9 (3.6)
Total cholesterol (mmol/l)	5.9 (1.0)	5.4 (1.0)†	5.9 (1.3)◊
HDL-cholesterol (mmol/l)	1.4 (0.4)	1.2 (0.3)†	1.3 (0.3)§
Serum glucose (mmol/l)	5.9 (1.4)	6.4 (2.3)†	6.2 (2.1)
Cardiovascular medication (%)	38.3	86.3†	62.8§,◊
Peripheral arterial disease (%)	15.6	32.2†	40.9§
Carotid artery plaques (%)	14.4	30.4†	29.0§
Distensibility coefficient (10-3/kPa)	10.6 (4.4)	10.5 (4.8)	8.0 (3.3)§,◊
Pulse wave velocity (m/s)	13.4 (3.0)	14.8 (3.3)†	14.6 (3.1)

Values are means(standard deviation) in case of continuous variables or percentages. *p<0.05 subjects with myocardial infarction versus subjects without myocardial infarction or stroke. †p<0.05 idem, adjusted for age. ‡p<0.05 subjects with stroke versus subjects without myocardial infarction or stroke. §p<0.05 idem, adjusted for age. ◊p<0.05 subjects with stroke versus subjects with myocardial infarction, adjusted for age. HDL-cholesterol: High-density lipoprotein cholesterol.

Table 2

Risk (OR) and 95% confidence interval(CI) of myocardial infarction per quartile of risk indicator.

	Model 1*		Model 2†	
Measure	n/events	OR(95% CI)	n/events	OR(95% CI)
PWV(m/s)	3175/79		2858/70	
1st(≤11.3)	795/11	1.0(reference)	652/10	1.0(reference)
2nd(11.3-13.1)	794/15	1.7(0.7-3.8)	747/13	1.5(0.6-3.6)
3rd(13.1-15.1)	795/23	2.6(1.2-5.6)	736/21	2.4(1.0-5.4)
4th(>15.1)	791/30	4.0(1.8-9.2)	723/26	3.5(1.5-8.5)
DC(10^{-3}/kPa)	2825/77		2663/70	
1st(≤7.4)	706/23	2.3(1.1-5.1)	572/23	2.0(0.9-4.5)
2nd(7.4-10.0)	706/20	1.8(0.9-3.8)	586/17	1.4(0.6-2.9)
3rd(10.0-13.2)	707/15	1.1(0.5-2.3)	604/11	0.8(0.4-1.7)
4th(>13.2)	706/19	1.0(reference)	628/19	1.0(reference)
ABPI	3302/87		2428/57	
1st(≤0.95)	787/36	3.2(1.6-6.4)	586/19	2.2(1.0-5.1)
2nd(0.95-1.05)	848/24	2.2(1.1-4.7)	595/18	2.2(1.0-5.0)
3rd(1.05-1.14)	832/16	1.6(0.7-3.5)	613/11	1.3(0.5-3.3)
4th(>1.14)	835/11	1.0(reference)	634/9	1.0(reference)
CA Plaques	3285/90		2485/61	
1st(0)	1074/11	1.0(reference)	814/8	1.0(reference)
2nd(1)	601/8	1.3(0.5-3.2)	453/8	1.6(0.6-4.3)
3rd(2-3)	815/25	2.7(1.3-5.6)	633/18	2.4(1.0-5.6)
4th(4-12)	795/46	4.3(2.4-8.6)	585/27	3.3(1.4-7.6)

*Model 1: adjusted for age, sex, mean arterial pressure and heart rate. †Model 2: as model 1, except models with PWV and DC additionally adjusted for ABPI and CA plaques and models with ABPI and CA plaques additionally adjusted for PWV and DC.

n: number of subjects; OR: Odds ratio; CI: Confidence interval; PWV: Pulse wave velocity; DC: Distensibility coefficient; ABPI: Ankle-brachial pressure index; CA: Carotid artery.

Table 3

Risk (OR) and 95% confidence interval(CI) of stroke per quartile of risk indicator.

