The role of atherosclerosis, hormones and genes in stroke

M. Hollander

The role of atherosclerosis, hormones and genes in stroke

Acknowledgements

The work presented in this thesis was supported by grant 904-61-091 of the Netherlands Organization of Scientific Research (N.W.O.) and conducted at the Department of Epidemiology & Biostatistics of the Erasmus Medical Center Rotterdam in close collaboration with the Department of Neurology of the Erasmus Medical Center Rotterdam.

The author gratefully acknowledges the collaboration with the Julius Center for Patient Oriented Research and General Practice (Prof. dr. D.E. Grobbee and Dr. M.L. Bots) and with the Department of Internal Medicine of the Erasmus Medical Center Rotterdam (Prof. dr. H.A.P. Pols).

The Department of Epidemiology & Biostatistics of the Erasmus Medical Center Rotterdam and the Julius Center for Patient Oriented Research and General Practice supported the publication of this thesis.

Bristol-Myers Squibb supported the printing of this thesis.

Cover design: Susan Vos, Vlijmen
Printed by: Print Partners Ipskamp, Enschede

ISBN 90-9016562-2

The role of atherosclerosis, hormones and genes in stroke

De rol van atherosclerose, hormonen en genen in relatie tot beroerte

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam op gezag van de
Rector Magnificus
Prof.dr.ir. J.H. van Bemmel
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 22 januari 2003 om 15:45 uur

door

Monika Hollander

geboren te Groningen.

Promotiecommissie

Promotoren:	Prof.dr. A. Hofman
	Prof.dr. D.E. Grobbee
Overige leden:	Prof.dr. C. De Carli
	Prof.dr. P.J. Koudstaal
	Prof.dr. C.M. van Duijn
Copromotor:	Dr. M.M.B. Breteler

CONTENTS

Papers and manuscripts based on the studies presented in this thesis

Chapter 2.1

M Hollander, PJ Koudstaal, ML Bots, DE Grobbee, A Hofman, MMB Breteler. Incidence, risk and case fatality of stroke in the elderly; the Rotterdam Study. (J Neurol Neurosurg Psychiatry 2003, accepted).

Chapter 3.1

M Hollander, MMB Breteler. Markers of subclinical vascular disease and stroke. In: Gorelick PB, Alter M, eds. Stroke Prevention. London, United Kingdom, Parthenon Publishing, 2002, page 105-114.

Chapter 3.2

M Hollander, ML Bots, A Iglesias del Sol, PJ Koudstaal, A Hofman, DE Grobbee, JCM Witteman, MMB Breteler. Carotid artery intima-media thickness and risk of intracerebral hemorrhage and lacunar infarction; the Rotterdam Study. (submitted)

Chapter 3.3

M Hollander, ML Bots, A Iglesias del Sol, PJ Koudstaal, JCM Witteman, DE Grobbee, A Hofman, MMB Breteler. Carotid plaques increase the risk of stroke and subtypes of cerebral infarction in asymptomatic elderly; the Rotterdam Study. Circulation 2002;105:2872-7.

Chapter 3.4

R Vliegenthart, M Hollander, MMB Breteler, DAM van der Kuip, A Hofman, M Oudkerk, JCM Witteman. Stroke is associated with coronary calcifications as detected by electron-beam CT. The Rotterdam Coronary Calcification Study. Stroke 2002;33:462-465.

Chapter 3.5

NM van Popele, M Hollander, ML Bots, DAM van der Kuip, RS Reneman, APG Hoeks, R Asmar, A Hofman, MMB Breteler, DE Grobbee, JCM Witteman. Measures of arterial stiffness are associated with myocardial infarction and stroke. The Rotterdam Study. (submitted)

Chapter 3.6

M Hollander, AE Hak, PJ Koudstaal, ML Bots, DE Grobbee, A Hofman, JCM Witteman, MMB Breteler. A comparison between measures of atherosclerosis and risk of stroke; the Rotterdam Study. (submitted)

Chapter 4.1

M Hollander, HAP Pols, MI Geerlings, JCM Witteman, FJ de Jong, PJ Koudstaal, A Hofman, MMB Breteler. Endogenous sex hormone levels and risk of stroke; the Rotterdam Study. (submitted)

Chapter 5.1

M Hollander, F Sayed, PJ Koudstaal, A Hofman, CM van Duijn, MMB Breteler. The ACE gene polymorphism and risk of stroke in the elderly; the Rotterdam Study. (submitted)

Chapter 5.2

OT Njajou, M Hollander, PJ Koudstaal, A Hofman, JCM Witteman, MMB Breteler, CM van Duijn. Mutations in the hemochromatosis gene (HFE) and stroke. Stroke 2002;33:2363-6.

1
General introduction

Stroke is a major cause of death and a devastating disorder that puts a large burden on health care systems. Stroke occurs particularly in the elderly.[1] Most studies on the incidence of stroke have focused on persons aged younger than 85 years and limited data exist on the occurrence of stroke in the very old.[2] Since populations are growing older, an increase in the burden of stroke is expected in coming decades. A challenge for medical research is the question whether and how this devastating disease can be prevented. This requires identification of modifiable risk factors that are amenable to intervention. Moreover, it requires possibilities to recognize those who may benefit most from preventive interventions.

The main risk factor for stroke is atherosclerosis, which accumulates with age. Several non-invasive measures of atherosclerosis exist. Despite research that has been done in this field, the strength and nature of the relation between measures of atherosclerosis and stroke subtypes is not yet fully understood. Further, it is not clear whether other risk factors are related to stroke through unrelated mechanisms, or that they trigger the presence or progression of atherosclerosis. Besides atherosclerosis, there are other putative risk factors for stroke such as sex hormones and genetic factors.[3] The relevance of these factors in relation to stroke still needs to be established. The work described in this thesis aims to further quantify the incidence of stroke in the elderly and the relation between atherosclerosis, sex hormones and genetic markers and stroke. The studies were based on the Rotterdam Study, a large population-based cohort study among 7,983 persons aged 55 years or over that started in 1990. Since then, the cohort was followed for morbidity and mortality, including stroke.

Chapter 2 describes the occurrence of stroke in elderly men and women in this cohort. Chapter 3 focuses on the relation between non-invasive measures of atherosclerosis and risk of stroke. Structural as well as functional measures of atherosclerosis will be dealt with. For each measure we aim to assess the strength of the relation with stroke. In chapter 3.2, 3.3 and 3.4 the relation between structural measures of atherosclerosis and risk of stroke is assessed. In particular we examine the effect of carotid intima media thickness (chapter 3.2), plaques in the carotid artery (chapter 3.3) and coronary calcifications (chapter 3.4). In chapter 3.5 en we describe the relation between functional measures of atherosclerosis and stroke risk, in particular measures of arterial stiffness. In the last paragraph of this chapter we evaluate and compare the strength of the relation between different measures of atherosclerosis in relation to stroke

(chapter 3.6). In chapter 4, we investigate the relation between sex hormone levels and stroke in men and in women. Both estrogen and testosterone are evoked to be related to the risk of cardiovascular disease, but the relation with stroke is not yet clear.[4-6] In chapter 5 we investigate the role of two candidate genes, namely mutations in the hemochromatosis gene and the angiotensin-converting enzyme polymorphism. Finally in chapter 6 we review our findings and we comment on the strength and limitation of our studies. Further, we discuss the clinical relevance of our findings and make recommendations for further research.

REFERENCES

1. Feinleib M, Ingster L, Rosenberg H, Maurer J, Singh G, Kochanek K. Time trends, cohort effects, and geographic patterns in stroke mortality--United States. Ann Epidemiol 1993;3(5):458-65.
2. Bonita R. Epidemiology of stroke. Lancet 1992;339(8789):342-4.
3. Hassan A, Markus HS. Genetics and ischaemic stroke. Brain 2000;123(Pt 9):1784-812.
4. Barrett-Connor E, Goodman-Gruen D. Prospective study of endogenous sex hormones and fatal cardiovascular disease in postmenopausal women. Bmj 1995;311(7014):1193-6.
5. English KM, Mandour O, Steeds RP, Diver MJ, Jones TH, Channer KS. Men with coronary artery disease have lower levels of androgens than men with normal coronary angiograms. Eur Heart J 2000;21(11):890-4.
6. Jeppesen LL, Jorgensen HS, Nakayama H, Raaschou HO, Olsen TS, Winther K. Decreased serum testosterone in men with acute ischemic stroke. Arterioscler Thromb Vasc Biol 1996;16(6):749-54.

2

Incidence of stroke in the elderly

2.1 Incidence, risk and case fatality of stroke in the elderly

Abstract

Objective - To estimate the incidence, survival and life-time risk of stroke in the elderly.
Methods - We conducted a study in 7,721 participants from the population-based Rotterdam Study who were free from stroke at baseline (1990-1993) and were followed up for stroke until January 1 1999. We calculated age and sex specific incidence, case fatality rates and lifetime risks of stroke.
Results - Mean follow-up was 6.0 years and 432 strokes occurred. The incidence rate of stroke per 1,000 personyears increased with age and ranged from 1.7 (95% CI 0.4-6.6) in men aged 55 to 59 years to 69.8 (95% CI 22.5-216.6) in men aged 95 years or over. Corresponding figures for women were 1.2 (95% CI 0.3-4.7) and 33.1 (9% CI 17.8-61.6). Men and women had similar absolute lifetime risks of stroke (21% for those aged 55 years). The survival after stroke did not differ according to sex.
Conclusions - Stroke incidence increases with age, also in the very old. Although the incidence rate is higher in men than in women over the entire age range, the lifetime risks were similar for both sexes.

INTRODUCTION

In recent decades a decreasing trend in mortality of stroke has been observed.[1-6] Nevertheless, stroke is still a leading cause of death in the western countries and puts a large burden on health care systems.[7,8] Stroke incidence increases with age, but information on the incidence and survival of stroke in the very old in the general population is limited. Since populations are growing older this information is of importance, particularly for health care planners.[9] Also, data on the risk of stroke during one's life is lacking and is of interest on the individual level. We performed a large prospective study on the incidence of stroke in a population-based cohort of elderly subjects aged 55 years or over, which enabled us to calculate incidence rates, survival, case fatality and period and lifetime risks of stroke.

METHODS

Study population

The present study was performed in the framework of the Rotterdam Study, a population-based, single-center 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.[10] 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 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. During the 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 and a previous stroke was considered to have occurred if medical records confirmed it.[11] The present study comprises a cohort of 7,721 subjects who were free from stroke at baseline. Follow up for stroke was complete until January 1, 1999.

Assessment of stroke

Once subjects enter the Rotterdam Study they are continuously monitored for major events through automated linkage with files from the general practitioners. For the present study information on stroke and death was used. Stroke is defined as rapidly developing clinical signs of focal or global disturbance of cerebral function with no apparent cause other than a vascular origin. When an event or death had been reported, additional information was obtained by interviewing the general practitioner and by scrutinizing information from hospital discharge records in case of admittance or referral. Information from reports on all possible strokes was reviewed by two research physicians and a neurologist (PJK) who classified the stroke as definite, probable or possible stroke.[12] The stroke was definite if the diagnosis was based on typical clinical symptoms and neuro-imaging excluded other diagnoses. The 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. All reported transient ischemic attacks also were reviewed in order to screen and classify all strokes. Subarachnoid hemorrhages were excluded. Until the end of follow up 202 definite, 130 probable and 96 possible strokes occurred. We used definite and probable strokes in the analyses (n=432). For subjects with a stroke, follow-up was complete until January 1, 1999. Case fatality within 28 days was defined as death occurring within 28 days after onset of the stroke.

Subtypes of stroke

If the CT or MRI scan showed a cerebral hemorrhage or infarction the type of stroke was coded accordingly. In case of no abnormality on CT or MRI, the stroke was classified as cerebral infarction. Strokes without neuro-imaging could be classified as possible hemorrhagic stroke or a cerebral infarction on the basis of the following symptoms. A possible hemorrhagic stroke was coded in case of sudden hemiplegia or other focal signs with permanent unconsciousness or death within hours. The stroke was classified as possible cerebral infarction if there was limited impairment (isolated aphasia, isolated weakness of one limb,

isolated facial weakness or isolated hemianopia), complete improvement within 72 hours or documented atrial fibrillation at time of the stroke.

Data analysis

The age-specific incidence rate was obtained for each 5-year band by dividing the number of strokes by the total amount of person-years attributed to a specific age category. The follow-up ended either at occurrence of stroke, or at death, or at January 1, 1999, whichever came first. Participants who suffered from a subarachnoid hemorrhage were censored on the date of the event. Incidence rates are given with 95% confidence intervals (CI), assuming Poisson distribution. We calculated incidence and 28 days case fatality rates for subtypes of stroke in 10-years age strata. For the calculation of 52 weeks case fatality rates we restricted ourselves to cases that occurred before December 31, 1998. We used Cox proportional hazards regression to investigate gender differences in risk of stroke and survival after stroke. In these analyses we adjusted for age at baseline and stroke, respectively. Survival after stroke was calculated using the Kaplan Meier method. In order to calculate the absolute risk of stroke over time we took the competing risk of dying into account. First we obtained the stroke free survival at different ages from the cohort with the Kaplan Meier method. Age at baseline was used as entry time and age at end of follow-up, occurrence of stroke or death as failure time. Then the cumulative absolute risk of stroke over a period was calculated as the integrated product of the age-specific stroke incidences and the stroke-free survival.[13] The risk of stroke over time was calculated separately for men and women at ages 55, 65, 75 and 85 years.

RESULTS

The mean follow-up time was 6.0 years. We had 46,011 person-years of observation and 432 suffered from a first-ever stroke. Of these 39 were primary intracerebral hemorrhages (9.0%), 233 cerebral infarctions (53.9%) and 160 unspecified strokes (37.0%). A CT or MRI scan was performed in 256 (59.3%) cases and in 2 cases necropsy was performed. The hemorrhages and infarctions were confirmed by neuro-imaging in 74.4% and respectively 94.4%. A total of 256 persons (59.3%) were hospitalized.

Table 1

Age and gender specific incidence rate of stroke in the elderly.

Age	Men				Women				Total			
	No. of strokes	Person-years	Incidence rate*	95% CI	No. of strokes	Person-years	Incidence rate*	95% CI	No. of strokes	Person-years	Incidence rate*	95% CI
55-59	2	1205.1	1.7	0.4-6.6	2	1691.2	1.2	0.3-4.7	4	2896.3	1.4	0.5-3.7
60-64	8	3428.6	2.3	1.2-4.7	10	4725.0	2.1	1.1-3.9	18	8153.6	2.2	1.4-3.5
65-69	31	4067.9	7.6	5.4-10.8	16	5230.4	3.1	1.9-5.0	47	9298.3	5.1	3.8-6.7
70-74	32	3565.5	9.0	6.3-12.7	29	5072.1	5.7	4.0-8.2	61	8637.7	7.1	5.5-9.1
75-79	48	2656.9	18.1	13.6-24.0	65	4557.0	14.3	11.1-18.1	113	7213.9	15.7	13.0-18.8
80-84	31	1556.9	19.9	14.0-28.3	41	3495.0	11.7	8.6-15.9	72	5051.8	14.3	11.3-18.0
85-89	17	665.6	25.5	15.9-41.1	49	2379.5	20.6	15.6-27.2	66	3045.0	21.7	17.0-27.6
90-94	8	239.6	33.4	16.7-66.8	30	1130.3	26.5	18.6-38.0	38	1369.9	27.7	20.2-38.1
95+	3	43.0	69.8	22.5-216.6	10	301.7	33.1	17.8-61.6	13	344.7	37.7	21.9-65.0
All	180	17429.0	10.3	8.9-12.0	252	28582.2	8.8	7.8-10.0	432	46011.2	9.4	8.5-10.3

* Rate per 1000 person-years.

The incidence rate of stroke increased with age and ranged from 1.7 (95% CI 0.4-6.6) in men aged 55-59 years to 69.8 (95% CI 22.5-216.6) in men aged 95 or over (table 1). Corresponding rates for women were 1.2 (95% CI 0.3-4.7) and 33.1 (95% CI 17.8-61.6), respectively. Incidence rates were higher in men than in women over the entire age range. Adjusted for age at baseline the relative risks of stroke for men compared to women in partipants aged younger and older than 75 years were respectively 1.64 (95% CI 1.26-2.15) and 1.36 (95% CI 1.02-1.83).

Table 2

Incidence rate of subtypes of stroke.

Age	Cerebral infarction			Intracerebral hemorrhage			Unspecified stroke		
	No. of cases	IR*	95% CI†	No. of cases	IR*	95% CI†	No. of cases	IR*	95% CI†
55-64	17	1.5	1.0-2.5	2	0.2	0.0-0.7	3	0.3	0.1-0.8
65-74	72	4.0	3.2-5.1	10	0.6	0.3-1.0	26	1.4	1.0-2.1
75-84	111	9.0	7.5-10.9	22	1.8	1.2-2.7	52	4.2	3.2-5.6
85+	33	6.9	4.9-9.8	5	1.1	0.4-2.5	79	16.6	13.3-20.7
All	233	5.1	4.5-5.8	39	0.8	0.6-1.2	160	3.5	3.0-4.1

* IR: Incidence rate per 1000 person-years.
† CI: Confidence interval.

Table 2 shows that the incidence rate increased with age until the age of 85 for all subtypes of stroke. After age 85, the proportion of unspecified strokes became very high and estimates of the frequency of subtypes of stroke less reliable. The proportion of hospitalized strokes decreased with age in both sexes (Figure 1).

Figure 1

Incidence rate per 1000 person-years for stroke and hospitalized stroke in relation to age in men and in women.

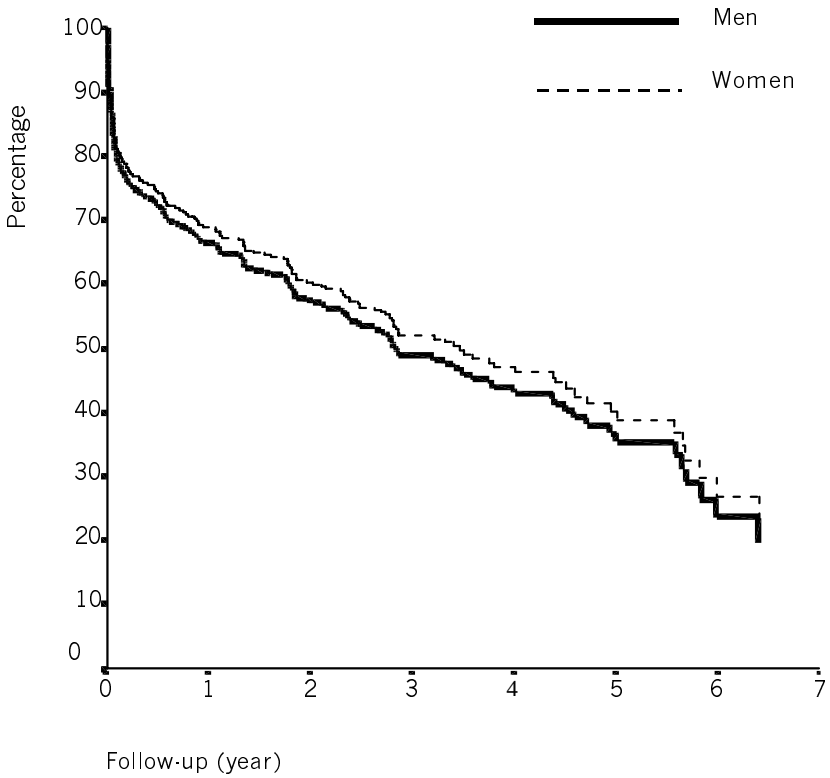

Figure 2

Cumulative survival after stroke according to gender, adjusted for age at stroke.

Case fatality

The overall 28-days case fatality rate was 32.5% for all strokes, 12.4% for cerebral infarctions and 33.3% for cerebral hemorrhages. Corresponding 52-weeks case fatality rates were 40.4, 23.6 and 62.9%. We observed no difference in survival after stroke between men and women (figure 2). Case fatality rates of stroke, cerebral infarction and intracerebral hemorrhage increased with age (table 3).

Table 3

28-Days case-fatality for stroke, subtypes of stroke and hospitalized strokes.

Age	Stroke		Cerebral infarction		Intracerebral hemorrhage		Hospitalized stroke	
	Number/ total	% fatal	Number/ total	% fatal	Number/ total	% fatal	Number/ total	% fatal
55-64	2/22	9.0	0/17	0.0	1/2	50.0	2/16	12.5
65-74	15/108	13.9	5/72	6.9	1/10	10.0	12/76	15.8
75-84	33/105	31.4	15/111	13.5	8/22	36.4	27/119	22.7
85+	61/117	52.1	9/33	27.2	3/5	60.0	22/45	48.9
All	111/342	32.5	29/233	12.4	13/39	33.3	63/256	24.6

Lifetime risk of stroke

Figure 3 shows survival and stroke-free survival for 55 and 75 years old participants as observed in the total study cohort. The areas between the survival and stroke free survival curves represent the average time subjects live after a stroke. The area comprised 3.45 (95% CI 2.93-3.97) years for men and 3.12 (95% CI 2.69-3.55) years for women aged 55 years. Corresponding figures for persons aged 75 years were 2.16 (95% CI 1.59-2.73) and 2.10 (95% CI 1.63-2.57) years, respectively. Table 4 shows the cumulative incidence of stroke over time for men and women at different ages. The absolute risk of getting a stroke is nearly similar for men and women in all age categories, reflecting higher incidence rates, but shorter life expectancy in men as compared to women.

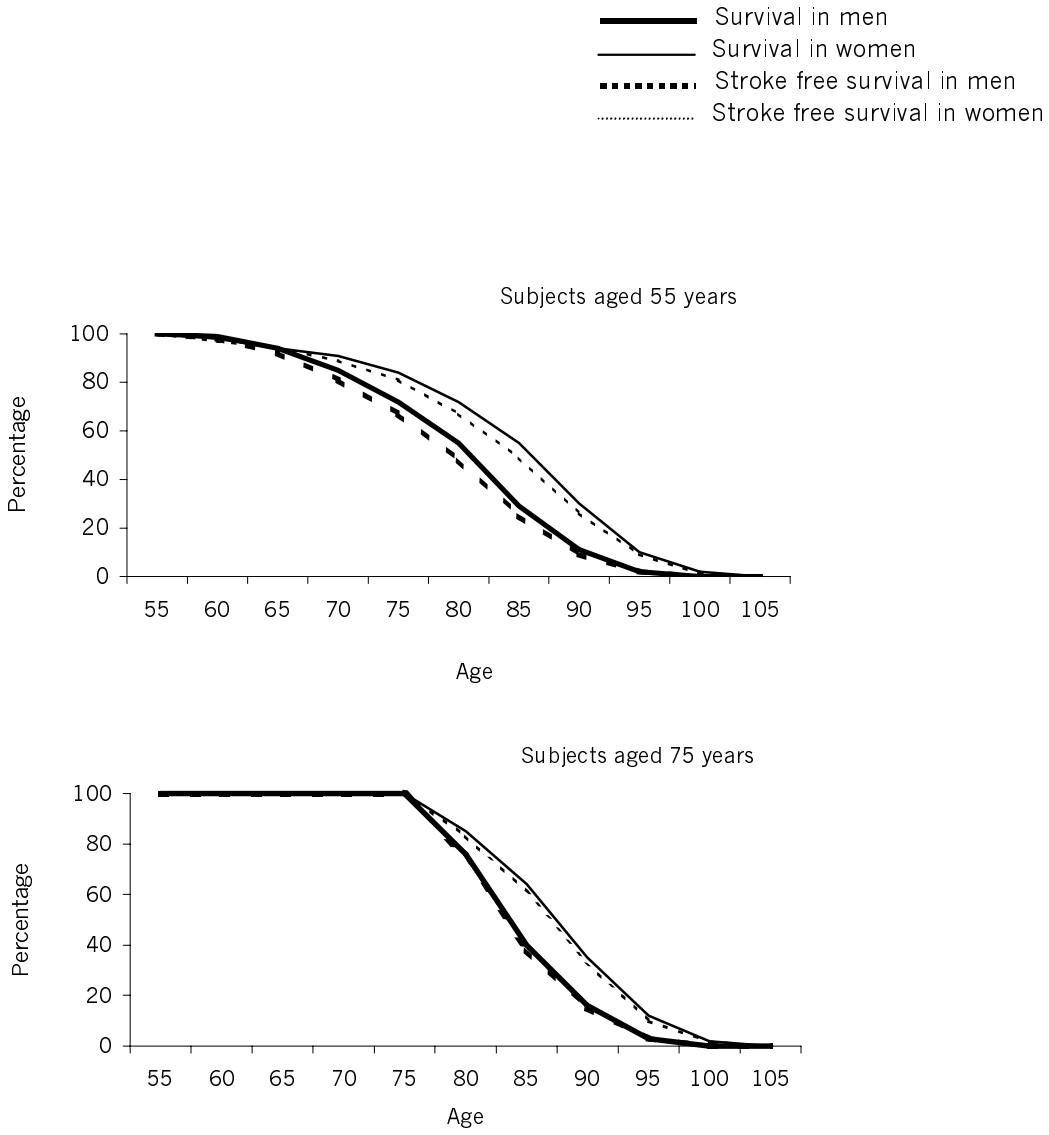

Figure 3
Survival and stroke-free survival according to age and gender.

Table 4

Period and lifetime risk of stroke for 55, 65, 75 and 85 years old men and women taking competing risk of death into account.

Age	Period risk (%)								Lifetime risk
	5yr	10yr	15 yr	20 yr	25 yr	30 yr	35 yr	40 yr	
Men									
55	0.7	1.8	4.8	8.5	13.7	17.3	19.3	20.2	20.5
65	2.2	6.0	11.8	15.9	18.2	19.1	19.4		19.4
75	6.9	12.5	15.7	17.0	17.4				17.4
85	6.0	8.3	9.0						9.0
Women									
55	0.6	1.7	2.9	5.6	10.7	14.2	18.1	20.3	21.0
65	1.1	3.9	9.4	13.1	17.2	19.6	20.3	20.4	20.4
75	5.3	9.6	14.4	17.1	17.9	18.0	18.0		18.0
85	6.8	11.1	12.4	12.6					12.6

DISCUSSION

We found that the incidence rate of stroke was higher in men than in women over the entire age range. Nevertheless, as a net result of a shorter life expectancy and a higher incidence rate of stroke in men as compared to women, lifetime risks of stroke in men and women were similar at different ages.

Case ascertainment

Studies on incidence of stroke are vulnerable to selection bias. Although our participation rate was high (78%), it is conceivable that non-participants had a higher risk profile for stroke. Consequently, our incidence figures most likely are conservative estimates of the true population incidence of stroke. Complete and accurate case ascertainment is crucial for the validity of the results of an incidence study. The prospective study design, standard definitions, defined study population and multiple methods of case finding were in accordance with core criteria proposed by Malmgren and colleagues.[14] Furthermore, the work-up and characteristics of stroke in the present study fulfill criteria for optimal case ascertainment defined by researchers from the WHO MONICA stroke study.[15] The follow-up was complete for all participants and we used extensive and

overlapping case finding methods to reduce selection bias in follow-up. The proportion of non-hospitalized strokes in our study was 39%. This is in accordance with the proportion (46%) found by the Oxfordshire Stroke Project.[16] Rotterdam with nearly 700,000 inhabitants did not have a specific stroke admission strategy during the study period. The low admission rate was mainly explained by a shortage of hospital beds and by the fact that Stroke Units and Stroke Services were not yet widespread available and thrombolytic therapy not yet an established treatment. A weakness of our study that results from the fact that not all strokes were hospitalized is the relatively low proportion of CT or MRI-confirmed strokes. We observed that the proportion of unspecified strokes increased with age. Restriction to only neuro-imaging confirmed strokes would have led to an underestimation of the incidence, especially in the very old. Since only 75% of the intracerebral hemorrhages were confirmed by CT or MRI, the overall results on hemorrhages should be regarded carefully. Almost 95% of the cerebral infarctions were CT confirmed. Therefore, we feel that the overall figures on cerebral infarctions are reliable. However, incidence figures of stroke subtypes in the very old are less reliable.

Stroke incidence studies

One hospital-based study in the Netherlands (1978-1980) reported comparable, though slightly higher incidence rates of stroke than the present study, but did not analyze the very old.[17] Possibly the incidence has declined during the time interval between both studies. The incidence rates found in our study are in accordance with the results from other studies in Europe, although some studies reported lower incidence rates in subjects >85 years.[16,18-24] Studies in Eastern Europe reported higher incidence rates in subjects younger than 85 years, and lower rates in subjects older than 85.[25-28] Incidence rates in Eastern European countries are known to be higher than in Western Europe. The lower rates in the very old in other studies might be explained by under-report of stroke in the elderly, as we have shown that the proportion of hospitalized strokes declines with age. Incidence rates of stroke in subjects with a European background in Auckland, New Zealand (1992) were higher than in the Rotterdam Study.[29] However, the North East Melbourne Stroke Incidence Study (1996-1997) reported similar rates.[30] In our study the survival after stroke was equal in men and women. Most other studies did not find gender differences either.[29,31-34] We observed an increasing case fatality with age, and a higher case fatality of

cerebral hemorrhages as compared to cerebral infarctions. These results are in accordance with results from other studies.[21,29,33,35,36]

Lifetime risk of stroke

Our calculations of lifetime risks of stroke were based on the assumption that characteristics of the cohort remain constant over time. They showed that although the incidence rate of stroke in men was higher than in women, the lifetime risk to get a stroke was similar when we took the competing risk of dying into account. The higher incidence rate of stroke is counterbalanced by shorter survival in men as compared to women. As a consequence, women on average are older when they get a stroke. In conclusion, stroke incidence increases with age, also in the very old. Although incidence rates in men were higher than in women over the entire age range, lifetime risks were similar.

REFERENCES

1. Feinleib M, Ingster L, Rosenberg H, Maurer J, Singh G, Kochanek K. Time trends, cohort effects, and geographic patterns in stroke mortality--United States. Ann Epidemiol 1993;3(5):458-65.
2. Wolf PA, RB DA, MA ON, Sytkowski P, Kase CS, Belanger AJ, Kannel WB. Secular trends in stroke incidence and mortality. The Framingham Study. Stroke 1992;23(11):1551-5.
3. Broderick JP. Stroke trends in Rochester, Minnesota, during 1945 to 1984. Ann Epidemiol 1993;3(5):476-9.
4. Howard G. Decline in stroke mortality in North Carolina. Description, predictions, and a possible underlying cause. Ann Epidemiol 1993;3(5):488-92.
5. Tuomilehto J, Sarti C, Torppa J, Salmi K, Puska P. Trends in stroke mortality and incidence in Finland in the 1970s and 1980s. Ann Epidemiol 1993;3(5):519-23.
6. Bonita R. Stroke trends in Australia and New Zealand: mortality, morbidity, and risk factors. Ann Epidemiol 1993;3(5):529-33.
7. Bonita R, Beaglehole R. The enigma of the decline in stroke deaths in the United States: the search for an explanation. Stroke 1996;27(3):370-2.
8. Bonita R. Epidemiology of stroke. Lancet 1992;339(8789):342-4.
9. Bonita R, Beaglehole R. Monitoring stroke. An international challenge. Stroke 1995;26(4):541-2.
10. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol 1991;7(4):403-22.
11. Bots ML, Looman SJ, Koudstaal PJ, Hofman A, Hoes AW, Grobbee DE. Prevalence of stroke in the general population. The Rotterdam Study. Stroke 1996;27(9):1499-501.

12. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 1997;96(5):1432-7.
13. Kalbfleisch JD, Prentice RL. The statistical analysis of failure time data. New York: John Wiley & Sons, Inc, 1980.
14. Malmgren R, Warlow C, Bamford J, Sandercock P. Geographical and secular trends in stroke incidence. Lancet 1987;2(8569):1196-200.
15. Asplund K, Bonita R, Kuulasmaa K, Rajakangas AM, Schaedlich H, Suzuki K, Thorvaldsen P, Tuomilehto J. Multinational comparisons of stroke epidemiology. Evaluation of case ascertainment in the WHO MONICA Stroke Study. World Health Organization Monitoring Trends and Determinants in Cardiovascular Disease. Stroke 1995;26(3):355-60.
16. Bamford J, Sandercock P, Dennis M, Warlow C, Jones L, McPherson K, Vessey M, Fowler G, Molyneux A, Hughes T, et al. A prospective study of acute cerebrovascular disease in the community: the Oxfordshire Community Stroke Project 1981-86. 1. Methodology, demography and incident cases of first-ever stroke. J Neurol Neurosurg Psychiatry 1988;51(11):1373-80.
17. Herman B, Leyten AC, van Luijk JH, Frenken CW, Op de Coul AA, Schulte BP. Epidemiology of stroke in Tilburg, the Netherlands. The population- based stroke incidence register: 2. Incidence, initial clinical picture and medical care, and three-week case fatality. Stroke 1982;13(5):629-34.
18. Wolfe CD, Giroud M, Kolominsky-Rabas P, Dundas R, Lemesle M, Heuschmann P, Rudd A. Variations in stroke incidence and survival in 3 areas of Europe. European Registries of Stroke (EROS) Collaboration. Stroke 2000;31(9):2074-9.
19. Kolominsky-Rabas PL, Sarti C, Heuschmann PU, Graf C, Siemonsen S, Neundoerfer B, Katalinic A, Lang E, Gassmann KG, von Stockert TR. A prospective community-based study of stroke in Germany--the Erlangen Stroke Project (ESPro): incidence and case fatality at 1, 3, and 12 months. Stroke 1998;29(12):2501-6.
20. Ellekjaer H, Holmen J, Indredavik B, Terent A. Epidemiology of stroke in Innherred, Norway, 1994 to 1996. Incidence and 30-day case-fatality rate. Stroke 1997;28(11):2180-4.
21. Ricci S, Celani MG, La Rosa F, Vitali R, Duca E, Ferraguzzi R, Paolotti M, Seppoloni D, Caputo N, Chiurulla C, et al. SEPIVAC: a community-based study of stroke incidence in Umbria, Italy. J Neurol Neurosurg Psychiatry 1991;54(8):695-8.
22. Laurora G, Cesarone MR, De Sanctis MT, Incandela L, Belcaro G. Delayed arteriosclerosis progression in high risk subjects treated with mesoglycan. Evaluation of intima-media thickness. J Cardiovasc Surg (Torino) 1993;34(4):313-8.
23. Di Carlo A, Launer LJ, Breteler MM, Fratiglioni L, Lobo A, Martinez-Lage J, Schmidt R, Hofman A. Frequency of stroke in Europe: A collaborative study of population- based cohorts. ILSA Working Group and the Neurologic Diseases in the Elderly Research Group. Italian Longitudinal Study on Aging. Neurology 2000;54(11):S28-33.
24. Thorvaldsen P, Davidsen M, Bronnum-Hansen H, Schroll M. Stable stroke occurrence despite incidence reduction in an aging population: stroke trends in the danish monitoring trends and determinants in cardiovascular disease (MONICA) population. Stroke 1999;30(12):2529-34.
25. Korv J, Roose M, Kaasik AE. Changed incidence and case-fatality rates of first-ever stroke between 1970 and 1993 in Tartu, Estonia. Stroke 1996;27(2):199-203.
26. Feigin VL, Wiebers DO, Whisnant JP, WM OF. Stroke incidence and 30-day case-fatality rates in Novosibirsk, Russia, 1982 through 1992. Stroke 1995;26(6):924-9.