	Model 1*		Model 2†	
Measure	n/events	OR(95% CI)	n/events	OR(95% CI)
PWV(m/s)	3175/72		2858/60	
1st(≤11.3)	795/8	1.0(reference)	744/6	1.0(reference)
2nd(11.3-13.1)	794/11	1.0(0.4-2.6)	722/7	0.9(0.3-2.7)
3rd(13.1-15.1)	795/30	2.4(1.0-5.5)	704/27	3.1(1.2-7.9)
4th(>15.1)	791/23	1.4(0.5-3.4)	688/20	1.8(0.6-5.1)
DC(10^{-3}/kPa)	2825/62		2663/53	
1st(≤7.4)	706/32	12.6(2.7-58.1)	648/28	9.9(2.1-46.6)
2nd(7.4-10.0)	706/13	5.9(1.3-27.3)	664/9	3.7(0.8-18.1)
3rd(10.0-13.2)	707/15	7.2(1.6-32.0)	669/14	6.7(1.5-30.0)
4th(>13.2)	706/2	1.0(reference)	682/2	1.0(reference)
ABPI	3302/69		2428/49	
1st(≤0.95)	787/29	2.0(1.0-3.9)	544/18	1.7(0.8-3.8)
2nd(0.95-1.05)	848/17	1.3(0.6-2.7)	634/12	1.2(0.5-2.9)
3rd(1.05-1.14)	832/10	0.8(0.3-1.8)	628/9	1.0(0.4-2.5)
4th(>1.14)	835/13	1.0(reference)	622/10	1.0(reference)
CA plaques	3285/76		2485/55	
1st(0)	1074/11	1.0(reference)	814/8	1.0(reference)
2nd(1)	601/17	3.0(1.3-6.7)	453/11	2.3(0.9-5.7)
3rd(2-3)	815/17	2.0(0.9-4.9)	633/13	1.5(0.6-3.7)
4th(4-12)	795/31	3.0(1.4-6.5)	585/23	2.5(1.1-5.8)

*Model 1: adjusted for age, sex, mean arterial pressure and heart rate. †Model 2: as model 1, except models with PWV and DC additionally adjusted for ABPI and CA plaques and models with ABPI and CA plaques additionally adjusted for PWV and DC.

n: number of subjects; OR: Odds ratio; CI: Confidence interval; PWV: Pulse wave velocity; DC: Distensibility coefficient; ABPI: Ankle-brachial pressure index; CA: Carotid artery.

PWV and carotid plaques had four-times more often a myocardial infarction as compared to subjects in the respective reference categories. Subjects in the lowest quartile of the DC had two-times more often a myocardial infarction and subjects in the lowest quartile of the ABPI had three-times more often a myocardial infarction as compared to their reference categories.

Previous stroke was most strongly associated with the DC (Table 3, Model 1). Subjects in the lowest quartile of the DC had twelve-times more often a stroke as compared to the reference category. Subjects in the highest quartile of carotid plaques had three-times more often a stroke and subjects in the lowest quartile of the ABPI had two-times more often a stroke as compared to the respective reference categories. PWV was not clearly associated with stroke.

Results did not change after additional adjustment for cardiovascular risk factors (data not shown). After adjustment for the use of cardiovascular medication, results for PWV and DC did not materially change except for the association of DC with myocardial infarction which was no longer significant (odds ratio, 95% confidence interval: 1.6, 0.7-3.7).

The odds ratios for previous myocardial infarction or stroke associated with arterial stiffness and atherosclerosis, respectively, decreased slightly when adjusted for the presence of the other, though results generally remained significant (Table 2, Model 2 and Table 3, Model 2).

Discussion

Our results show that aortic stiffness is associated with previous myocardial infarction. The strength of the association of aortic stiffness with myocardial infarction is comparable with that of atherosclerosis. CCA stiffness is also associated with previous myocardial infarction, though less strongly. CCA stiffness is strongly associated with previous stroke. The association is stronger than that of measures of atherosclerosis with previous stroke. All observed associations of arterial stiffness with cardiovascular disease were independent of atherosclerosis.

Some methodological aspects need to be discussed. Firstly, assessment of vascular events from baseline examination onwards was complete for both myocardial infarction and stroke until January 1998. Because arterial stiffness was measured until July 1999, recent occurrences of a myocardial infarction or stroke were unknown, resulting in incorrectly classifying some subjects as free of myocardial infarction or stroke. This misclassification of disease, however,