27. Czlonkowska A, Ryglewicz D, Weissbein T, Baranska-Gieruszczak M, Hier DB. A prospective community-based study of stroke in Warsaw, Poland. Stroke 1994;25(3):547-51.
28. Eisenblatter D, Heinemann L, Classen E. Community-based stroke incidence trends from the 1970s through the 1980s in East Germany. Stroke 1995;26(6):919-23.
29. Bonita R, Broad JB, Beaglehole R. Ethnic differences in stroke incidence and case fatality in Auckland, New Zealand. Stroke 1997;28(4):758-61.
30. Thrift AG, Dewey HM, Macdonell RA, McNeil JJ, Donnan GA. Stroke incidence on the east coast of Australia: the North East Melbourne Stroke Incidence Study (NEMESIS). Stroke 2000;31(9):2087-92.
31. Vemmos KN, Bots ML, Tsibouris PK, Zis VP, Takis CE, Grobbee DE, Stamatelopoulos S. Prognosis of stroke in the south of greece: 1 year mortality, functional outcome and its determinants: the arcadia stroke registry. J Neurol Neurosurg Psychiatry 2000;69(5):595-600.
32. Truelsen T, Bonita R, Gronbaek M, Sehnohr P, Boysen G. Stroke incidence and case fatality in two populations: the Auckland Stroke Study and the Copenhagen City Heart Study. Neuroepidemiology 1998;17(3):132-8.
33. Bonita R, Broad JB, Beaglehole R. Changes in stroke incidence and case-fatality in Auckland, New Zealand, 1981-91. Lancet 1993;342(8885):1470-3.
34. Lauria G, Gentile M, Fassetta G, Casetta I, Agnoli F, Andreotta G, Barp C, Caneve G, Cavallaro A, Cielo R, et al. Incidence and prognosis of stroke in the Belluno province, Italy. First- year results of a community-based study. Stroke 1995;26(10):1787-93.
35. Vemmos KN, Bots ML, Tsibouris PK, Zis VP, Grobbee DE, Stranjalis GS, Stamatelopoulos S. Stroke incidence and case fatality in southern Greece: the Arcadia stroke registry. Stroke 1999;30(2):363-70.
36. Brown RD, Whisnant JP, Sicks JD, O'Fallon WM, Wiebers DO. Stroke incidence, prevalence, and survival: secular trends in Rochester, Minnesota, through 1989. Stroke 1996;27(3):373-80.

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.

References

1. Yao ST, Hobbs JT, Irvine WT. Ankle systolic pressure measurements in arterial disease affecting the lower extremities. Br J Surg. 1969;56:676-9.
2. Fowkes FG, Housley E, Macintyre CC, Prescott RJ, Ruckley CV. Variability of ankle and brachial systolic pressures in the measurement of atherosclerotic peripheral arterial disease. J Epidemiol Community Health. 1988;42:128-33.
3. Fowkes FG, Housley E, Cawood EH, Macintyre CC, Ruckley CV, Prescott RJ. Edinburgh Artery Study: prevalence of asymptomatic and symptomatic peripheral arterial disease in the general population. Int J Epidemiol. 1991;20:384-92.
4. Newman AB, Siscovick DS, Manolio TA, Polak J, Fried LP, Borhani NO, Wolfson SK. Ankle-arm index as a marker of atherosclerosis in the Cardiovascular Health Study. Cardiovascular Heart Study (CHS) Collaborative Research Group. Circulation. 1993;88:837-45.
5. Leng GC, Fowkes FG, Lee AJ, Dunbar J, Housley E, Ruckley CV. Use of ankle brachial pressure index to predict cardiovascular events and death: a cohort study. Bmj. 1996;313:1440-4.
6. Newman AB, Shemanski L, Manolio TA, Cushman M, Mittelmark M, Polak JF, Powe NR, Siscovick D. Ankle-arm index as a predictor of cardiovascular disease and mortality in the Cardiovascular Health Study. The Cardiovascular Health Study Group. Arterioscler Thromb Vasc Biol. 1999;19:538-45.
7. Tsai AW, Folsom AR, Rosamond WD, Jones DW. Ankle-brachial index and 7-year ischemic stroke incidence: the ARIC study. Stroke. 2001;32:1721-4.
8. Meijer T. Peripheral arterial disease in the elderly. In: Epidemiology & Biostatistics. Rotterdam: Erasmus Medical Centre Rotterdam; 1999:135.
9. Abbott RD, Rodriguez BL, Petrovitch H, Yano K, Schatz IJ, Popper JS, Masaki KH, Ross GW, Curb JD. Ankle-brachial blood pressure in elderly men and the risk of stroke: the Honolulu Heart Program. J Clin Epidemiol. 2001;54:973-8.
10. Bots ML, Hofman A, de Bruyn AM, de Jong PT, Grobbee DE. Isolated systolic hypertension and vessel wall thickness of the carotid artery. The Rotterdam Elderly Study. Arterioscler Thromb. 1993;13:64-9.
11. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation. 1997;96:1432-7.
12. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK, Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med. 1999;340:14-22.
13. Chambless LE, Folsom AR, Clegg LX, Sharrett AR, Shahar E, Nieto FJ, Rosamond WD, Evans G. Carotid wall thickness is predictive of incident clinical stroke: the Atherosclerosis Risk in Communities (ARIC) study. Am J Epidemiol. 2000;151:478-87.
14. Sabetai MM, Tegos TJ, Nicolaides AN, El-Atrozy TS, Dhanjil S, Griffin M, Belcaro G, Geroulakos G. Hemispheric symptoms and carotid plaque echomorphology. J Vasc Surg. 2000;31:39-49.
15. Golledge J, Cuming R, Ellis M, Davies AH, Greenhalgh RM. Carotid plaque characteristics and presenting symptom. Br J Surg. 1997;84:1697-701.
16. Gomez RG. Carotid plaque morphology and risk for stroke. Stroke. 1990;21:148-151.
17. Polak JF, Shemanski L, O'Leary DH, Lefkowitz D, Price TR, Savage PJ, Brant WE, Reid C. Hypoechoic plaque at US of the carotid artery: an independent risk factor for incident

stroke in adults aged 65 years or older. Cardiovascular Health Study. Radiology. 1998;208:649-54.
18. Mathiesen EB, Bonaa KH, Joakimsen O. Echolucent plaques are associated with high risk of ischemic cerebrovascular events in carotid stenosis : the tromso study. Circulation. 2001;103:2171-5.
19. Gronholdt ML, Nordestgaard BG, Schroeder TV, Vorstrup S, Sillesen H. Ultrasonic echolucent carotid plaques predict future strokes. Circulation. 2001;104:68-73.
20. Tenenbaum A, Fisman EZ, Schneiderman J, Stroh CI, Shemesh J, Schwammenthal E, Vered Z, Motro M. Disrupted mobile aortic plaques are a major risk factor for systemic embolism in the elderly. Cardiology. 1998;89:246-51.
21. Cohen A, Tzourio C, Bertrand B, Chauvel C, Bousser MG, Amarenco P. Aortic plaque morphology and vascular events: a follow-up study in patients with ischemic stroke. FAPS Investigators. French Study of Aortic Plaques in Stroke. Circulation. 1997;96:3838-41.
22. Iribarren C, Sidney S, Sternfeld B, Browner WS. Calcification of the aortic arch: risk factors and association with coronary heart disease, stroke, and peripheral vascular disease. Jama. 2000;283:2810-5.
23. Mitusch R, Doherty C, Wucherpfennig H, Memmesheimer C, Tepe C, Stierle U, Kessler C, Sheikhzadeh A. Vascular events during follow-up in patients with aortic arch atherosclerosis. Stroke. 1997;28:36-9.
24. Sen S, Wu K, McNamara R, Lima J, Piantadosi S, Oppenheimer SM. Distribution, severity and risk factors for aortic atherosclerosis in cerebral ischemia. Cerebrovasc Dis. 2000;10:102-9.
25. Rundek T, Di Tullio MR, Sciacca RR, Titova IV, Mohr JP, Homma S, Sacco RL. Association between large aortic arch atheromas and high-intensity transient signals in elderly stroke patients. Stroke. 1999;30:2683-6.
26. Rumberger JA, Simons DB, Fitzpatrick LA, Sheedy PF, Schwartz RS. Coronary artery calcium area by electron-beam computed tomography and coronary atherosclerotic plaque area. A histopathologic correlative study. Circulation. 1995;92:2157-62.
27. Vliegenthart R, Hollander M, Breteler MMB, van der Kuip DAM, Hofman A, Oudkerk M, Witteman JCM. Stroke is associated with coronary calcification as detected on electron-beam computed tomography: The Rotterdam Coronary Calcification Study. In press.
28. Ekpo EB, Ashworth IN, Fernando MU, White AD, Shah IU. Prevalence of mixed hypertension, isolated systolic hypertension and isolated diastolic hypertension in the elderly population in the community. J Hum Hypertens. 1994;8:39-43.
29. Aguero Torres H, Fratiglioni L, Lindberg J, Winblad B. Hypertension in the elderly population: prevalence data from an urban area in Sweden. Aging (Milano). 1994;6:249-55.
30. Reneman RS, van Merode T, Hick P, Muytjens AM, Hoeks AP. Age-related changes in carotid artery wall properties in men. Ultrasound Med Biol. 1986;12:465-71.
31. Lehmann ED. Terminology for the definition of arterial elastic properties. Pathol Biol (Paris). 1999;47:656-64.
32. Popele van N. Causes and consequences of arterial stiffness. An epidemiological approach. In: Department of Epidemiology & Biostatistics. Rotterdam: Erasmus Medical Center Rotterdam; 2000:185.
33. Lee RT, Kamm RD. Vascular mechanics for the cardiologist. J Am Coll Cardiol. 1994;23:1289-95.
34. Lehmann ED, Hopkins KD, Jones RL, Rudd AG, Gosling RG. Aortic distensibility in patients with cerebrovascular disease. Clin Sci (Colch). 1995;89:247-53.

35. Valton L, Larrue V, le Traon AP, Massabuau P, Geraud G. Microembolic signals and risk of early recurrence in patients with stroke or transient ischemic attack. Stroke. 1998;29:2125-8.
36. Valton L, Larrue V, Pavy Le Traon A, Geraud G. Cerebral microembolism in patients with stroke or transient ischaemic attack as a risk factor for early recurrence. J Neurol Neurosurg Psychiatry. 1997;63:784-7.
37. Serena J, Segura T, Castellanos M, Davalos A. Microembolic signal monitoring in hemispheric acute ischaemic stroke: a prospective study. Cerebrovasc Dis. 2000;10:278-82.
38. Lund C, Rygh J, Stensrod B, Sandset PM, Brucher R, Russell D. Cerebral microembolus detection in an unselected acute ischemic stroke population. Cerebrovasc Dis. 2000;10:403-8.
39. Eicke BM, von Lorentz J, Paulus W. Embolus detection in different degrees of carotid disease. Neurol Res. 1995;17:181-4.
40. Baracchini C, Manara R, Ermani M, Meneghetti G. The quest for early predictors of stroke evolution: can TCD be a guiding light? Stroke. 2000;31:2942-7.
41. Bakker SL, de Leeuw FE, Koudstaal PJ, Hofman A, Breteler MM. Cerebral CO2 reactivity, cholesterol, and high-density lipoprotein cholesterol in the elderly. Neurology. 2000;54:987-9.
42. Bakker SL, de Leeuw FE, de Groot JC, Hofman A, Koudstaal PJ, Breteler MM. Cerebral vasomotor reactivity and cerebral white matter lesions in the elderly. Neurology. 1999;52:578-83.
43. Vermeer SE, Koudstaal PJ, Oudkerk M, Hofman A, Breteler MMB. Prevalence and risk factors of silent brain infarcts in the population-based Rotterdam Scan Study. Stroke 2002;33:21-25.
44. Longstreth WT, Jr., Manolio TA, Arnold A, Burke GL, Bryan N, Jungreis CA, Enright PL, O'Leary D, Fried L. Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people. The Cardiovascular Health Study. Stroke. 1996;27:1274-82.
45. Bernick C, Kuller L, Dulberg C, Longstreth WT, Jr., Manolio T, Beauchamp N, Price T. Silent MRI infarcts and the risk of future stroke: the cardiovascular health study. Neurology. 2001;57:1222-9.
46. De Groot JC DLFaBM. White matter changes: frequency and risk factors. In: Pantoni L InDaWA, ed. Clinical and pathophysiological aspects of white matter disease related to cognitive decline and vascular dementa; 1998.
47. Kobayashi S, Okada K, Koide H, Bokura H, Yamaguchi S. Subcortical silent brain infarction as a risk factor for clinical stroke. Stroke. 1997;28:1932-9.
48. Liao D, Cooper L, Cai J, Toole JF, Bryan NR, Hutchinson RG, Tyroler HA. Presence and severity of cerebral white matter lesions and hypertension, its treatment, and its control. The ARIC Study. Atherosclerosis Risk in Communities Study. Stroke. 1996;27:2262-70.
49. de Leeuw FE, de Groot JC, Achten E, Oudkerk M, Ramos LM, Heijboer R, Hofman A, Jolles J, van Gijn J, Breteler MM. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. J Neurol Neurosurg Psychiatry. 2001;70:9-14.
50. Kobayashi S, Okada K, Yamashita K. Incidence of silent lacunar lesion in normal adults and its relation to cerebral blood flow and risk factors. Stroke. 1991;22:1379-83.
51. Bots ML, van Swieten JC, Breteler MM, de Jong PT, van Gijn J, Hofman A, Grobbee DE. Cerebral white matter lesions and atherosclerosis in the Rotterdam Study. Lancet. 1993;341:1232-7.

52. de Leeuw FE, De Groot JC, Oudkerk M, Witteman JC, Hofman A, van Gijn J, Breteler MM. Aortic atherosclerosis at middle age predicts cerebral white matter lesions in the elderly. Stroke. 2000;31:425-9.
53. Silent brain infarction in nonrheumatic atrial fibrillation. EAFT Study Group. European Atrial Fibrillation Trial. Neurology. 1996;46:159-65.
54. Leys D, Englund E, Del Ser T, Inzitari D, Fazekas F, Bornstein N, Erkinjuntti T, Bowler JV, Pantoni L, Parnetti L, De Reuck J, Ferro J, Bogousslavsky J. White matter changes in stroke patients. Relationship with stroke subtype and outcome. Eur Neurol. 1999;42:67-75.
55. Tarvonen-Schroder S, Kurki T, Raiha I, Sourander L. Leukoaraiosis and cause of death: a five year follow up. J Neurol Neurosurg Psychiatry. 1995;58:586-9.
56. Inzitari D, Cadelo M, Marranci ML, Pracucci G, Pantoni L. Vascular deaths in elderly neurological patients with leukoaraiosis. J Neurol Neurosurg Psychiatry. 1997;62:177-81.
57. van Swieten JC, Kappelle LJ, Algra A, van Latum JC, Koudstaal PJ, van Gijn J. Hypodensity of the cerebral white matter in patients with transient ischemic attack or minor stroke: influence on the rate of subsequent stroke. Dutch TIA Trial Study Group. Ann Neurol. 1992;32:177-83.
58. Miyao S, Takano A, Teramoto J, Takahashi A. Leukoaraiosis in relation to prognosis for patients with lacunar infarction. Stroke. 1992;23:1434-8.
59. Inzitari D, Di Carlo A, Mascalchi M, Pracucci G, Amaducci L. The cardiovascular outcome of patients with motor impairment and extensive leukoaraiosis. Arch Neurol. 1995;52:687-91.
60. Touboul PJ, Elbaz A, Koller C, Lucas C, Adrai V, Chedru F, Amarenco P, Investigators f. Common carotid artery intima-media thickness and brain infarction: the etude du profil Genetique de l'Infarctus Cerebral (GENIC) case-control study. Circulation. 2000;102:313-8.

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.

REFERENCES

1. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK, Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med 1999;340(1):14-22.
2. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 1997;96(5):1432-7.
3. Chambless LE, Folsom AR, Clegg LX, Sharrett AR, Shahar E, Nieto FJ, Rosamond WD, Evans G. Carotid wall thickness is predictive of incident clinical stroke: the Atherosclerosis Risk in Communities (ARIC) study. Am J Epidemiol 2000;151(5):478-87.
4. Ay H, Oliveira-Filho J, Buonanno FS, Ezzeddine M, Schaefer PW, Rordorf G, Schwamm LH, Gonzalez RG, Koroshetz WJ. Diffusion-weighted imaging identifies a subset of lacunar infarction associated with embolic source. Stroke 1999;30(12):2644-50.
5. Touboul PJ, Elbaz A, Koller C, Lucas C, Adrai V, Chedru F, Amarenco P, Investigators f. Common carotid artery intima-media thickness and brain infarction : the etude du profil Genetique de l'Infarctus Cerebral (GENIC) case-control study. Circulation 2000;102(3):313-8.
6. Cupini LM, Pasqualetti P, Diomedi M, Vernieri F, Silvestrini M, Rizzato B, Ferrante F, Bernardi G. Carotid artery intima-media thickness and lacunar versus nonlacunar infarcts. Stroke 2002;33(3):689-94.
7. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol 1991;7(4):403-22.
8. Hollander M, Bots ML, Del Sol AI, Koudstaal PJ, Witteman JC, Grobbee DE, Hofman A, Breteler MM. Carotid plaques increase the risk of stroke and subtypes of cerebral infarction in asymptomatic elderly: the Rotterdam study. Circulation 2002;105(24):2872-7.

9. Bots ML, Hofman A, de Bruyn AM, de Jong PT, Grobbee DE. Isolated systolic hypertension and vessel wall thickness of the carotid artery. The Rotterdam Elderly Study. Arterioscler Thromb 1993;13(1):64-9.
10. Bots ML, Hofman A, Grobbee DE. Common carotid intima-media thickness and lower extremity arterial atherosclerosis. The Rotterdam Study. Arterioscler Thromb 1994;14(12):1885-91.
11. Bots ML, Mulder PG, Hofman A, van Es GA, Grobbee DE. Reproducibility of carotid vessel wall thickness measurements. The Rotterdam Study. J Clin Epidemiol 1994;47(8):921-30.
12. Kanters SD, Algra A, van Leeuwen MS, Banga JD. Reproducibility of in vivo carotid intima-media thickness measurements: a review. Stroke 1997;28(3):665-71.
13. Bots ML, Looman SJ, Koudstaal PJ, Hofman A, Hoes AW, Grobbee DE. Prevalence of stroke in the general population. The Rotterdam Study. Stroke 1996;27(9):1499-501.
14. Bots ML, van der Wilk EC, Koudstaal PJ, Hofman A, Grobbee DE. Transient neurological attacks in the general population. Prevalence, risk factors, and clinical relevance. Stroke 1997;28(4):768-73.
15. Rose GA, Blackburn H, Gillum RF, Prineas RJ. Cardiovascular survey methods. Geneva: World Health Organization, 1982. 1982.
16. The fifth report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure (JNC V). Arch Intern Med 1993;153(2):154-83.