can be considered to be independent of arterial stiffness and thus, if present, will have led to an underestimation of the observed association. Secondly, changes in life-style are likely to be induced by a cardiovascular event. This could diminish long-term contrast in arterial stiffness and atherosclerosis between subjects with and without a history of an event, which will lead to an underestimation of the association. Moreover, longer time between an event and measurement of arterial stiffness will result in more misclassification of arterial stiffness at the time of the event, especially as arteries become stiffer with age. To minimize these effects without losing too many events we included only myocardial infarctions and strokes that occurred after baseline, resulting in a history of five to ten years. Thirdly, we calculated the PWV by using the distance between carotid and femoral artery, which is longer as the 'true' distance belonging to the time-delay between the pulse waves resulting in an overestimation of the PWV. Because variations in anatomy are limited, this overestimation can be considered similar for all subjects and therefore will not have seriously affected our results. Further, we calculated the DC by adjusting the distension of the CCA for pulse pressure measured in the brachial artery. We thereby assume that pulse pressure measured in the brachial artery is representative of pulse pressure in CCA. Several studies support the validity of using of brachial pressures as a proxy of aortic.[14-16] To the best of our knowledge, there are no studies evaluating the validity of using the brachial pulse pressure in stead of carotid pulse pressure. However, the aorta is a more central artery than the carotid artery. If using brachial pressures instead of aortic pressures is reasonably valid, the bias introduced by using brachial pressures instead of carotid pressures probably will be limited also. Finally, some subjects could not be included for various reasons, e.g. evaluating the association of risk indicators with disease in subjects with prevalent disease means only including survivors of myocardial infarction and stroke. Additionally, survivors of a myocardial infarction or stroke with considerable physical impairment might not be willing to visit the research center. Further, information on measures of arterial stiffness and measures of atherosclerosis was not available for all subjects attending the third examination phase due to logistic reasons. We do not think that missing these subjects has seriously altered our results. Missing subjects with severe disease will probably have led to an underestimation of the association, while random loss of subjects due to logistic reasons will not have biased our results.

We found both aortic and CCA stiffness to be associated with previous myocardial infarction. Various previous studies showed a relation between arterial stiffness and coronary artery disease[1-3] but these studies included only a small number of subjects. We found CCA stiffness to be associated with previous stroke, while no such association was found for aortic stiffness. Previous studies on the association between measures of arterial stiffness and stroke are limited to one cross-sectional study on aortic stiffness. This study found, in contrast with our results, a strong association.[7] However, the observed association between aortic stiffness and stroke in this study may be confounded by blood pressure as the blood pressure corrected index of aortic distensibility *Cp* used in this study was still significantly correlated with blood pressure in subjects with stroke.

The association of arterial stiffness with cardiovascular disease may be explained partly through an association of arterial stiffness with atherosclerosis. Our results indicate, however, that the associations between measures of arterial stiffness and previous myocardial infarction and stroke were only slightly attenuated after including measures of atherosclerosis in the model. This suggests that additional mechanisms play a role. Arterial stiffness leads to an increased pulse pressure which has been shown to be strongly related to myocardial infarction.[17] In our data, however, there seemed to be no difference in pulse pressure between subjects with or without a previous myocardial infarction (table 1) but this is likely to be due to increased use of cardiovascular medication in subjects after an event occurred. The stronger association of aortic stiffness as compared to CCA stiffness with myocardial infarction can probably be explained by the larger influence of the thoracic aorta than the CCA on afterload of the heart. Recent evidence shows that an increased pulse pressure, is also a strong risk factor for stroke.[18] Our results show that CCA stiffness was strongly associated with previous stroke, comparable with the association of measures of atherosclerosis with previous stroke. Some authors suggest that the risk of embolisms due to rupture of plaques is increased in stiff arteries.[19] Especially when inhomogeneities in stiffness in and around the plaque are present, this is likely to result in increased strain on the plaque and subsequent rupture. If this mechanism adds to the association between CCA stiffness and previous stroke it would imply an interaction between common carotid artery plaques and stiffness in which the presence of one increases the risk associated with the other and vice versa. Unfortunately, we could not evaluate a possible

interaction because of too small numbers of events. The absence of a clear association between aortic stiffness and previous stroke is difficult to explain, as one would expect to find an association between aortic stiffness and previous stroke when arterial stiffening is a generalized process throughout the arterial tree. Also, aortic stiffness probably contributes to an increased carotid pulse pressure. To what extend difference in the employed method to assess aortic and carotid stiffness adds to the observed difference in association of aortic and carotid stiffness with myocardial infarction and stroke is difficult to establish.

This is the first large population-based study showing an association between arterial stiffness and cardiovascular disease. The strength of the association of arterial stiffness with cardiovascular disease is comparable with the strength of non-invasive measures of atherosclerosis with cardiovascular disease. The finding that the association of arterial stiffness with myocardial infarction and stroke remains after adjustment for atherosclerosis suggests that arterial stiffness is an independent risk factor for cardiovascular disease in a general population of elderly subjects.

In conclusion, our results showed that arterial stiffness is associated with cardiovascular disease in a general population of elderly subjects. Aortic stiffness was most strongly associated with myocardial infarction while carotid stiffness showed a stronger association with stroke.