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).

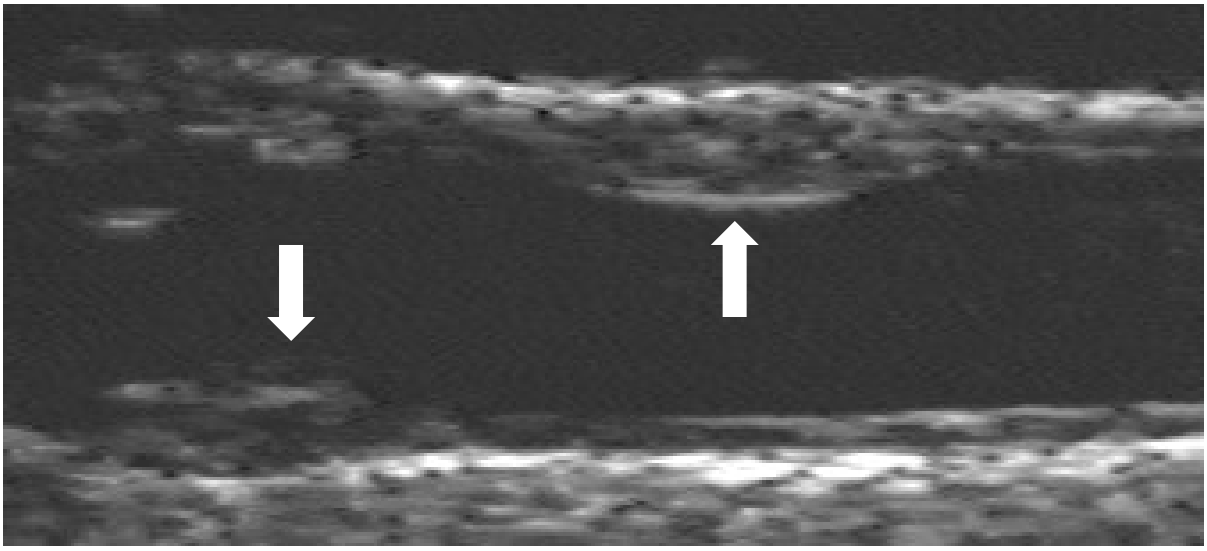

Figure 1
B-mode image of the carotid artery with plaques in the common carotid artery (right arrow) and bifurcation (left arrow).

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.

REFERENCES

1. Sabetai MM, Tegos TJ, Nicolaides AN et al. Hemispheric symptoms and carotid plaque echomorphology. J Vasc Surg. 2000;31:39-49.
2. Golledge J, Cuming R, Ellis M et al. Carotid plaque characteristics and presenting symptom. Br J Surg. 1997;84:1697-701.
3. Gomez RG. Carotid plaque morphology and risk for stroke. Stroke. 1990;21:148-151.
4. Polak JF, Shemanski L, O'Leary DH et al. Hypoechoic plaque at US of the carotid artery: an independent risk factor for incident stroke in adults aged 65 years or older. Cardiovascular Health Study. Radiology. 1998;208:649-54.

5. Rothwell PM. Carotid artery disease and the risk of ischaemic stroke and coronary vascular events. Cerebrovasc Dis. 2000;10:21-33.
6. Nagai Y, Kitagawa K, Sakaguchi M et al. Significance of earlier carotid atherosclerosis for stroke subtypes. Stroke. 2001;32:1780-5.
7. Dempsey RJ, Diana AL, Moore RW. Thickness of carotid artery atherosclerotic plaque and ischemic risk. Neurosurgery. 1990;27:343-8.
8. Manolio TA, Burke GL, O'Leary DH et al. Relationships of cerebral MRI findings to ultrasonographic carotid atherosclerosis in older adults : the Cardiovascular Health Study. CHS Collaborative Research Group. Arterioscler Thromb Vasc Biol. 1999;19:356-65.
9. Handa N, Matsumoto M, Maeda H et al. Ischemic stroke events and carotid atherosclerosis. Results of the Osaka Follow-up Study for Ultrasonographic Assessment of Carotid Atherosclerosis (the OSACA Study). Stroke. 1995;26:1781-6.
10. Hofman A, Grobbee DE, de Jong PT et al. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-22.
11. Bots ML, Hofman A, De Jong PT et al. Common carotid intima-media thickness as an indicator of atherosclerosis at other sites of the carotid artery. The Rotterdam Study. Ann Epidemiol. 1996;6:147-53.
12. Bots ML, Breslau PJ, Briet E et al. Cardiovascular determinants of carotid artery disease. The Rotterdam Elderly Study. Hypertension. 1992;19:717-20.
13. Taylor DC, Strandness DE, Jr. Carotid artery duplex scanning. J Clin Ultrasound. 1987;15:635-44.
14. Bots ML, Looman SJ, Koudstaal PJ et al. Prevalence of stroke in the general population. The Rotterdam Study. Stroke. 1996;27:1499-501.
15. Bots ML, van der Wilk EC, Koudstaal PJ et al. Transient neurological attacks in the general population. Prevalence, risk factors, and clinical relevance. Stroke. 1997;28:768-73.
16. Rose GA, Blackburn H, Gillum RF et al. Cardiovascular survey methods. Geneva: World Health Organization, 1982. 1982.
17. Rothman KJ, Greenland S. Modern Epidemiology. Philadelphia: Lippincott-Raven Publushers; 1998.
18. Adams HP, Jr., Bendixen BH, Kappelle LJ et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke. 1993;24:35-41.
19. Gronholdt ML, Nordestgaard BG, Schroeder TV et al. Ultrasonic echolucent carotid plaques predict future strokes. Circulation. 2001;104:68-73.
20. Hallerstam S, Carlstrom C, Zetterling M et al. Carotid atherosclerosis in relation to symptoms from the Territory supplied by the carotid artery. Eur J Vasc Endovasc Surg. 2000;19:356-61.
21. Ay H, Oliveira-Filho J, Buonanno FS et al. Diffusion-weighted imaging identifies a subset of lacunar infarction associated with embolic source. Stroke. 1999;30:2644-50.

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.

REFERENCES

1. Bots ML, Hoes AW, Hofman A, et al. Cross-sectionally assessed carotid intima-media thickness relates to long-term risk of stroke, coronary heart disease and death as estimated by available risk functions. J Intern Med. 1999;245:269-276.
2. Bots ML, Hoes AW, Koudstaal PJ, et al. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation. 1997;96:1432-1437.
3. Bots ML, Looman SJ, Koudstaal PJ, et al. Prevalence of stroke in the general population. The Rotterdam Study. Stroke. 1996;27:1499-1501.
4. Inzitari D, Eliasziw M, Gates P, et al. The causes and risk of stroke in patients with asymptomatic internal- carotid-artery stenosis. North American Symptomatic Carotid Endarterectomy Trial Collaborators. N Engl J Med. 2000;342:1693-1700.
5. Rothwell PM, Gibson R, Warlow CP. Interrelation between plaque surface morphology and degree of stenosis on carotid angiograms and the risk of ischemic stroke in patients with symptomatic carotid stenosis. On behalf of the European Carotid Surgery Trialists' Collaborative Group. Stroke. 2000;31:615-621.
6. Leng GC, Fowkes FG, Lee AJ, et al. Use of ankle brachial pressure index to predict cardiovascular events and death: a cohort study. BMJ. 1996;313:1440-1444.
7. Zheng ZJ, Sharrett AR, Chambless LE, et al. Associations of ankle-brachial index with clinical coronary heart disease, stroke and preclinical carotid and popliteal atherosclerosis: the Atherosclerosis Risk in Communities (ARIC) Study. Atherosclerosis. 1997;131:115-125.
8. Touboul PJ, Elbaz A, Koller C, et al. Common carotid artery intima-media thickness and brain infarction : the Etude du Profil Genetique de l'Infarctus Cerebral (GENIC) case-control study. The GENIC Investigators. Circulation. 2000;102:313-318.
9. Rumberger JA, Simons DB, Fitzpatrick LA, et al. Coronary artery calcium area by electron-beam computed tomography and coronary atherosclerotic plaque area. A histopathologic correlative study. Circulation. 1995;92:2157-2162.
10. Arad Y, Spadaro LA, Goodman K, et al. Predictive value of electron beam computed tomography of the coronary arteries. 19-month follow-up of 1173 asymptomatic subjects. Circulation. 1996;93:1951-1953.
11. Arad Y, Spadaro LA, Goodman K, et al. Prediction of coronary events with electron beam computed tomography. J Am Coll Cardiol. 2000;36:1253-1260.
12. Detrano RC, Wong ND, Doherty TM, et al. Coronary calcium does not accurately predict near-term future coronary events in high-risk adults Circulation. 1999;99:2633-2638.

13. Wong ND, Hsu JC, Detrano RC, et al. Coronary artery calcium evaluation by electron beam computed tomography and its relation to new cardiovascular events. Am J Cardiol. 2000;86:495-498.
14. Raggi P, Callister TQ, Cooil B, et al. Identification of patients at increased risk of first unheralded acute myocardial infarction by electron-beam computed tomography. Circulation. 2000;101:850-855.
15. Megnien JL, Sene V, Jeannin S, et al. Coronary calcification and its relation to extracoronary atherosclerosis in asymptomatic hypercholesterolemic men. The PCV METRA Group. Circulation. 1992;85:1799-1807.
16. Simon A, Giral P, Levenson J. Extracoronary atherosclerotic plaque at multiple sites and total coronary calcification deposit in asymptomatic men. Association with coronary risk profile. Circulation. 1995;92:1414-1421.
17. Hofman A, Grobbee DE, de Jong PT, et al. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-422.
18. Agatston AS, Janowitz WR, Hildner FJ, et al. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol. 1990;15:827-832.
19. World Health Organization. International Statistical Classification of Diseases and Related Health Problems, tenth Revision. 1992;
20. Van Gent CM, van der Voort HA, de Bruyn AM, et al. Cholesterol determinations. A comparative study of methods with special reference to enzymatic procedures. Clin Chim Acta. 1977;75:243-251.
21. Norris JW and Zhu CZ. Silent stroke and carotid stenosis. Stroke. 1992;23:483-485.
22. Nicolaides AN. Asymptomatic carotid stenosis and risk of stroke. Identification of a high risk group (ACSRS). A natural history study. Int Angiol. 1995;14:21-23.

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.

REFERENCES

1. Stefanadis C, Wooley CF, Bush CA, et al. Aortic distensibility abnormalities in coronary artery disease. Am J Cardiol. 1987;59:1300-1304.
2. Hirai T, Sasayama S, Kawasaki T, et al. Stiffness of systemic arteries in patients with myocardial infarction. A noninvasive method to predict severity of coronary atherosclerosis. Circulation. 1989;80:78-86.
3. Gatzka CD, Cameron JD, Kingwell BA, et al. Relation between coronary artery disease, aortic stiffness, and left ventricular structure in a population sample. Hypertension. 1998;32:575-578.
4. Blacher J, Pannier B, Guerin AP, et al. Carotid arterial stiffness as a predictor of cardiovascular and all- cause mortality in end-stage renal disease. Hypertension. 1998;32:570-574.
5. Blacher J, Guerin AP, Pannier B, et al. Impact of aortic stiffness on survival in end-stage renal disease. Circulation. 1999;99:2434-2439.
6. Laurent T, Boutouyrie P, Asmar R, et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension. 2001;37:1236-1241.

7. Lehmann ED, Hopkins KD, Jones RL, et al. Aortic distensibility in patients with cerebrovascular disease. Clin Sci (Colch). 1995;89:247-253.
8. Hofman A, Grobbee DE, de Jong PT, et al. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-422.
9. Asmar R, Benetos A, Topouchian J, et al. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. Hypertension. 1995;26:485-490.
10. Hoeks AP, Brands PJ, Smeets FA, et al. Assessment of the distensibility of superficial arteries. Ultrasound Med Biol. 1990;16:121-128.
11. Reneman RS, van Merode T, Hick P, et al. Age-related changes in carotid artery wall properties in men. Ultrasound Med Biol. 1986;12:465-471.
12. Vogt MT, Wolfson SK, Kuller LH. Lower extremity arterial disease and the aging process: a review. J Clin Epidemiol. 1992;45:529-542.
13. Meijer WT, Hoes AW, Rutgers D, et al. Peripheral arterial disease in the elderly: The Rotterdam Study. Arterioscler Thromb Vasc Biol. 1998;18:185-192.
14. Stefanadis C, Stratos C, Boudoulas H, et al. Distensibility of the ascending aorta: comparison of invasive and non-invasive techniques in healthy men and in men with coronary artery disease. Eur Heart J. 1990;11:990-996.
15. Stefadouros MA, Dougherty MJ, Grossman W, et al. Determination of systemic vascular resistance by a noninvasive technic. Circulation. 1973;47:101-107.
16. Imura T, Yamamoto K, Kanamori K, et al. Non-invasive ultrasonic measurement of the elastic properties of the human abdominal aorta. Cardiovasc Res. 1986;20:208-214.
17. Vaccarino V, Holford TR, Krumholz HM. Pulse pressure and risk for myocardial infarction and heart failure in the elderly. Journal of the American College of Cardiology. 2000;36:130-138.
18. Domanski MJ, Davis BR, Pfeffer MA, et al. Isolated systolic hypertension : prognostic information provided by pulse pressure. Hypertension. 1999;34:375-380.
19. Lee RT, Kamm RD. Vascular mechanics for the cardiologist. Journal of the American College of Cardiology. 1994;23:1289-1295.

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.

REFERENCES

1. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 1997;96(5):1432-7.
2. Polak JF, Shemanski L, O'Leary DH, Lefkowitz D, Price TR, Savage PJ, Brant WE, Reid C. Hypoechoic plaque at US of the carotid artery: an independent risk factor for incident stroke in adults aged 65 years or older. Cardiovascular Health Study. Radiology 1998;208(3):649-54.
3. Tsai AW, Folsom AR, Rosamond WD, Jones DW. Ankle-brachial index and 7-year ischemic stroke incidence: the ARIC study. Stroke 2001;32(8):1721-4.
4. Iribarren C, Sidney S, Sternfeld B, Browner WS. Calcification of the aortic arch: risk factors and association with coronary heart disease, stroke, and peripheral vascular disease. Jama 2000;283(21):2810-5.
5. O'Rourke RA, Brundage BH, Froelicher VF, Greenland P, Grundy SM, Hachamovitch R, Pohost GM, Shaw LJ, Weintraub WS, Winters WL, Jr. American College of Cardiology/American Heart Association Expert Consensus Document on electron-beam computed tomography for the diagnosis and prognosis of coronary artery disease. J Am Coll Cardiol 2000;36(1):326-40.
6. Bots ML, Hofman A, de Bruyn AM, de Jong PT, Grobbee DE. Isolated systolic hypertension and vessel wall thickness of the carotid artery. The Rotterdam Elderly Study. Arterioscler Thromb 1993;13(1):64-9.
7. Hollander M, Bots ML, Del Sol AI, Koudstaal PJ, Witteman JC, Grobbee DE, Hofman A, Breteler MM. Carotid plaques increase the risk of stroke and subtypes of cerebral infarction in asymptomatic elderly: the Rotterdam study. Circulation 2002;105(24):2872-7.
8. Vliegenthart R, Hollander M, Breteler MM, van Der Kuip DA, Hofman A, Oudkerk M, Witteman JC. Stroke Is Associated With Coronary Calcification as Detected by Electron-Beam CT: The Rotterdam Coronary Calcification Study. Stroke 2002;33(2):462-465.
9. Witteman JC, Grobbee DE, Valkenburg HA, van Hemert AM, Stijnen T, Burger H, Hofman A. J-shaped relation between change in diastolic blood pressure and progression of aortic atherosclerosis. Lancet 1994;343(8896):504-7.

10. del Sol AI, Moons KG, Hollander M, Hofman A, Koudstaal PJ, Grobbee DE, Breteler MM, Witteman JC, Bots ML. Is carotid intima-media thickness useful in cardiovascular disease risk assessment? The Rotterdam Study. Stroke 2001;32(7):1532-8.
11. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol 1991;7(4):403-22.
12. Bots ML, Hofman A, De Jong PT, Grobbee DE. Common carotid intima-media thickness as an indicator of atherosclerosis at other sites of the carotid artery. The Rotterdam Study. Ann Epidemiol 1996;6(2):147-53.
13. Meijer WT, Hoes AW, Rutgers D, Bots ML, Hofman A, Grobbee DE. Peripheral arterial disease in the elderly: The Rotterdam Study. Arterioscler Thromb Vasc Biol 1998;18(2):185-92.
14. Hyman JB, Epstein FH. A study of the correlation between roentgoenographic and postmortem calcifications of the aorta. Am Heart J 1954;48:540-3.
15. Bots ML, Looman SJ, Koudstaal PJ, Hofman A, Hoes AW, Grobbee DE. Prevalence of stroke in the general population. The Rotterdam Study. Stroke 1996;27(9):1499-501.
16. Bots ML, van der Wilk EC, Koudstaal PJ, Hofman A, Grobbee DE. Transient neurological attacks in the general population. Prevalence, risk factors, and clinical relevance. Stroke 1997;28(4):768-73.
17. Rose GA, Blackburn H, Gillum RF, Prineas RJ. Cardiovascular survey methods. Geneva: World Health Organization, 1982. 1982.
18. Technical report series 727. Diabetes mellitus. Geneva: W.H.O., 1985.
19. Harrell FE, Jr., Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med 1996;15(4):361-87.
20. Lehmann ED, Hopkins KD, Jones RL, Rudd AG, Gosling RG. Aortic distensibility in patients with cerebrovascular disease. Clin Sci (Colch) 1995;89(3):247-53.
21. Popele van N. Causes and consequences of arterial stiffness. An epidemiological approach. Erasmus Medical Center Rotterdam, 2000.