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3.6 A comparison between measures of atherosclerosis and risk of stroke

Abstract

Background - Several measures of atherosclerosis predict the risk of stroke. However, a comparison between various measures of atherosclerosis is lacking and limited information exists on the added value of individual measures of atherosclerosis to cardiovascular risk factors. We compared different measures of atherosclerosis in relation to the risk of stroke.
Methods and results - The study was based on the prospective cohort of the Rotterdam Study and included 6,913 participants who did not suffer from a previous stroke. At baseline, carotid intima-media thickness and plaques, ankle arm index and presence of aortic calcifications were assessed. After a mean follow-up of 6.1 years, 378 strokes occurred. Cox proportional hazards regression and Akaike's information criteria scores were used to evaluate the strength of the relation between each measure of atherosclerosis and stroke and to assess the contribution to classical cardiovascular risk factors. Carotid intima-media thickness and aortic calcifications were strongest related to the risk of stroke (RR 2.23 (95% CI 1.48-3.36) and 1.89 (95% CI 1.28-2.80) for highest versus lowest tertile, respectively). The relations between intima-media thickness, aortic calcifications and carotid plaques and stroke remained after adjustment for cardiovascular risk factors. Intima-media thickness and aortic calcifications were independently of each other related to the risk of stroke. Ankle arm index was not a good predictor for stroke, since the relation disappeared after adjustment for cardiovascular risk factors.
Conclusions - Carotid intima-media thickness and aortic calcifications are the strongest risk factors for stroke. They have additional value to each other and to classical risk factors and may reflect different processes.

INTRODUCTION

Several non-invasive measures of atherosclerosis, including carotid artery intima-media thickness, carotid plaques, ankle arm index and aortic calcifications are related to the risk of stroke in the population.[1-4] Most studies have focused on individual measures of atherosclerosis and there are few studies that have made a comparison between measures of atherosclerosis in the prediction of stroke.[5] It is still unclear whether all measures of atherosclerosis reflect the same process. Carotid intima-media thickness is considered to be a marker of generalized atherosclerosis.[6] Carotid plaques are reported to be markers of generalized atherosclerosis as well as sources of emboli.[7] Recently it was reported that ankle arm index may only reflect an unfavorable cardiovascular risk profile.[3] Calcifications in the vessel wall are considered to reflect the extent of atherosclerosis elsewhere.[8] Therefore, measures reflecting the amount of calcification in the atherosclerotic plaque such as aortic atherosclerosis, visualized on X-ray,[9] may be strongly related to stroke. Data on the contribution of each individual measure of atherosclerosis to classical cardiovascular risk factors in relation to stroke are limited.[10] We evaluated and compared the strength of the relation between carotid plaques, carotid intima-media thickness, ankle arm index and aortic calcifications in relation to stroke in a population of elderly subjects.

POPULATION

The study is part of the Rotterdam Study, a population-based cohort study on chronic and disabling diseases in the elderly. All inhabitants of Ommoord, a district of Rotterdam, aged 55 years or over were invited. People living in homes for the elderly were included. Participation rate of those invited for the study was 78% and in total 7,983 subjects participated.[11] The Medical Ethics Committee of Erasmus University Rotterdam approved the study. Written informed consent to retrieve information from treating physicians was obtained from all participants. Baseline measurements were obtained from 1990 to 1993 and consisted of a home interview and two visits to the research center for physical examination. A total of 7,721 participants were free from previous stroke. At the baseline visit to the research center we assessed several measures of atherosclerosis, including carotid intima-media thickness, carotid plaques,

ankle arm index and aortic calcifications. The study population consisted of 6,913 persons who had assessment of at least one measure of atherosclerosis.

ASSESSMENT OF ATHEROSCLEROSIS AT BASELINE

Carotid intima-media thickness and carotid plaques

Participants underwent B-mode ultrasonography of both carotid arteries. We measured intima-media thickness of the common carotid artery according to a standardized protocol as published previously.[1] During ultrasonography, the left and right common carotid artery, bifurcation and internal carotid artery were visualized and examined for the presence of plaques which were defined as focal widenings of the vessel wall of more than 50% relative to adjacent segments, with protrusion into the lumen. The total plaque score reflected the total number of sites with plaques and ranged from 0 to 6 (left and right-sided common carotid artery, bifurcation and internal carotid artery).[7] When data on one or more sites were missing, a weighted plaque score was computed, based on the available number of sites. We divided the number of sites with a plaque by the number of sites with plaque assessment and multiplied by 6. A reproducibility study of the plaque assessment resulted in a kappa of 0.66 for the left, 0.68 for the right carotid artery and 0.67 for either side, indicating moderate agreement.[12]

Ankle arm index

The systolic blood pressure of the posterior tibial artery was measured on both sides, using an 8 MHz continuous-wave Doppler probe (Huntleigh 500 D, Huntleigh technology, Bedfordshire, UK) and a random-zero sphygmomanometer.[13] Sitting blood pressure of the right arm was measured twice with a random-zero sphygmomanometer. The mean blood pressure was used to calculate the ankle arm index for each leg. In the analyses we used the lowest measurement. Because of possible measurement artifacts reflecting the presence of rigid or calcified walls, 36 participants with an ankle arm index >1.5 were excluded.