4

Sex hormones and stroke

4.1 Endogenous sex hormone levels and risk of stroke in the elderly

Summary

Background - There is controversy as to whether sex hormones, and in particular estradiol, are related to the risk of stroke. The relation between endogenous sex hormones and risk of stroke in men and postmenopausal women is not yet clear.

Methods - We analyzed cohort of 6,735 participants from the population-based Rotterdam Study, who were free from previous stroke, reported no hormone therapy and had a blood sample taken at baseline (1990-1993). The cohort was followed for stroke until January 1 1998. A total of 217 strokes occurred. Total and bioavailable estradiol and testosterone were measured in stroke cases and a subcohort of 1,372 random participants. The relation between sex hormones and stroke in men and women was assessed using a case cohort approach and Cox regression.

Findings - Endogenous sex hormone levels were not related to the risk of stroke in women. In men, higher estradiol levels were related to a decreased risk of stroke, although not statistically significant. Higher testosterone levels were related to a lower risk of stroke and cerebral infarction (RR 0.44 (95% CI 0.26-0.77) and 0.39 (95% CI 0.22-0.71) per SD increase in bioavailable testosterone, respectively). A statistically significant interaction was found between current smoking and testosterone. Particularly in male non-smokers, higher testosterone levels were related to a lower risk of stroke.

Interpretation - Endogenous sex hormones are not related to the risk of stroke in postmenopausal women. Higher testosterone levels are related to a lower risk of stroke in men, particularly in those who do not smoke.

Introduction

Sex hormones, and in particular estradiol, may play a role in the differences between men and women in the occurrence of cardiovascular disease. Yet, several studies on estrogen replacement therapy in women failed to report a beneficial effect on stroke.[1,2] The role of endogenous sex hormones in elderly men and women is not yet clear. With ageing, hormone levels decline in both sexes, with women experiencing a rapid decline after menopause and men having a more gradual decrease with age.[3,4] To date, it is unclear whether lower endogenous sex hormone levels are related to an increased risk of stroke. Hospital-based studies have reported decreased levels of testosterone in men with coronary artery disease and acute stroke.[5,6] However, this relationship has not yet been investigated in a population. We hypothesized that a low estradiol level in women, and a low testosterone level in men is related to an increased risk of stroke. We investigated this relationship in an elderly population of elderly men and postmenopausal women.

Methods

Study population

The study was conducted in 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 older were invited to the study. People living in homes for the elderly were also included. 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. Participation rate of those invited to the study was 78% and in total 7,983 subjects participated. 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. For the present analysis, 6,732 persons (2,727 men and 4,005 women) were eligible who had a blood sample taken, had no history of stroke and did not use any hormone therapy at baseline.

Study design

We used a case cohort approach.[8] Among the 6,732 eligible persons, we selected a random subcohort of 1,372 participants, including 647 men and 725 women. All eligible persons were followed-up for stroke until January 1 1998. A total of 217 strokes had occurred. Among these, 51 occurred in the subcohort.

Steroid hormone measurements

We took non-fasting blood samples during the baseline-visit to the research center. Samples were taken between 8.30 AM and 4.00 PM. Platelet-free plasma was obtained by two-stage centrifugation, first for 10 minutes at 1,600 g at 4˚C and then for 30 minutes at 7,000 g. Platelet-free samples were immediately frozen in liquid nitrogen and transferred to the laboratory. At the laboratory the plasma samples were stored at -80°C until hormone assessment. Plasma levels of steroids (testosterone and estradiol) and sex hormone binding globulin (SHBG) were estimated in 12 batches using coated tube or double antibody radioimmunoasays, purchased from Diagnostic Systems Laboratories (Webster, Texas, USA). Because of the relatively small volumes of plasma available, all values reported are single sample estimations. Intra-assay coefficients of variation, determined on basis of duplicate results of internal quality control plasma pools with 3 different levels of each analyte, were below 4% (SHBG), 21% (estradiol) and 13% (testosterone). The results of all batches were normalized by multiplying all concentrations within a batch with a factor, which equalized results for the internal quality control pools. Assays were performed blinded for outcome. Bioavailable estradiol and testosterone (non-SHBG bound) were calculated on the basis of hormone and binding protein levels and respective affinity constants according to the method described by Södergård [9] and Van den Beld et al.[10] Total testosterone and estradiol were measured in 613 and respectively 494 men and in 718 and respectively 587 women. We were able to measure bioavailable testosterone and estradiol in 457 (74.6%) and 331 (67.0%) men, respectively. Corresponding numbers for women were 579 (80.6%) and 438 (74.6%), respectively. Missing hormone values did not differ according to case status.

Assessment of stroke

A previous stroke was assessed during the baseline interview 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.[11] Once subjects enter the Rotterdam Study they are continuously monitored for major cardiovascular events through automated linkage with the files from GP's. 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 GP and by scrutinizing information from hospital discharge records in case of admittance or referral. Information on all possible strokes was reviewed by a neurologist (PJK) who classified the stroke as definite, probable or possible and assessed subtypes, based on neuro-imaging.[12] 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 a reported stroke.

Cardiovascular risk factors

A computerized questionnaire was used to obtain information on current health status, medication use and medical history at baseline, including history of myocardial infarction, coronary bypass surgery (CABG) and coronary angioplasty (PTCA). All reported myocardial infarctions and TIAs were verified by using medical records. History of intermittent claudication and angina pectoris was assessed through the Rose questionnaire.[13] A history of cardiovascular disease was considered to be present if the participant had one of the following conditions: a history of myocardial infarction, TIA, CABG, PTCA, intermittent claudication or angina pectoris. Medication use was classified according to ATC-codes.[14] Functional status was assessed using the Disability Index of the Stanford Health Assessment Questionnaire.[15] Smoking status was assessed during the baseline interview and participants were classified as current, former or never smoker. In women, age at menopause was assessed during the interview. At the research center, height and weight was

measured and Quetelet's body mass index (kg/m^2) was calculated. Diabetes mellitus was defined as use of oral glucose lowering drugs or insulin or post-load serum glucose level higher than 11.0 mmol/L. Ultrasonography of both left and right carotid artery was performed using a 7.5 MHz linear array transducer (ATL UltraMark IV, Advanced Technology Laboratories, Bethel, Washington, U.S.A.). According to the protocol of the Rotterdam Study the mean common carotid intima-media thickness was measured.[16]

Data analysis

We evaluated the relation between endogenous sex hormones and risk of stroke and cerebral infarction with a Cox proportional hazards model, with modification of standard errors, based on robust variance estimates.[17] We used the method according to Barlow[17] in which the subcohort is weighted by the inverse of the sampling fraction from the source population. Men and women were analyzed separately. We examined total and bioavailable (non-SHBG bound) plasma levels of testosterone and estradiol in quintiles. Participants in the lowest quintile were taken as the reference. Subsequently we analyzed estradiol and testosterone as a continuous variable per standard deviation increase. In order to optimally adjust for age, we used age as timescale. Entry time was defined as age at study entry. Censoring age was defined as age at stroke, death or end of the study, whichever came first. We further adjusted for diabetes, history of cardiovascular disease, smoking, blood pressure, medication use, body mass index, disability index, blood pressure and common carotid intima-media thickness. Since smoking influences hormone levels, we performed separate analyses within the non-smokers. Results are presented as rate ratios with corresponding 95% confidence intervals. All analyses were performed using SAS software.

Results

Table 1 shows baseline characteristics of the study population. Mean follow-up time was 4.5 years. A total of 97 strokes occurred in men and 120 in women. Subtyping of stroke in men revealed 55 cerebral infarctions, 12 intracerebral hemorrhages and 30 unspecified strokes. Corresponding figures for women were 58, 10 and 52, respectively. Overall, hormone levels were higher in men than in women (table 1).

Table 1

Baseline characteristics of the subcohort.

	Men (n= 647)	Women (n=725)
Age (years)	69.4 (8.3)	71.5 (9.4)
Body mass index (kg/m^2)	25.7 (3.0)	26.6 (3.8)
Smoking (% current)	28.4	19.5
Systolic blood pressure (mm Hg)	139.2 (21.8)	140.5 (22.3)
Diastolic blood pressure (mm Hg)	75.4 (11.9)	74.0 (11.7)
Diabetes (%)	9.1	7.6
Total estradiol (mean) (pmol/L)	45.0 (23.6)	15.5 (14.4)
Bioavailable estradiol (mean) (pmol/L)	34.5 (18.2)	11.0 (10.7)
Total testosterone (nmol/L)	11.5 (4.0)	1.4 (0.8)
Bioavailable testosterone (nmol/L)	6.8 (2.8)	0.7 (0.4)
History of cardiovascular disease (%)	34.9	19.0
Medication use (number of categories)	1.5 (1.9)	1.9 (2.1)
Mean common carotid IMT (mm)	0.8 (0.1)	0.8 (0.2)
Disability Index	1.2 (0.4)	1.5 (0.6)

Values represent means (SD).

Women

In women, endogenous estradiol and testosterone were not related to the risk of stroke (figures 1 and 2). The results did not change when we used cerebral infarction as outcome. Restriction to non-smokers yielded similar results.

Men

Figure 1 shows that higher estradiol levels were related to a lower risk of stroke in men, although this was not statistically significant (RR per SD increase 0.73 (95% CI 0.42-1.27)). The results for cerebral infarction were similar. Higher levels of testosterone were related to a significantly decreased risk of stroke. Men in the highest two quintiles of bioavailable testosterone had an 11-fold and respectively 5-fold decreased risk of stroke, as compared to the lowest quintile (figure 2). Corresponding relative risks of cerebral infarction were 0.08 (95% CI 0.01-0.45) and 0.15 (95% CI 0.03-0.76), respectively. The risks of stroke and cerebral infarction decreased by respectively 56% and 61% per standard deviation increase in bioavailable testosterone (RR 0.44 (95% CI 0.26-0.77) and 0·39 (95% CI 0.22-0.71), respectively).

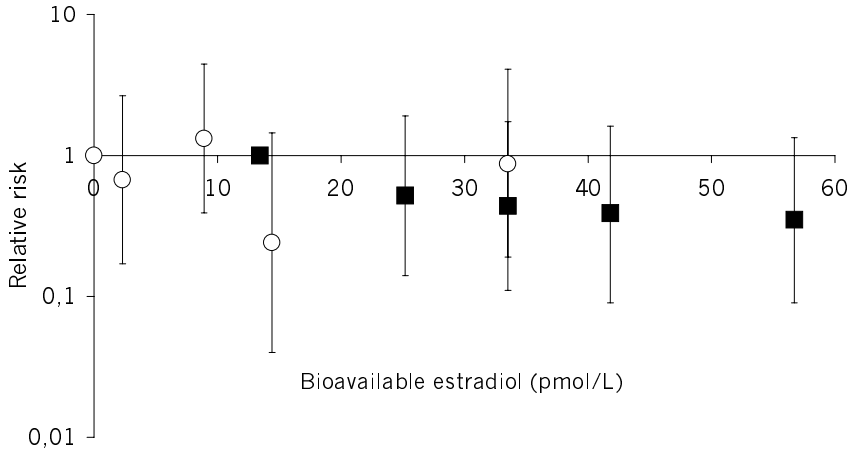

Figure 1

Relative risk of stroke in relation to quintiles of bioavailable estradiol (plotted at its median) in men (black squares) and women (white rounds), adjusted for age, diabetes, systolic and diastolic blood pressure, smoking, body mass index, history of cardiovascular disease, medication use, carotid IMT and disability index.

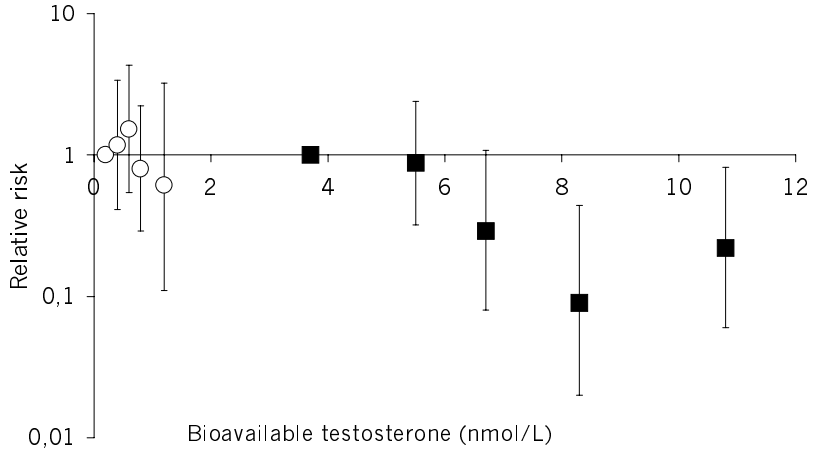

Figure 2

Relative risk of stroke in relation to quintiles of bioavailable testosterone (plotted at its median) in men (black squares) and women (white rounds), adjusted for age, diabetes, systolic and diastolic blood pressure, smoking, body mass index, history of cardiovascular disease, medication use, carotid IMT and disability index.

In men, a statistically significant interaction was found between current smoking and testosterone ($p<0.01$). With restriction to non-smokers, the relationship between testosterone and stroke in men became even stronger, in particular in the highest quintile. Using the same overall cut-off points, men in the fourth and fifth quintile of bioavailable testosterone had respectively 8 fold and 20-fold decreased risk of stroke, as compared to the first quintile (table 2).

Table 2

Risk ratio of stroke in relation to quintiles of testosterone (nmol/L) and estradiol (pmol/L) in non-smokers.

Quintile	Men (n=491)			
	Range	No. of cases	RR*	RR¶
Total testosterone				
Quintile				
1st (reference)	<8.3	19	1.00	1.00
2nd	8.3-10.7	13	0.66 (0.26-1.66)	0.62 (0.20-1.90)
3rd	10.7-12.2	4	0.23 (0.07-0.73)	0.19 (0.04-0.87)
4th	12.2-14.2	3	0·21 (0.05-0.77)	0.12 (0.02-0.71)
5th	>14.2	5	0.39 (0.12-1.27)	0.31 (0.07-1.34)
Per SD increase			0.56 (0.38-0.81)	0.43 (0.25-0.76)
Bioavailable testosterone				
Quintile				
1st (reference)	0-4.71	16	1.00	1.00
2nd	4.71-6.12	10	0.91 (0.36-2.28)	0.91 (0.25-3.26)
3rd	6.13-7.16	8	0.54 (0.20-1.47)	0.31 (0.05-1.90)
4th	7.17-8.66	2	0.17 (0.03-0.82)	0.12 (0.02-0.72)
5th	>8.66	1	0.09 (0.01-0.83)	0·05 (0·00-0·60)
Per SD increase			0.49 (0.32-0.75)	0.32 (0.16-0.64)
Total estradiol				
Quintile				
1st (reference)	<26.8	7	1.00	1.00
2nd	26.8-37.2	7	1.57 (0.44-5.53)	1.52 (0.33-7.01)
3rd	37.2-48.1	7	1.52 (0.44-5.23)	0.99 (0.23-4.32)
4th	48.1-60.3	7	1.32 (0.39-4.48)	0.83 (0.17-4.14)
5th	>60.3	8	1.67 (0.44-6.42)	0.53 (0.11-2.67)
Per SD increase			1.08 (0.73-1.59)	0.72 (0.44-1.18)
Bioavailable estradiol				
1st (reference)	0-18.97	8	1.00	1.00
2nd	18.98-28.91	4	0.67 (0.19-2.37)	0.75 (0.14-3.93)
3rd	28.92-37.55	6	0.80 (0.22-2.89)	0.51 (0.12-2.29)
4th	37.56-47.13	6	0.67 (0.19-2.39)	0.44 (0.07-3.03)
5th	>47.14	6	0.77 (0.19-3.13)	0.30 (0.05-1.79)
Per SD increase			0.85 (0.53-1.34)	0.61 (0.35-1.08)

* Adjusted for age and gender.

¶ Adjusted for age, diabetes, systolic and diastolic blood pressure, smoking, body mass index, history of cardiovascular disease, medication use, carotid IMT and disability index.

In male non-smokers, the risk of stroke significantly decreased by 68% per standard deviation increase of bioavailable testosterone (table 2). The corresponding risk for cerebral infarction decreased by 71% (RR 0.29 (95% CI 0.11-0.75)). Higher estradiol levels decreased the risk of stroke in male non-smokers. The risk of stroke and cerebral infarction decreased by 39% and 50%, respectively. The risk reduction for cerebral infarction was statistically significant (RR 0.50 (95% CI 0.25-0.98)). Within the male smokers, the relative risks of stroke per SD increase in total and free testosterone were 1.23 (0.38-3.95) and 0.82 (0.18-3.75), respectively. Corresponding relative risks for total and free estradiol 1.14 (0.49-2.64) and 0.86 (0.16-4.37), respectively.

DISCUSSION

We found that endogenous estradiol and testosterone levels were not related to the risk of stroke in postmenopausal women. In men, higher testosterone and estradiol levels were related to stroke, particularly in the non-smokers. The strengths of the present study are its prospective and population-based design. Further, the study was large and based on a relatively long follow-up period. A possible limitation is the impaired long-term stability of hormone levels in blood stored at low temperatures. Deviation of hormone levels due to long storage is independent of cases status. Therefore, misclassification of hormone levels would have led to an underestimation of the associations that we found. Another possible limitation is that we had missing values of hormone levels. However, because missingness of hormone levels was independent of case status, incompleteness of hormone assessment probably has not led to a selection bias. The classification of strokes was done blinded for hormone status. Therefore, misclassification of strokes, if present, would have led to an underestimation of the associations.[18]

Sex hormones and stroke in women

We failed to observe an effect from endogenous estradiol and testosterone on the risk of stroke in women. All women in our study were postmenopausal and hormone levels were very low. It is conceivable that only premenopausal levels are effective. To date, there is controversy about the effect of hormone replacement therapy in women on stroke. Despite positive reports,[19-25] a recent case-control study and the Heart and Estrogen-progestin Replacement Study failed to find a protective effect on stroke.[1,2] It has even been reported that

hormone replacement therapy has no beneficial effect on the secondary prevention of stroke.[26] Although endogenous hormone levels are much lower than the levels achieved by hormone substitution, our findings are in agreement with these negative studies.