Aortic calcification

Aortic calcification was diagnosed by radiographic detection of calcified deposits in the abdominal aorta.[9] At baseline, lateral abdominal films (T12-S1)

were made from a fixed distance while the subject was seated. Aortic calcifications were considered to be present when linear densities were seen in an area parallel and anterior to the lumbar spine (L1-L4). Baseline values for the extent of calcification were scored into 5 categories according to the length of the involved area (one plaque 0.5-1.0 cm, several disseminated plaques <2.5 cm, 2.5-4.9 cm, 5.0-9.9 cm and >=10 cm). Readers were blinded to clinical information. X-rays of the lumbar spine were available for 5,796 participants who visited the research center (84%). Logistic reasons accounted for most of the missingness of X-rays (n=1,925). Missing calcification scores (n=165) were due to visualization problems, e.g. aorta not clearly depicted on X-ray. The validity of radiographic assessment of aortic calcification has been studied by comparing results of this method with data obtained at autopsy. Radiographic assessment was shown to be highly specific, and in most cases visible calcification represented advanced intimal atherosclerosis.[14] Intimal calcification was also shown to be clearly distinguishable from medial calcification. A comparison study involving computed tomograph (CT) was performed at our department. In 56 unselected elderly persons, aortic calcifications were independently assessed by radiography and CT. Calcifications were detected on abdominal radiography in 32 subjects. In all but 1 person, these calcifications were shown to be located in the aorta on the corresponding CT images.[9]

The number of participants with data available on carotid intima-media thickness, plaques, ankle-arm index and aortic calcifications were 5,479, 5,440, 6,196 and 5,631, respectively. Data on all measurements of atherosclerosis were available for 3,996 participants. Participants with data available on all measurements were on average 4.6 years younger (95% CI 4.2-5.1), were more often male (41% vs. 38%), had 2.6 mm Hg (95% CI 1.5-3.7) higher systolic blood pressure, and 0.09 mmol/L (95% CI 0.0-0.1) higher total cholesterol level, compared with participants with missing values for one or more measures of atherosclerosis (adjusted for age and/or gender). We observed no differences in diastolic blood pressure, diabetes, smoking and cholesterol.

Assessment of stroke

During the baseline interview a previous stroke was assessed by asking “Did you ever suffer from a stroke, diagnosed by a physician?”. Medical records of subjects who answered ‘yes’ were checked in order to verify the diagnosis.[15] A

history of TIA was also assessed during the baseline interview. All reported TIAs were reviewed by a neurologist.[16] Once subjects enter the Rotterdam Study they are continuously monitored for major events through automated linkage of the study database with the files from general practitioners. Information on vital status is obtained at regular intervals from the municipal authorities in Rotterdam. When an event or death has been reported, additional information is obtained by interviewing the general practitioner and scrutinizing information from hospital discharge records in case of admittance or referral. A neurologist (P.J.K.) reviewed information on all possible strokes and classified the stroke as definite, probable or possible. A stroke was definite if the diagnosis was based on typical clinical symptoms and neuro-imaging excluded other diagnoses. A stroke was considered probable in case typical clinical symptoms were present but neuro-imaging was not performed. For fatal strokes, other causes of death, especially cardiac, should have been excluded. A stroke was classified as possible if clinical symptoms were less typical and neuro-imaging was not performed, or if a cardiac cause of death could not be excluded in case of reported stroke. Subclassification in hemorrhagic or ischemic stroke was based on neuro-imaging, which was available for 61% of all cases. Follow-up was available for all participants until January 1, 1999.

Cardiovascular disease status and risk factors

Information on current health status and medical history at baseline was obtained using a computerized questionnaire. Participants smoking status was asked and subjects were classified as current, former or never smoker. At baseline, information on current health status, medication use and medical history including previous myocardial infarction, coronary bypass surgery and coronary angioplasty was obtained using a computerized questionnaire. All reported myocardial infarctions were verified by using medical records. History of intermittent claudication and angina pectoris was assessed through the Rose questionnaire.[17] Non-fasting blood samples were taken and serum total cholesterol and high-density lipoprotein (HDL) cholesterol were measured using an automated enzymatic procedure. Sitting blood pressure was measured twice on the right arm with a random-zero sphygmomanometer. In the analyses we used the average of the two measurements. Diabetes mellitus was defined as use of oral blood glucose lowering drugs or insulin or random or post-load serum glucose level higher than 11.0 mmol/L, according to the World Health

Organization criteria.[18] Atrial fibrillation was assessed by an electrocardiogram. A history of cardiovascular disease was coded if participants had a history of TIA, myocardial infarction, angina pectoris, intermittent claudication, atrial fibrillation, coronary bypass surgery and/or coronary angioplasty.