Sex hormones and stroke in men

Our finding that a higher testosterone level protects against stroke in men, particularly in non-smokers, remained even when we corrected for atherosclerosis, comorbidity and markers of good health. Estrogen showed a similar, though less strong relationship. Hospital-based studies have reported decreased testosterone levels in men with coronary artery disease and acute stroke. Due to the cross-sectional design of these studies, it could not be established whether low testosterone levels preceded or followed the stroke. The underlying mechanisms of this relationship are not yet clear. One possible explanation is that testosterone is involved in vasodilatation and increased blood flow, as was shown by infusion of physiological concentrations of testosterone in coronary heart disease patients.[27] Experimental studies have hypothesized that the dilating effect of testosterone on the vessel wall might be through binding to testosterone receptors, local conversion into estradiol, antioxidant effects or influence on the endothelium.[27-29] Another explanation is that the effect of low testosterone levels is through increase of arterial stiffness. Studies in prostate cancer patients have shown that a low testosterone level due to orchidectomy is related to an increase in arterial stiffness.[30] Alternatively, a low testosterone level could also simply reflect a worse general health. Although we corrected for medication use, history of cardiovascular disease and functional disability, it is possible that we could not optimally adjust for general health. Our finding that higher estradiol levels are also related to lower risks of stroke in men is most likely explained by the fact that 80% of the estradiol originates from peripheral conversion of testosterone into estradiol. Therefore, the relationship with estradiol is most likely a reflection of the testosterone-effect. In recent years, testosterone replacement therapy in men is increasingly being discussed and suggested to have beneficial effects on mood, muscle strength, libido and well being.[31] Our findings suggest that a low testosterone level is a novel risk factor for stroke in men. This finding merits further research on the effect of testosterone on cardiovascular disease. Confirmation is needed before studies on

the protective effect of testosterone replacement therapy on stroke in men are advocated.

REFERENCES

1. Petitti DB, Sidney S, Quesenberry CP, Jr., Bernstein A. Ischemic stroke and use of estrogen and estrogen/progestogen as hormone replacement therapy. Stroke 1998;29(1):23-8.
2. Simon JA, Hsia J, Cauley JA, Richards C, Harris F, Fong J, Barrett-Connor E, Hulley SB. Postmenopausal hormone therapy and risk of stroke: The Heart and Estrogen-progestin Replacement Study (HERS). Circulation 2001;103(5):638-42.
3. Lamberts SW, van den Beld AW, van der Lely AJ. The endocrinology of aging. Science 1997;278(5337):419-24.
4. Ferrini RL, Barrett-Connor E. Sex hormones and age: a cross-sectional study of testosterone and estradiol and their bioavailable fractions in community-dwelling men. Am J Epidemiol 1998;147(8):750-4.
5. English KM, Mandour O, Steeds RP, Diver MJ, Jones TH, Channer KS. Men with coronary artery disease have lower levels of androgens than men with normal coronary angiograms. Eur Heart J 2000;21(11):890-4.
6. Jeppesen LL, Jorgensen HS, Nakayama H, Raaschou HO, Olsen TS, Winther K. Decreased serum testosterone in men with acute ischemic stroke. Arterioscler Thromb Vasc Biol 1996;16(6):749-54.
7. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol 1991;7(4):403-22.
8. Barlow WE, Ichikawa L, Rosner D, Izumi S. Analysis of case-cohort designs. J Clin Epidemiol 1999;52(12):1165-72.
9. Sodergard R, Backstrom T, Shanbhag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol- 17 beta to human plasma proteins at body temperature. J Steroid Biochem 1982;16(6):801-10.
10. van den Beld AW, de Jong FH, Grobbee DE, Pols HA, Lamberts SW. Measures of bioavailable serum testosterone and estradiol and their relationships with muscle strength, bone density, and body composition in elderly men. J Clin Endocrinol Metab 2000;85(9):3276-82.
11. Bots ML, Looman SJ, Koudstaal PJ, Hofman A, Hoes AW, Grobbee DE. Prevalence of stroke in the general population. The Rotterdam Study. Stroke 1996;27(9):1499-501.
12. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 1997;96(5):1432-7.
13. Rose GA, Blackburn H, Gillum RF, Prineas RJ. Cardiovascular survey methods. Geneva: World Health Organization, 1982. 1982.
14. Anonymous. Anatomical Therapeutical Chemical (ATC) Classification Index. Oslo: World Health Organization Collaborating Centre for Drug Statistics Methodology, 1993.
15. Fries JF, Spitz P, Kraines RG, Holman HR. Measurement of patient outcome in arthritis. Arthritis Rheum 1980;23(2):137-45.

16. Bots ML, Hofman A, de Bruyn AM, de Jong PT, Grobbee DE. Isolated systolic hypertension and vessel wall thickness of the carotid artery. The Rotterdam Elderly Study. Arterioscler Thromb 1993;13(1):64-9.
17. Barlow WE. Robust variance estimation for the case-cohort design. Biometrics 1994;50(4):1064-72.
18. Rothman KJ, Greenland S. Modern Epidemiology. Philadelphia: Lippincott-Raven Publushers, 1998.
19. Stampfer MJ, Colditz GA, Willett WC, Manson JE, Rosner B, Speizer FE, Hennekens CH. Postmenopausal estrogen therapy and cardiovascular disease. Ten-year follow-up from the nurses' health study. N Engl J Med 1991;325(11):756-62.
20. Grady D, Rubin SM, Petitti DB, Fox CS, Black D, Ettinger B, Ernster VL, Cummings SR. Hormone therapy to prevent disease and prolong life in postmenopausal women. Ann Intern Med 1992;117(12):1016-37.
21. Falkeborn M, Persson I, Terent A, Adami HO, Lithell H, Bergstrom R. Hormone replacement therapy and the risk of stroke. Follow-up of a population-based cohort in Sweden. Arch Intern Med 1993;153(10):1201-9.
22. Finucane FF, Madans JH, Bush TL, Wolf PH, Kleinman JC. Decreased risk of stroke among postmenopausal hormone users. Results from a national cohort. Arch Intern Med 1993;153(1):73-9.
23. Lindenstrom E, Boysen G, Nyboe J. Lifestyle factors and risk of cerebrovascular disease in women. The Copenhagen City Heart Study. Stroke 1993;24(10):1468-72.
24. Pedersen AT, Lidegaard O, Kreiner S, Ottesen B. Hormone replacement therapy and risk of non-fatal stroke. Lancet 1997;350(9087):1277-83.
25. Grodstein F, Stampfer MJ, Manson JE, Colditz GA, Willett WC, Rosner B, Speizer FE, Hennekens CH. Postmenopausal estrogen and progestin use and the risk of cardiovascular disease. N Engl J Med 1996;335(7):453-61.
26. Viscoli CM, Brass LM, Kernan WN, Sarrel PM, Suissa S, Horwitz RI. A clinical trial of estrogen-replacement therapy after ischemic stroke. N Engl J Med 2001;345(17):1243-9.
27. Webb CM, McNeill JG, Hayward CS, de Zeigler D, Collins P. Effects of testosterone on coronary vasomotor regulation in men with coronary heart disease. Circulation 1999;100(16):1690-6.
28. Hanke H, Lenz C, Hess B, Spindler KD, Weidemann W. Effect of testosterone on plaque development and androgen receptor expression in the arterial vessel wall. Circulation 2001;103(10):1382-5.
29. Ong PJ, Patrizi G, Chong WC, Webb CM, Hayward CS, Collins P. Testosterone enhances flow-mediated brachial artery reactivity in men with coronary artery disease. Am J Cardiol 2000;85(2):269-72.
30. Smith JC, Bennett S, Evans LM, Kynaston HG, Parmar M, Mason MD, Cockcroft JR, Scanlon MF, Davies JS. The effects of induced hypogonadism on arterial stiffness, body composition, and metabolic parameters in males with prostate cancer. J Clin Endocrinol Metab 2001;86(9):4261-7.
31. Tenover JL. Experience with testosterone replacement in the elderly. Mayo Clin Proc 2000;75 Suppl:S77-81; discussion S82.

5
Genetic factors and stroke

5.1 The ACE-gene polymorphism and risk of stroke

Abstract

Background and objectives - It has been suggested that the D-allele of the ACE-gene may be related to stroke. Limited prospective data exist on this relation. We investigated the relation between the ACE gene polymorphism and stroke and the role of hypertension in this relation in a large population-based cohort among the elderly.
Methods - The study was performed among 5,312 participants of the Rotterdam Study who were free from previous stroke at baseline (1990-1993) and were followed for stroke until January 1, 1999. The relation between ACE genotype and risk of stroke and subtypes of cerebral infarction was assessed using Cox regression. Adjustments were made for age and sex, and additionally for smoking, diabetes mellitus and carotid intima-media thickness.
Results - Overall, the D-allele was not related to the risk of stroke or cerebral infarction. In normotensives the D-allele increased the risk of cerebral infarction (RR 2.01 (95% CI 0.96-4.21)), whereas it decreased the risk in hypertensives (RR 0.58 (95% CI 0.34-1.00)).The interaction between hypertension and the D-allele in relation to cerebral infarction was statistically significant ($p<0.005$). Hypertension was less strongly related to cerebral infarction in carriers of the D-allele as compared to non-carriers (RR 1.31 (95% CI 0.70-2.44) and 7.14 (95% CI 2.33-21.81), respectively).
Conclusion - Overall, the D-allele is not related to the risk of stroke or cerebral infarction. Presence of the D-allele may modify the effect of hypertension, or vice versa, in relation to stroke.

INTRODUCTION

Genetic factors are considered to play a role in the etiology of stroke. They may be independently related to the risk of stroke, or by modulating the effect of risk factors such as hypertension.[1,2] The D-allele of the angiotensin converting enzyme (ACE) gene is reported to be related to elevated ACE levels, possibly leading to hypertension and increased risk of vascular disease.[3-9] The D-allele could also directly effect stroke risk, or through other factors e.g. influence on the endothelium.[10] Studies on the relation between ACE polymorphism and stroke thus far have reported conflicting results.[3-9,11-14] Some studies reported a relationship,[3,4,6,9,11] whereas others did not find any relation between the ACE gene polymorphism and stroke.[5,7,8,12-14] The majority of these studies were case control studies. The only prospective cohort study among 14,916 persons found that the D-allele was not related to the risk of stroke or cerebral infarction in normotensives without diabetes.[13] Among the positive studies, some have reported that the D-allele is particularly related to an increased risk of lacunar infarction,[11,14] but this relation has not yet been explored in a prospective cohort. We investigated the existence of a relationship between the ACE gene polymorphism and risk of stroke and subtypes of cerebral infarction in a large cohort of elderly persons. The further aim was to identify possible underlying mechanisms.

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.[15] 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,

216 had experienced a previous stroke. Among those without previous stroke, blood samples were taken in 6,846 persons. Missing blood samples were due to logistic reasons. In 1,534 persons no DNA was available or genotyping failed, resulting in a study population of 5,312 persons.

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.[16] A history of TIA was also assessed during the baseline interview. A neurologist reviewed all TIAs.[17] 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 considered lacunar if consciousness and higher cerebral function were 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. For the present study, follow up for stroke was complete for all participants until January 1, 1999.

Assessment of cardiovascular risk factors

At baseline, information on current health status, medication use and medical history was obtained using a computerized questionnaire. Participants were

classified as current, former or never smokers. 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. Hypertension was defined as a systolic blood pressure of 140 mm Hg or over, or a diastolic blood pressure of 90 mmHg or over, or current use of antihypertensive drugs for the indication of hypertension. The pulse pressure was calculated as the difference between systolic and diastolic blood pressure. 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. Participants underwent B-mode ultrasonography of both carotid arteries during the baseline visit to the research center. We measured intima-media thickness of the common carotid artery according to a standard protocol.[18]

Assessment of ACE-gene polymorphism

Peripheral venous blood samples were drawn using standard techniques and genomic DNA was isolated from whole blood. Genotypes for the dinucleotide polymorphism in the ACE gene were identified on the basis of multiplex polymerase chain reaction (PCR) amplification of the respective fragments of intron 16 of the ACE gene according to Lindpaintner et al[19] followed by visualization by electrophoresis. Two independent investigators scored the PCR results. Because in heterozygous status D allele is preferentially amplified, each sample that was found to be DD genotype was subjected to a second PCR amplification with a primer pair that recognizes an insertion specific sequence. No intra-individual variability was found on repeated readings from the same gel.

Data analysis

First, we tested whether the cohort was in Hardy Weinberg equilibrium regarding ACE genotype using analysis of covariance. We used Cox regression to examine the overall relation between ACE polymorphism and risk of stroke and subtypes of cerebral infarction. The ACE polymorphism was analyzed as an additive (DD vs. II and ID vs. II) and as a dominant (DD and ID vs. II) model of inheritance, respectively. Analyses were adjusted for age and sex and additionally for hypertension, smoking, diabetes and carotid intima-media thickness. We performed analyses in strata of hypertension for two reasons. The first reason was to explore any effect modification by hypertension. Secondly, it

allowed us to investigate the relation in a low risk group, namely normotensives. We further investigated whether there was an interaction between hypertension and presence of the D-allele in relation to stroke. To minimize the effect that antihypertensive medication e.g. ACE inhibitors may have on the relationship, we excluded persons who used antihypertensive medication at baseline in the following analyses. All anti-hypertensive medication was excluded since no distinction was made between type of anti-hypertensive drug at baseline. In order to investigate the effects of hypertension and the D-allele separately, we split up the cohort in groups according to presence of hypertension (yes/no) and D-allele (yes/no). We analyzed the risk of stroke and cerebral infarction in the subgroups, taking persons without hypertension and D-allele as reference group. Finally we assessed the relation between hypertension and risk of stroke in strata of the D-allele (present/absent), adjusting for age and sex.

RESULTS

The mean follow-up was 6.1 years and a total of 268 strokes occurred. Subtyping revealed 138 cerebral infarctions, 39 intracerebral hemorrhages and 91 unspecified strokes. The cerebral infarctions were non-lacunar in 102 and lacunar in 36 cases.

Table 1

Baseline characteristics of the study population.

	Cohort (n=5312)
Age	69.3 (9.2)
Sex (% female)	61.7%
SBP (mm Hg)	139.1 (22.4)
DBP (mm Hg)	73.7 (11.6)
Diabetes	9.9%
Smoking (% current)	21.9%
Common carotid IMT (mm)	0.80 (0.16)

SBP: Systolic blood pressure, DBP: Diastolic blood pressure, IMT: Intima-media thickness. Values represent means (SD).

Table 1 shows baseline characteristics of the cohort. The genotype frequencies in the cohort were in Hardy-Weinberg equilibrium ($p>0.05$) (table 2). Overall, the D-allele was not related to the risk of stroke or subtypes of cerebral infarction (table 3).

Table 2

Frequencies of the ACE D/I polymorphism.

Genotype						
Outcome,	Stroke		Cerebral infarction		Cohort	
P value for HWE	P=0.65		P=0.49		P=0.87	
	number	frequency	number	frequency	number	frequency
DD	75	0.28	34	0.25	1484	0.28
ID	137	0.51	73	0.53	2653	0.50
II	56	0.21	31	0.22	1175	0.22
All	268	1.00	138	1.00	5312	1.00

HWE: Hardy Weinberg Equilibrium.

Adjustment for cardiovascular risk factors including hypertension did not change the results. When we stratified for hypertension, presence of the D-allele was also not related to the risk of stroke in normotensives. However, presence of the D-allele in normotensives nearly significantly doubled risk of cerebral infarction and significantly more than doubled the risk of non-lacunar infarction, as compared to II individuals (RR 2.01 (95% CI 0.96-4.21) and 2.51 (95% CI 1.00-6.34), respectively) (table 3). The risk estimates remained largely similar, but lost statistical significance with adjustment for carotid intima-media thickness (RR 2.17 (95% CI 0.93-5.08) and 2.60 (95% CI 0.93-7.30), respectively). We found no relation between the D-allele and risk of lacunar infarction in normotensives. In hypertensive persons, presence of the D-allele seemed to have a protective effect on the risk of cerebral infarction (RR 0.57 (95% CI 0.34-0.96)) and in particular on non-lacunar infarction (RR 0.50 (95% CI 0.27-0.92)). When we tested for interaction between hypertension and presence of the D-allele, the interaction term was significant for cerebral infarction ($P<0.005$) and non-lacunar infarction ($P<0.005$), but not for stroke ($P=0.08$).

Table 5 shows that the effect of having both hypertension and the D-allele did not increase the risk more, as compared to only having hypertension. The risks did not change after adjustment for diabetes, smoking and carotid IMT.

Table 3

Relative risk of stroke and cerebral infarction in relation to ACE polymorphism, adjusted for age and sex.

ACE polymorphism	No. at risk	Stroke		Cerebral infarction		Non-lacunar infarction		Lacunar infarction	
		Cases	RR (95% CI)	Cases	RR (95% CI)	Cases	RR (95% CI)	Cases	RR (95% CI)
II (reference)	1175	56	1.00	31	1.00	23	1.00	8	1.00
Additive model									
ID	2653	137	1.02 (0.75-1.39)	73	1.00 (0.66-1.52)	55	1.01 (0.62-1.64)	18	0.97 (0.42-2.24)
DD	1484	75	0.98 (0.70-1.39)	34	0.84 (0.52-1.37)	24	0.79 (0.45-1.41)	10	0.97 (0.38-2.76)
Dominant model									
ID/DD	4137	212	1.01 (0.75-1.35)	107	0.94 (0.63-1.40)	79	0.93 (0.58-1.48)	28	0.97 (0.44-2.13)

Table 4

Relative risk of stroke and cerebral infarction in relation to ACE polymorphism in strata of hypertension, adjusted for age and sex.

ACE polymorphism	No at risk	Stroke		Cerebral infarction		Non-lacunar infarction		Lacunar infarction	
		Cases	RR (95% CI)	Cases	RR (95% CI)	Cases	RR (95% CI)	Cases	RR (95% CI)
Non-hypertensives									
II (reference)	776	22	1.00	8	1.00	5	1.00	3	1.00
Additive model									
ID	1696	69	1.35 (0.84-2.18)	40	2.20 (1.03-4.69)	32	2.78 (1.08-7.15)	8	1.20 (0.32-4.53)
DD	944	37	1.26 (0.74-2.14)	17	1.68 (0.72-3.87)	13	2.03 (0.72-5.69)	4	1.06 (0.24-4.76)
Dominant model									
ID/DD	2640	106	1.31 (0.83-2.09)	57	2.01 (0.96-4.21)	45	2.51 (1.00-6.34)	12	1.15 (0.32-4.08)
Hypertensives									
II (reference)	349	30	1.00	20	1.00	15	1.00	5	1.00
Additive model									
ID	889	67	0.80 (0.52-1.24)	32	0.59 (0.34-1.04)	22	0.54 (0.28-1.05)	10	0.75 (0.26-2.20)
DD	498	35	0.76 (0.47-1.24)	15	0.52 (0.27-1.02)	9	0.42 (0.18-0.95)	6	0.83 (0.25-2.74)
Dominant model									
ID/DD	1387	102	0.79 (0.53-1.19)	47	0.58 (0.34-1.00)	31	0.50 (0.27-0.92)	16	0.78 (0.29-2.13)

When we analyzed the relation between hypertension and stroke in strata of ACE genotypes we found that hypertension increased the risk of stroke and cerebral infarction in carriers of the D-allele (RR 1.69 (95% CI 1.14-2.52) and 1.31 (0.70-2.44), respectively). The corresponding risks in II individuals were 2.69 (95% 1.12-6.46) and 7.14 (95% CI 2.33-21.81), respectively.

Table 5

Relative risk of stroke and cerebral infarction in relation to presence of hypertension and D-allele, adjusted for age and sex.

	No. at risk	Pulse pressure (SD)	No. at risk	Stroke		Cerebral infarction	
				No. of cases	RR (95% CI)	No. of cases	RR (95% CI)
Ht– D–	682	59.8 (13.8)	682	15	1.00 (reference)	6	1.00 (reference)
Ht+ D–	112	85.6 (5.7)	112	8	2.64 (1.12-6.24)	7	6.67 (2.23-19.96)
Ht– D+	2340	59.5 (6.3)	2340	89	1.60 (0.92-2.76)	48	2.24 (0.96-5.23)
Ht+ D+	422	87.7 (5.9)	422	35	2.72 (1.48-5.00)	13	2.96 (1.12-7.84)

RR: relative risk; Ht+: hypertension present; Ht–: hypertension absent; D+: D-allele present; D–: D-allele absent; Pulse pressure in mm Hg.

DISCUSSION

In our prospective population-based study among 5,312 elderly persons we found that overall, the D-allele was not related to the risk of stroke or cerebral infarction. The D-allele increased the risk of cerebral infarction in normotensives and decreased the risk in hypertensives. We further found a significant interaction between hypertension and presence of the D-allele in relation to cerebral infarction. Before we interpret our results, some methodological issues need to be addressed.

We may have misclassified some strokes or ACE genotypes. We took extra care to avoid misclassification in genotyping. A second PCR has been performed whenever scoring of two investigators were not consistent. Also to avoid mistyping of ID to DD genotype another PCR with an insertion specific primer has been done. However, since classification of stroke was performed blinded to information on ACE genotype and vice versa, misclassification, if any, would have led to an underestimation of the relations that we have found.

The Physician's Health Study reported that there was no relation between the ACE polymorphism and risk of stroke or cerebral infarction.[13] In that study,

348 strokes occurred during 12 years of follow-up. In men without hypertension and diabetes, presence of the D-allele was related to a 1.58-fold (95% CI 0.88-2.82) increased risk of cerebral infarction. In contrast, the present study was based on both men and women and we analyzed subtypes of cerebral infarction. We found no relation between the D-allele and lacunar infarction, which contrasts with studies that did find such a relation. Those studies had a cross-sectional design and were based on limited numbers of cases.[11,14,20] A major pitfall of cross-sectional studies is selection bias, since only stroke survivors are included. This may have distorted the results.

We found a significant relation between the D-allele and non-lacunar infarction in normotensive persons. The majority of non-lacunar infarctions are caused by large artery disease. One possible explanation is that carotid atherosclerosis is the intermediate factor in this relationship, since the D-allele is considered to be related to atherosclerosis.[8] However, the relation remained largely similar after adjustment for carotid IMT, which undermines this explanation. Alternatively, the D-allele may influence the vascular system by inducing hypertension, influencing endothelial function or regulation of smooth muscle cell proliferation.[10,13,21]

In contrast to the normotensives, the D-allele was not related to an increased risk of stroke in hypertensive persons. Several mechanisms may explain this finding. First, hypertensive carriers of the D-allele may be more susceptible for coronary heart disease which may have resulted in a selective non-response in the study, leading to less stroke cases in this group. Second, in hypertensives, the II genotype is possibly related to an increased arterial stiffness. Benetos et al reported a relation between II genotype and arterial stiffness in hypertensive, but not in normotensive persons.[22] Hypertensive persons with the II genotype may be exposed to chronically low levels of angiotensin converting enzyme, which may upregulate the angiotensin II type 1 receptor, or be related to insulin resistence.[22,23] Both upregulation of the angiotensin II receptor and insulin resistance are reported to increase arterial stiffness.[22] The combination of hypertension and increased stiffness in hypertensive persons with the II genotype may explain why they not at lower risk than persons carrying the D-allele. However, this explanation is still controversial and it needs further study, as our data showed no difference in pulse-pressure between the ACE genotypes in strata of hypertension.

In summary, presence of the D-allele overall is not related to an increased risk of stroke. Presence of the D-allele may modify the effect of hypertension or vice versa. Further studies are needed to verify our findings and to investigate whether assessment of ACE genotype is helpful in selecting people who may benefit most from preventive therapy, such as use of ACE inhibitors.

REFERENCES

1. Hassan A, Markus HS. Genetics and ischaemic stroke. Brain 2000;123(Pt 9):1784-812.
2. Rubattu S, Giliberti R, Volpe M. Etiology and pathophysiology of stroke as a complex trait. Am J Hypertens 2000;13(10):1139-48.
3. Tiret L, Blanc H, Ruidavets JB, Arveiler D, Luc G, Jeunemaitre X, Tichet J, Mallet C, Poirier O, Plouin PF, Cambien F. Gene polymorphisms of the renin-angiotensin system in relation to hypertension and parental history of myocardial infarction and stroke: the PEGASE study. Projet d'Etude des Genes de l'Hypertension Arterielle Severe a moderee Essentielle. J Hypertens 1998;16(1):37-44.
4. Seino Y, Ikeda U, Maeda Y, Haga Y, Yashima H, Shimada K. Angiotensin-Converting Enzyme Gene Polymorphism and Plasminogen Activator Inhibitor 1 Levels in Subjects with Cerebral Infarction. J Thromb Thrombolysis 1998;5(3):263-267.
5. Szolnoki Z, Somogyvari F, Kondacs A, Szabo M, Fodor L. Evaluation of the roles of the Leiden V mutation and ACE I/D polymorphism in subtypes of ischaemic stroke. J Neurol 2001;248(9):756-61.
6. Catto A, Carter AM, Barrett JH, Stickland M, Bamford J, Davies JA, Grant PJ. Angiotensin-converting enzyme insertion/deletion polymorphism and cerebrovascular disease. Stroke 1996;27(3):435-40.
7. Ueda S, Weir CJ, Inglis GC, Murray GD, Muir KW, Lees KR. Lack of association between angiotensin converting enzyme gene insertion/deletion polymorphism and stroke. J Hypertens 1995;13(12 Pt 2):1597-601.
8. Agerholm-Larsen B, Nordestgaard BG, Tybjaerg-Hansen A. ACE gene polymorphism in cardiovascular disease: meta-analyses of small and large studies in whites. Arterioscler Thromb Vasc Biol 2000;20(2):484-92.
9. Sharma P. Meta-analysis of the ACE gene in ischaemic stroke. J Neurol Neurosurg Psychiatry 1998;64(2):227-30.
10. Butler R, Morris AD, Burchell B, Struthers AD. DD angiotensin-converting enzyme gene polymorphism is associated with endothelial dysfunction in normal humans. Hypertension 1999;33(5):1164-8.
11. Kario K, Kanai N, Saito K, Nago N, Matsuo T, Shimada K. Ischemic stroke and the gene for angiotensin-converting enzyme in Japanese hypertensives. Circulation 1996;93(9):1630-3.
12. Pfohl M, Fetter M, Koch M, Barth CM, Rudiger W, Haring HU. Association between angiotensin I-converting enzyme genotypes, extracranial artery stenosis, and stroke. Atherosclerosis 1998;140(1):161-6.

13. Zee RY, Ridker PM, Stampfer MJ, Hennekens CH, Lindpaintner K. Prospective evaluation of the angiotensin-converting enzyme insertion/deletion polymorphism and the risk of stroke. Circulation 1999;99(3):340-3.
14. Markus HS, Barley J, Lunt R, Bland JM, Jeffery S, Carter ND, Brown MM. Angiotensin-converting enzyme gene deletion polymorphism. A new risk factor for lacunar stroke but not carotid atheroma. Stroke 1995;26(8):1329-33.
15. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol 1991;7(4):403-22.
16. Bots ML, Looman SJ, Koudstaal PJ, Hofman A, Hoes AW, Grobbee DE. Prevalence of stroke in the general population. The Rotterdam Study. Stroke 1996;27(9):1499-501.
17. Bots ML, van der Wilk EC, Koudstaal PJ, Hofman A, Grobbee DE. Transient neurological attacks in the general population. Prevalence, risk factors, and clinical relevance. Stroke 1997;28(4):768-73.
18. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 1997;96(5):1432-7.
19. Lindpaintner K, Pfeffer MA, Kreutz R, Stampfer MJ, Grodstein F, LaMotte F, Buring J, Hennekens CH. A prospective evaluation of an angiotensin-converting-enzyme gene polymorphism and the risk of ischemic heart disease. N Engl J Med 1995;332(11):706-11.
20. Takami S, Imai Y, Katsuya T, Ohkubo T, Tsuji I, Nagai K, Satoh H, Hisamichi S, Higaki J, Ogihara T. Gene polymorphism of the renin-angiotensin system associates with risk for lacunar infarction. The Ohasama study. Am J Hypertens 2000;13(2):121-7.
21. Carluccio M, Soccio M, De Caterina R. Aspects of gene polymorphisms in cardiovascular disease: the renin- angiotensin system. Eur J Clin Invest 2001;31(6):476-88.
22. Benetos A, Gautier S, Ricard S, Topouchian J, Asmar R, Poirier O, Larosa E, Guize L, Safar M, Soubrier F, Cambien F. Influence of angiotensin-converting enzyme and angiotensin II type 1 receptor gene polymorphisms on aortic stiffness in normotensive and hypertensive patients. Circulation 1996;94(4):698-703.
23. Taniwaki H, Kawagishi T, Emoto M, Shoji T, Hosoi M, Kogawa K, Nishizawa Y, Morii H. Association of ACE gene polymorphism with arterial stiffness in patients with type 2 diabetes. Diabetes Care 1999;22(11):1858-64.

5.2 Mutations in the hemochromatosis gene (HFE) and stroke

Abstract

Background and objectives - Increased serum iron is found to be a risk factor for stroke. Carriers of *HFE* C282Y and H63D mutations have elevated serum iron levels and may have an increased risk for stroke. We studied the association between *HFE* gene mutations, carotid atherosclerosis and stroke.
Methods - We compared the frequency of the *HFE* C282Y and H63D gene mutations in 202 cases (onset before the age of 75 years) of stroke to that of 2,730 controls from a population-based study, the Rotterdam Study. The relationship between *HFE* mutations and stroke and its influence on the relation between hypertension, smoking and stroke were studied using a logistic regression model adjusted for age and sex. We computed the mean intima-media thickness of the common carotid artery and evaluated the effect of hypertension and smoking by *HFE* genotype.
Results - The percentage of both C282Y and H63D carriers in cases (43.7%, n=87) did not differ significantly (P=0.09) from that of controls (37.6%, n=986). The odds ratios for stroke (95% CI) for *HFE* carriers who also suffered from hypertension was 3.0 (1.9-4.6) and for those *HFE* carriers who were also smokers, the odds ratio for stroke was 2.6 (1.4-5.0). The mean (SD) intima-media thickness of the carotid artery was 0.77 (0.14) for non carriers without a history of hypertension or smoking compared to 0.81 (0.17) for *HFE* carriers who were smokers (P<0.004) and 0.84 (0.20) for *HFE* carriers who were hypertensives (P<0.001).
Conclusions - Mutations in the *HFE* gene were not significantly related to stroke or atherosclerosis in the carotid artery. The *HFE* gene may modify the relationship between smoking and stroke.

Introduction

Studies of the role of mutations in the hemochromatosis (*HFE*) gene and the risk of atherosclerosis and stroke have yielded controversial results.[1-4] Two major mutations are known in the *HFE* gene, i.e. the C282Y and the H63D mutation. These mutations determine serum iron, ferritin and transferrin saturation.[5,6] Rossi et al, (2000)[7] reported that the C282Y mutation did not influence the formation of plaques or the mean intima-media thickness (IMT) of the carotid artery. However, they found that serum ferritin levels were independently associated with the formation of plaques in the carotid artery of carriers of the C282Y mutation. Mortality from cerebrovascular disease was found to be significantly related to the C282Y mutation in women heterozygous for the C282Y mutation.[3] The association was strongest in women with a history of hypertension and/or smoking, which are important risk factors for stroke.

Up until now, the influence of the H63D on the pathogenesis of atherosclerosis and stroke has been given little attention. We studied the association between the C282Y and H63D mutations in the *HFE* gene in relation to atherosclerosis and stroke in a population-based sample of elderly people aged 55 years and over.

Methods

Study population

This study was conducted within the Rotterdam Study, an ongoing population-based cohort study for which all inhabitants aged 55 years or over, living in a suburb of Rotterdam, The Netherlands, were invited. The rationale and design of the Rotterdam study have been described elsewhere.[8] Baseline data collection was performed between 1990 and 1993. Written informed consent and permission to retrieve information from medical records were obtained from every participant. The study has been approved by the medical ethics committee of the Erasmus Medical Center. A total of 7,983 subjects participated (response rate 78%) in the study which includes individuals from the general population and those living in nursing homes. At baseline interview, information on current medication, alcohol intake and smoking habits was obtained. People who smoked were asked for the age at first smoking, for the duration of interval

periods without smoking, and for the average daily number of cigarettes smoked. For the purpose of this study, only current smokers (n=761, 26%) and those who never smoked (n=792, 27%) were considered, whereas past smokers (n=1,353, 46%) were excluded.

Two blood pressure measurements were taken with a random zero sphygmomanometer with the subject in sitting position and the average of these two measurements was taken. Hypertension was defined as a systolic blood pressure $\geq$160 mm Hg or a diastolic blood pressure $\geq$95 mm Hg on two consecutive measurements or current use of blood pressure lowering drugs for indication of hypertension.

Assessment of stroke

During the interview at baseline, a previous stroke was assessed by asking the question, “did you ever suffer from a stroke, diagnosed by a physician?" Medical records of subjects who answered ‘yes’ were checked and a previous stroke was considered to have occurred if confirmed by medical records.[9] Once subjects enter the Rotterdam Study they are continuously monitored for major events through automated linkage with files from the general practitioners. When an event or death had been reported, additional information was obtained by interviewing the general practitioner and by scrutinizing information from hospital discharge records in case of admittance or referral. Information from reports on all possible strokes was reviewed by two research physicians and a neurologist (PJK) who classified the stroke as definite, probable or non-stroke. The stroke was definite if the diagnosis was based on typical clinical symptoms and neuro-imaging excluded other diagnoses. The 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. Since a mixture of multiple genetic and environmental factors may determine stroke at late age, the present study focused on early stroke (age at onset $\leq$75). In total 202 stroke cases (110 prevalent, 92 incident cases) were considered. The controls consisted of a group of 2,730 subjects (aged $\leq$75 years) without any history of stroke and selected randomly from the total cohort.

The intima-media thickness (IMT) and the presence of atherosclerotic plaques of the common carotid artery (CCA) were assessed with ultrasound.[10] For each subject, the mean intima media thickness ((left + right)/2) was taken as

measure of wall thickness of the distal common carotid artery. Both CCAs were evaluated for the presence of atherosclerotic plaques. Both presence of plaques and increased CCA intima-media wall thickness were considered to be indicators of generalized atherosclerosis.

Laboratory procedures

From all the subjects, blood samples were collected by venepuncture and kept frozen until analysis. Genomic DNA was extracted from frozen buffy coat using the salting out procedure. Fragments of DNA were amplified by the Polymerase Chain Reaction (PCR) and genotyped using oligonucleotides primers as described elsewhere.[11]

Statistical Analysis

The chi-square statistic was used to compare categorical variables and the two sample t test to study normally distributed and continuous variables. The carrier frequencies for the C282Y and H63D mutations were estimated by counting gene and calculating sample proportions. As heterozygosity for C282Y and H63D were found to have a similar effect on serum iron levels in our population (submitted for publication elsewhere), we pooled the data on both mutations. We used logistic regression methods to estimate the odds ratios for stroke with 95% confidence interval adjusted for age and sex. Effect modification of the relation between smoking, hypertension and stroke by *HFE* was explored by stratifying the data by absence of both risk factors (reference group), presence of any risk factor or both factors simultaneously. Interaction was evaluated according to an additive model and using the synergy index which is defined as the ratio of the relative risk of both measurements indicating severe outcome minus 1 divided by the sum of the risk of each exposure minus 2. A synergy index of 1 indicates no interaction.

Results

Table 1 shows the baseline characteristics of the study population. Mean age of cases (69.3 years) was significantly ($P<0.001$) different to that of controls (65.1 years). There were significantly more men, hypertensives and smokers among the cases compared to the controls ($P=0.03$). When pooling the two mutations,

among cases, 43.7% were *HFE* carriers compared to 37.6% of controls but this difference was not significant (P=0.09).

Table 1

Baseline characteristics of the study population.