Data analysis

We evaluated the relationship between carotid intima-media thickness, carotid plaques, ankle-arm index and aortic calcifications and risk of first-ever stroke and cerebral infarction using Cox regression analysis. The data were analyzed in several ways. First, we assessed the risks in participants in tertiles, taking the least severe atherosclerosis as the reference. A composite atherosclerosis score was obtained by adding up the scores for the separate measures of atherosclerosis (range 0-8). We further analyzed carotid intima-media thickness and ankle-arm index per standard deviation increase, and carotid plaques and aortic calcifications per unit increase. The analyses were adjusted for age and gender and additionally for diabetes mellitus (yes, no), smoking (current, former or never), systolic and diastolic blood pressure, total and HDL cholesterol level. Results are presented as relative risks with corresponding 95% confidence intervals.

We used Akaike optimal information criteria (AIC) to evaluate the prognostic ability of the measures of atherosclerosis as compared to a reference model.[19] This method allows to take both follow-up time and differences in numbers of degrees of freedom into account. The height of the AIC score reflects the prognostic value and a positive score indicates that adding the measure of atherosclerosis results in an improvement of the reference model. The Akaike information criterium was calculated as the Chi square statistic of the significant change for the extended model, as compared to a reference model, minus 2 times the number of degrees of freedom. For these analyses we restricted ourselves to participants with information available on all measures of atherosclerosis. We entered carotid intima-media thickness and ankle arm index as continuous variables and carotid plaques and aortic calcifications as categorical variables into the model. First, we used a model including age and gender as reference. Then, the reference model was expanded with diabetes mellitus, smoking, diastolic and systolic blood pressure, total and HDL cholesterol and additionally with history of cardiovascular disease. We further used stepwise regression analysis to assess which measures of atherosclerosis

were independently related to the risk of stroke and cerebral infarction. Age and gender were forced into the model.

RESULTS

Table 1 shows baseline characteristics of the study population. A total of 378 strokes occurred during a mean follow-up time of 6.1 years (42,272 person-years). Of these, 198 were cerebral infarctions (52%). Table 2 shows that participants in the highest tertile of carotid intima-media thickness had an approximately 2.5-fold increased risk of stroke as compared to the lowest tertile.

Table 1

Baseline characteristics of the study population.

	Study population n=6913
Age (years)	69.5 (9.2)
Gender (% female)	60.3
Diastolic blood pressure (mm Hg)	73.6 (11.7)
Systolic blood pressure (mm Hg)	139.2 (22.4)
Total cholesterol (mmol/L)	6.6 (1.2)
HDL-cholesterol (mmol/L)	1.3 (0.4)
Diabetes (%)	10.0
Smoking (%)	
(current)	22.7
(former)	41.6
History of cardiovascular disease (%)	24.4
Mean common carotid IMT (mm)	0.80 (0.16)
Carotid plaque score	1.4
Ankle-arm index	1.06 (0.23)
Aortic calcifications (%)	
No	33.4
0.5-1.0 cm	9.5
1.0-2.5 cm	26.6
2.5-4.9 cm	17.7
5.0-9.9 cm	10.7
>= 10 cm	2.2

Values represent means (SD), IMT: Intima-media thickness.

Table 2

Relative risk of stroke in relation to measures of atherosclerosis.

Measure of Atherosclerosis		No. at risk	No. of cases	Relative risk*	Relative risk¶
IMT (mm)					
Tertiles	Low	1777	35	1.00	1.00
	Intermediate	1820	90	1.66 (1.10·2.51)	1.64 (1.01·2.66)
	High	1882	169	2.23 (1.48·3.36)	2.42 (1.51·3.89)
Per SD increase				1.29 (1.15·1.44)	1.28 (1.15·1.44)
Carotid plaques					
Tertiles	Low	2225	72	1.00	1.00
	Intermediate	1826	101	1.24 (0.89·1.71)	1.18 (0.82·1.71)
	High	1389	122	1.61 (1.16·2.23)	1.47 (1.02·2.13)
Per category increase				1.13 (1.05·1.21)	1.15 (1.07·1.24)
Ankle-arm index					
Tertiles	High	2069	78	1.00	1.00
	Intermediate	2061	87	1.08 (0.79·1.47)	0.99 (0.66·1.46)
	Low	2066	160	1.55 (1.16·2.07)	1.28 (0.87·1.88)
Per SD decrease				1.10 (0.98·1.24)	1.13 (1.00·1.26)
Aortic calcification					
Tertiles	Low	1881	40	1.00	1.00
	Intermediate	2032	96	1.45 (0.99·2.14)	1.21 (1.06·1.52)
	High	1718	133	1.89 (1.28·2.80)	1.63 (1.06·2.52)
Per category increase				1.20 (1.09·1.32)	1.21 (1.10·1.33)
Composite score					
Tertiles	Low	1336	20	1.00	1.00
	Intermediate	1263	48	2.05 (1.21·3.48)	1.52 (0.83·2.80)
	High	1397	129	4.20 (2.55·6.90)	2.71 (1.50·4.90)
Per category increase				1.24 (1.14·1.35)	1.26 (1.16·1.38)

Ranges for tertiles were <0.72, 0.72·0.84, >0.84 for IMT, 0, 1·2, 3·6 for carotid plaques, <1.01, 1.01·1.17, >1.17 for ankle arm index, 0, 1·2, 3·5 for aortic calcifications, 0·2, 3·4, 5·8 for compound score. IMT: Intima·media thickness, * Adjusted for age and gender, ¶ adjusted for age, gender, diabetes mellitus, smoking, systolic and diastolic blood pressure, cholesterol and HDL cholesterol level and history of cardiovascular disease.

Table 3

Relative risk of cerebral infarction in relation to measures of atherosclerosis.

Measure of Atherosclerosis		No. at risk	No. of cases	Relative risk*	Relative risk¶
IMT (mm)					
Tertiles	Low	1777	22	1.00	1.00
	Intermediate	1820	59	2.30 (1.40-3.78)	2.26 (1.34-3.81)
	High	1882	82	2.95 (1.79-4.87)	2.30 (1.34-3.94)
Per SD increase				1.33 (1.15-1.54)	1.20 (1.02-1.41)
Carotid plaques					
Tertiles	Low	2225	42	1.00	1.00
	Intermediate	1826	59	1.56 (1.04-2.33)	1.38 (0.91-2.09)
	High	1389	63	2.14 (1.42-3.23)	1.55 (1.00-2.40)
Per category increase				1.18 (1.08-1.30)	1.09 (0.99-1.20)
Ankle-arm index					
Tertiles	High	2069	45	1.00	1.00
	Intermediate	2061	42	0.97 (0.63-1.48)	0.77 (0.50-1.20)
	Low	2066	78	1.71 (1.16-2.52)	1.02 (0.66-1.56)
Per SD decrease				1.28 (1.12-1.47)	1.09 (0.92-1.28)
Aortic calcification					
Tertiles	Low	1881	24	1.00	1.00
	Intermediate	2032	60	1.95 (1.21-3.15)	1.73 (1.04-2.85)
	High	1718	78	2.70 (1.67-4.35)	2.10 (1.26-3.50)
Per category increase				1.30 (1.16-1.45)	1.21 (1.07-1.37)
Composite score					
Tertiles	Low	1336	14	1.00	1.00
	Intermediate	1263	31	2.03 (1.07-3.85)	1.72 (0.90-3.30)
	High	1397	74	3.96 (2.16-7.26)	2.63 (1.38-5.05)
Per category increase				1.35 (1.22-1.48)	1.22 (1.10-1.36)

Ranges for tertiles were <0.72, 0.72-0.84, >0.84 for IMT, 0, 1-2, 3-6 for carotid plaques, <1.01, 1.01-1.17, >1.17 for ankle arm index, 0, 1-2, 3-5 for aortic calcifications, 0-2, 3-4, 5-8 for compound score. IMT: Intima-media thickness, * Adjusted for age and gender, ¶ adjusted for age, gender, diabetes mellitus, smoking, systolic and diastolic blood pressure, cholesterol and HDL cholesterol level and history of cardiovascular disease.

Table 4

Akaike's information criteria (AIC) scores for measures of atherosclerosis, and percentages of the maximal AIC score.

Outcome	Measure of atherosclerosis	AIC compared to model I		AIC compared to model II		AIC compared to model III	
		% of maximal AIC score	AIC score	% of maximal AIC score	AIC score	% of maximal AIC score	AIC score
Stroke	IMT	100%	31.84*	100%	18.43*	100%	11.53*
	Carotid plaques	58%	18.38*	34%	6.19*	16%	1.81 ¶
	Ankle arm index	36%	11.49*	33%	0.77 NS	0%	<0 NS
	Aortic calcifications	69%	22.09*	66%	12.18*	52%	5.99 *
Cerebral infarction	IMT	95%	11.30*	82%	4.17¶	54%	0.97 NS
	Carotid plaques	100%	11.94*	91%	4.63 ¶	100%	1.81 ¶
	Ankle arm index	56%	6.68*	12%	0.61 NS	0%	<0 NS
	Aortic calcifications	96%	11.41*	100%	5.09¶	31%	0.57 NS

Model I includes age and gender, Model II includes age, gender, diabetes mellitus, smoking, systolic and diastolic blood pressure, cholesterol and HDL cholesterol level, Model III includes age, gender, diabetes mellitus, smoking, systolic and diastolic blood pressure, cholesterol and HDL cholesterol level and history of cardiovascular disease, * $P<0.01$, ¶ $P<0.05$, NS $P>0.05$.

The corresponding risks in the severest tertiles of ankle arm index and carotid plaques were approximately 1.5-fold increased. Severe aortic calcifications approximately doubled the risk of stroke. We observed a four-fold increased risk of stroke for participants in the highest tertile of the composite atherosclerosis score. Additional adjustment for cardiovascular risk factors and history of cardiovascular disease attenuated the risk estimates, but the risks remained significantly increased, except for those related to a low ankle arm index. Table 3 shows that the results were largely similar for cerebral infarctions.

Table 4 shows the additional values for the different measures of atherosclerosis beyond traditional cardiovascular risk factors. It shows that intima media thickness and aortic calcifications had the highest added value to predict stroke if compared to a model including age and gender (model I). The additional value of both measures remained if compared to a model with cardiovascular disease (model II). The AIC scores diminished when the reference model was expanded with history of cardiovascular disease (model III). Ankle arm index performed worst of all and lost the additional value for stroke if compared to a reference model including age, gender and cardiovascular risk factors (model II).

Finally, we analyzed all measures of atherosclerosis in a stepwise regression model in which we forced the variables age and gender. Both for stroke and cerebral infarction, carotid intima-media thickness and aortic calcifications were included ($P<0.05$).

Discussion

In our population-based study among 6,943 participants, we found that carotid intima-media thickness and aortic calcifications were the strongest predictors for stroke. Carotid plaques and ankle arm index were less strong predictors. The relation between ankle-arm index and stroke was no longer statistically significant when cardiovascular risk factors were taken into account. Carotid intima-media thickness and aortic calcifications predict the risk of stroke independently of each other. This indicates that they may represent different pathophysiological mechanisms.

Before we interpret our results some methodological issues need to be addressed. First, some analyses were restricted to participants with assessment of all measures of atherosclerosis. Since the overall vascular risk profile was not very different between people with and without complete data on all measures of

atherosclerosis, we think that this has not caused a selection bias. Furthermore, misclassification of atherosclerosis could have occurred and we may have misclassified some strokes or subtypes of stroke. We restricted our analysis to CT or MRI confirmed cerebral infarctions to reduce misclassification. However, classification of atherosclerosis was done blinded for stroke status and vice versa. Hence, if misclassification had occurred, it is likely to be non-differential, leading to underestimation of the observed effects.

The main issue in our study was to determine which measures of atherosclerosis are strongest predictors of the risk of stroke and cerebral infarction. Carotid intima-media thickness and ankle-arm index were continuous measures and carotid plaques and aortic calcifications were categorical measures. Evaluation of relative risks in tertiles allowed us to compare the relative risks.

Intima-media thickness has widely been investigated as a measure of generalized atherosclerosis. Our results show the robustness of the relation between intima-media thickness and stroke. Presence of aortic calcifications was an important risk indicator for stroke, even when information on carotid intima-media thickness or cardiovascular risk factors was taken into account. Two mechanisms could explain the persistence of such association. First, calcifications could reflect arterial stiffness, leading to hypertension and stroke. The relationships remained significant after adjustment for blood-pressure and arterial stiffness has not consistently been shown to be a risk factor for stroke.[20,21] Still, this hypothesis should not be turned down, since one measurement of blood-pressure is a poor reflection of the lifetime exposure. Another factor to consider is that calcifications may represent presence of atherosclerotic lesions in the aortic arch, or carotid arteries, from which emboli can be released. A plausible explanation for our finding that intima-media thickness and aortic calcifications are related to stroke, independent of each other, is that both markers of atherosclerosis reflect different processes, as we described.

Ankle arm index was the poorest predictor for stroke, since it lost the predictive ability after taking cardiovascular risk factors into account. This result supports the findings by the Atherosclerosis Risk In Communities Study in which the relation between ankle arm index and stroke also diminished after adjustment for cardiovascular risk factors.[3] Our study confirms that ankle-arm

index has little prognostic ability beyond traditional risk factors for stroke and cerebral infarction.

In summary, carotid intima-media thickness and aortic calcifications are the most strong predictors for stroke. Carotid intima-media thickness, plaques and aortic calcifications have additional value to traditional cardiovascular risk factors. The results of our study merit further research on individual stroke-risk assessment that includes measures of atherosclerosis. In addition to carotid intima-media thickness, information on calcifications in the vessel wall may help in identifying people at high risk of stroke.

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