	Stroke (n=202)	Controls (n=2730)
Mean age (years)	69.3 (6.5)	65.1 (5.4)†
Men (%)	114 (56.4)	1323 (48.5)
Hypertensives (%)	128 (64.0)	1004 (38.2)†
Smoking		
Current (%)	62 (57.4)	699 (48.4)
Past (%)	94 (30.7)	1259 (46.6)
Never (%)	46 (22.8)	746 (27.6)
Mean IMT in mm (SD)	0.873 (0.218)	0.771 (0.144)
HFE carriers		
C282Y (%)	28 (14.1)	323 (12.2)
H63D (%)	62 (31.2)	707 (26.5)
HFE (%)	87 (43.7)	986 (37.6)

Values are means (SD) or number (%) based on all available data.
† P<0.05.

The effect of *HFE* on the relation between stroke, hypertension and smoking is shown in table 2. Hypertension was significantly associated with stroke in the absence of the *HFE* mutations (adjusted odds ratio: 2.3, 95% CI 1.5-3.4). By themselves, mutations in the *HFE* gene show only a weak association with stroke (odds ratio: 1.3, 95% CI: 0.8-2.2). Patients with hypertension who were also carriers of *HFE* mutations showed a significant relationship with stroke (adjusted odds ratio: 3.0, 95% CI: 1.9-4.6). The synergy index was 1.25. Neither smoking nor *HFE* mutations were significantly associated with stroke if the other factor was not present. But in those subjects who smoked and who were also *HFE* carriers, there was a significant relationship observed (odds ratio: 2.6, 95% CI: 1.4-5.0). The synergy index was 2.67. We obtained similar findings when comparing men and women and when we analyzed prevalent and incident cases of stroke separately (data not shown).

Table 2

Interaction between HFE C282Y and H63D mutations, hypertension, smoking and stroke.

HFE and hypertension

HFE carrier	Hypertensive	Stroke	Controls	Odds ratios (95% CI)	
				Unadjusted	Adjusted*
No	No	41 (21.0)	968 (39.5)	1.0 Reference	
No	Yes	71 (36.4)	601 (24.5)	2.8 (1.9-4.2)	2.3 (1.5-3.4)†
Yes	No	30 (15.4)	552 (22.5)	1.3 (0.8-2.1)	1.3 (0.8-2.2)
Yes	Yes	53 (27.2)	332 (13.5)	3.8 (2.5-5.8)	3.0 (1.9-4.6)†

HFE and smoking

HFE carrier	Smoker	Cases	Controls	Odds ratios (95% CI)	
				Unadjusted	Adjusted*
No	No	24 (22.9)	422 (31.4)	1.0 Reference	
No	Yes	29 (27.6)	435 (32.4)	1.2 (0.7-2.1)	1.3 (0.7-2.3)
Yes	No	22 (21.0)	261 (19.4)	1.5 (0.8-2.7)	1.3 (0.7-2.4)
Yes	Yes	30 (28.6)	224 (16.7)	2.4 (1.3-4.1)	2.6 (1.4-5.0)†

Values are number of individuals (percentage).

* Adjusted for age and sex

† P<0.05.

Table 3 shows the effect of *HFE* mutations and its interaction with hypertension and smoking on the mean IMT. There was no significant difference in the mean IMT between *HFE* carriers and non carriers. Hypertension was associated with a significant increase in mean IMT (P=0.001) both in the presence and absence of *HFE* mutations. Smoking was also significantly associated with an increased mean IMT in the presence (P<0.004) or absence (P=0.01) of *HFE* mutations. The association was strongest in mutation carriers. The effect of smoking and *HFE* on IMT was additive.

Table 3

Interaction between HFE C282Y and H63D mutations, hypertension, smoking and intima-media thickness.

HFE and hypertension			
HFE carrier	Hypertensive	Mean intima-media thickness (SD)	P-value
No	No (n=904)	0.761 (0.136)	Reference
No	Yes (n=671)	0.831 (0.163)	<0.001
Yes	No (n=530)	0.774 (0.151)	0.10
Yes	Yes (n=386)	0.836 (0.190)	<0.001
HFE and smoking			
HFE carrier	Smoker	Mean intima-media thickness (SD)	P-value
No	No (n=458)	0.771 (0.143)	Reference
No	Yes (n=419)	0.796 (0.150)	0.01
Yes	No (n=280)	0.784 (0.151)	0.24
Yes	Yes (n=226)	0.807 (0.173)	0.004

DISCUSSION

In our study, the C282Y and H63D mutations were not significantly associated with stroke or carotid atherosclerosis by themselves. However, the presence of *HFE* mutations modified the association between hypertension, smoking and stroke.

Our data are based on a mixture of prevalent and incident cases and remain to be confirmed, preferably using a set of incident cases. The findings on stroke and IMT show an additive effect of smoking and *HFE*, suggesting that *HFE* is involved in atherosclerosis, a major risk factor for stroke. The main advantage of our study is its population-based design.

Our findings are compatible with those of Roest et al, (1999)[3] who found that in women carriers of the C282Y mutation, mortality for cerebrovascular disease was 2.4 times increased. Roest et al, (1999)[3] found a strong effect modification by hypertension and smoking. In our study, the odds ratios for stroke (95% CI) in *HFE* carriers who were also hypertensives was 3.0 (1.9-4.6) and for those *HFE* carriers who were also smokers, the odds ratio for stroke was 2.6 (1.4-5.0). The mean IMT was not different between *HFE* carriers and non

carriers nor was this modified by hypertension or smoking. There have been various hypotheses as to why mutations in the *HFE* gene may be a risk factor for stroke. Among the most powerful ideas is the iron hypothesis which stipulates that iron depletion decreases and iron overload increases the risk of cardiovascular diseases.[12] High iron concentrations have been found in human atherosclerotic lesions[13] and it has been experimentally observed that iron overload contributes to atherogenesis.[14] Increased blood iron concentration may lead to an increase in the viscosity of blood, which may result in thrombosis. We and others have indeed found that carriers of *HFE* mutations do have significantly increased levels of iron.[5,6,15] Since we have data on serum iron, ferritin and transferrin in only a very limited sub-sample of this population, we cannot verify this hypothesis in the statistical analysis.

The modification of the relationship between smoking and *HFE* in relation to stroke and the additive effect opens another possible mechanism. Smoking and *HFE* mutations may both result in increased oxidation and thus cause damage to the vessel walls. Since *HFE* mutations are associated with iron overload, the effect of *HFE* mutation on the risk of stroke might be through excess of iron in carriers of the *HFE* mutations. Adding the oxidative effect of smoking to that of high iron levels in *HFE* carriers may increase the risk of atherosclerosis according to an additive model. Interestingly, with regard to the risk of stroke, the effect of these two risk factors may be more than additive, i.e. these risk factors may interact with regard to the outcome of stroke.

In conclusion, the C282Y and H63D mutations by themselves are not strongly related to stroke or atherosclerosis. In the presence of smoking, these mutations increase the risk of carotid atherosclerosis and stroke in carriers of *HFE* mutations.

References

1. Franco RF, Zago MA, Trip MD, ten Cate HEA, Prins MH, Kastelein JJ, et al. Prevalence of hereditary hemochromatosis in premature atherosclerotic vascular disease. Br J Haematol 1998;102:1272-5.
2. Roest M, van der Schouw YT, de Valk B, Marx JJM, Tempelman MJ, de Groot PG, Sixma JJ, Banga DJ. Heterozygosity for a hereditary hemochromatosis gene is associated with cardiovascular death in women. Circulation 1999;100:1268-73.
3. Gillum RF, Sempos CT, Makuc DM, Looker AC, Chien CY, Ingram D. Serum transferrin, stroke incidence and Mortality in women and men. The NHANES I epidemiologic follow-up study. Am J Epid 1996;144:59-68.
4. Calado RT, Franco RF, Pazin-Filho A, Simoes MV, Marin-Neto JA, Zago MA. HFE gene mutations in coronary atherothrombotic disease. Braz J Med Biol Res 2000;33:301-6.
5. Datz C, Haas T, Rinner H, Sandhoffer F, Patsch W, Paulweber B. Heterozygosity for the C282Y mutationin the hemochromatosis gene is associated with increased serum iron, transferrin saturation, and hemoglobin in young women: a protective role against iron deficiency? Clinical Chemistry 1998;44:2429-32.
6. Whitfield JB, Cullen LM, Jazwinska EC, Powell LW, Heath AC, Zhu G, Duffy DL and Martin NG. Effects of HFE C282Y and H63D polymorphism and polygenic background on iron stores in a large community sample of twins. Am J Hum Genet 2000;66:1246-58.
7. Rossi E, McQuillam BM, Hung J, Thompson PL, Kuek C, Beilby JP. Serum ferritin and C282Y mutation of the hemochromatosis gene as predictor of asymptomatic atherosclerosis in a community population. Stroke 2000;31:3015-20.
8. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of diseases and disability in the elderly: The Rotterdam Elderly Study. Eur J Epidemiol 1991;4:40322.
9. Bots ML, de Jong PT, Hofman A, Grobbee DE. Left, right, near or far wall common carotid intima-media thickness measurements: associations with cardiovascular disease and lower extremity arterial atherosclerosis. J Clin Epidemiol 1997;50:801-7.
10. Bots ML, Looman SJ, Koudstaal PJ, Hofman A, Hoes AW, Grobbee DE. Prevalence of stroke in the general population. The Rotterdam Study. Stroke 1996;27:1499-501.
11. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, et al. A novel MHCclass I-like gene is mutated in patients with hereditary hemochromatosis. Nature Genet 1996;13:399-408.
12. Sullivan JL. Iron and the genetics of cardiovascular disease. Circulation 1999;100:1260-3.
13. Swain J, Gutteridge JM. Prooxidant iron and copper, with ferroxidase and xanthine oxidase activities in human atherosclerotic material. FEBS Lett 1995;368:513-5.
14. Araujo JA, Romano EL, Brito BE, Parthe V, Romano M, Bracho M, Montano RF, Cardier J. Iron overload augments the development of atherosclerotic lesions in rabbits. Arteriosclerosis Thrombosis and vascular Biolog 1995;15:1172-80.
15. Burt MJ, George PM, Upton JD, Collett JA, Frampton CMA, Chapman TM, Walmsley TA, Chapman BA. The significance of hemochromatosis gene mutations in the general population: implications for screening. Gut 1988;43:830-6.

6
General discussion

The work described in this thesis was based on studies that have been performed in the Rotterdam Study, a large population-based cohort study that started in 1990 and included 7,983 persons aged 55 years and older of whom 7,721 had not experienced a previous stroke. The cohort was continuously monitored for incident stroke by linkage of the Rotterdam Study database with medical records from general practitioners and by regular visits to the general practitioners who were not included in the automated database (15%). Until January 1, 1999, including 46,011 person-years of follow up, we identified 432 first-ever stroke cases. We investigated risk factors for stroke. The main focus was on atherosclerosis in relation to stroke. Besides, we studied putative risk factors such as sex hormones and genetic factors, namely the Angiotensin Converting Enzyme (ACE) polymorphism and mutations in the genes for hemochromatosis (HFE genes). In the following we will discuss some methodological and conceptual issues that relate to the studies described in this thesis, summarize our findings and comment on the clinical relevance of our findings. Further, we will make recommendations for future research.

METHODOLOGICAL CONSIDERATIONS

Atherosclerosis

Atherosclerosis is the main risk factor for stroke and is involved in more than 70% of all strokes. To the extent that non-invasive measures of subclinical atherosclerosis can predict stroke risk, they can be used to identify high-risk persons who may benefit most from preventive interventions.

A number of issues merit attention if we consider measures of atherosclerosis in relation to stroke risk. Atherosclerosis in general can be related to stroke in several ways. First, it can be causally related to stroke, either directly, e.g. by occlusion of vessels or release of thrombo-emboli, or indirectly, e.g. by contributing to an unfavorable risk profile that causes the stroke. For example, atherosclerosis can be associated with cardiac disorders like atrial fibrillation, leading to emboli or hypoperfusion of the brain. But apart from playing a role in the etiology of stroke, atherosclerosis can also predict the risk of stroke because it marks the presence of other, causally related, factors. Moreover, different non-invasive measures of atherosclerosis exist and a specific measure of atherosclerosis can be related to the risk of stroke as a reflection of atherosclerosis elsewhere.

An other issue to consider is that atherosclerosis can be divided into two distinct pathophysiological entities, namely atherosis and sclerosis.[1] Atherosis refers to the structural changes that occur during the atherosclerotic process, whereas sclerosis causes functional changes in the vessel bed such as an increased arterial stiffness. These different entities may have distinct pathophysiological mechanisms in relation to stroke.

Stroke classification

Our follow-up was virtually complete which helped to reduce misclassification of stroke cases. We classified strokes as cerebral infarction, intracerebral hemorrhage or unspecified. In the majority of our studies we classified stroke subtypes based on CT or MRI scan, which was available for approximately 60% of the cases. Subtyping of cerebral infarctions was done based on size (lacunar infarctions in small perforating vessels or larger, non-lacunar infarctions) and location (anterior and posterior circulation). Investigation of the relation between atherosclerosis and subtypes of cerebral infarction may give more insight in the underlying mechanisms. For example, in order to investigate the causal role of carotid atherosclerosis in stroke, we analyzed infarctions anterior and posterior circulation of the brain separately. Blood flowing through the carotid arteries mainly supplies the anterior circulation of the brain. Therefore, a causal relation between carotid atherosclerosis and stroke would result in a stronger relation with anterior as compared to posterior circulation infarction. The fact that a considerable proportion of strokes remained unspecified reduced the power in our study. To the extent that the lack of additional diagnostic information was related to the determinants under study, it may have biased our findings.

In several clinical studies the TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification is used, which classifies cerebral infarctions according to etiology.[2] We did not use this classification because it is doubtful whether such a classification is useful in etiologic research since it would induce a circular argument. For example, atherosclerosis would by definition be related to infarctions that are caused by large vessel disease. Another drawback of the TOAST classification is that it so too simplistic. For example, it is doubtful whether all strokes related to atrial fibrillation are cardioembolic as the TOAST criteria dictate. It has been reported that hypoperfusion may also play a role in

strokes that occur in persons with atrial fibrillation, as may other co-existing vascular pathology.[3]

MAIN FINDINGS

Incidence of stroke in the elderly

In the last decades, a decreasing trend in stroke mortality has been observed.[4-9] Yet, stroke is still the third leading cause of death in many countries. Decreasing trends for stroke morbidity are less clear. Cerebrovascular diseases put a large burden on health care budgets. Limited data existed on the incidence and lifetime risk of stroke in the elderly. We found that the incidence rate of stroke increased with age. Further, the incidence rate was higher in men than in women over the entire age range. However, as a net result of shorter survival and higher incidence rates of stroke in men as compared to women, lifetime risks were similar for both sexes (21% for men and women aged 55 years). This study suggests that depletion of susceptibles for stroke with age does not occur.

Markers of structural vessel wall change and generalized atherosclerosis

Ankle-arm index

Ankle arm index is a simple measure of peripheral arterial disease and atherosclerosis. It has been suggested that ankle arm index can be used to select persons at high risk of cardiovascular disease.[10] 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.[11] In line with their observation we found that ankle arm index lost the additional value for the prediction of stroke if compared to a model including cardiovascular risk factors. When we compared the predictive value of several measures of atherosclerosis, we found similar results in the sense of little value of adding ankle arm index. These results suggest that a low ankle arm index acts as a marker of an unfavorable cardiovascular risk profile but does not directly reflect etiologically relevant pathology.

Carotid intima-media thickness

Carotid intima-media thickness has been widely studied as a marker of generalized atherosclerosis. It has been confirmed that an increased intima-media thickness is related to the risk of stroke and cerebral infarction, independently of traditional cardiovascular risk factors.[12-14] However, the relation between stroke subtypes, and in particular cerebral hemorrhages was not yet clear. We have shown that an increased intima-media thickness is related to all subtypes of stroke, including intracerebral hemorrhages and lacunar infarction. This opens the possibility that intima-media thickness is also reflecting small vessel disease, since both lacunar infarction and intracerebral hemorrhage are reported to be related to arteriolosclerosis of small intracerebral vessels.[15-17]

Compared to other measures of atherosclerosis, intima-media thickness was most strongly related to stroke, even when a history of cardiovascular disease was taken into account. Most likely, an increased intima-media thickness is a marker of generalized atherosclerosis, possibly including small vessel pathology. An increased intima-media thickness may be used to select persons at high risk for stroke, irrespective of other cardiovascular risk factors.

Carotid plaques

The significance of carotid plaques in relation to stroke in persons without previous stroke or TIA was not clear. Knowledge on the relation with subtypes of cerebral infarction and on the importance of plaque location was limited. Furthermore, it had not been established whether carotid plaques are merely sources of emboli, or markers of generalized atherosclerosis. Our study on the relation between carotid plaques at six locations in the carotid arteries and subtypes of cerebral infarction yielded several new insights. First, plaques increased the risk of stroke and cerebral infarction, irrespective of plaque location. We could not confirm that plaques at locations with a lot of turbulence like the bifurcation carried a higher risk than plaques elsewhere. Secondly, plaques were related to anterior but not to posterior circulation infarctions, which suggests that plaques may just be sources of thrombo-emboli. Thirdly, carotid plaques were related to the risk of lacunar infarction, which suggests that carotid plaques are also related to small vessel disease. Another explanation might be that part of the lacunar infarctions are related to emboli. In the last

decade, more evidence is accumulating that lacunar infarctions may be caused by emboli.[18-22] Our conclusion is that carotid plaques are both markers of generalized atherosclerosis and sources of thrombo-emboli.

When compared to other markers of atherosclerosis, carotid plaques were not the strongest predictor of stroke. Increased carotid intima-media thickness for example was a stronger predictor. Just counting number of plaques, as we did, may be too crude a measure. Possibly, measurement of the thickness of all plaques and measurement of plaque characteristics like echolucency, ulceration and intraplaque hemorrhage may help to more precisely measure plaques.

Calcifications in the vessel wall

In this thesis we studied two measures of calcification, namely aortic calcifications and coronary calcifications. We showed that presence of aortic calcifications is related to an increased risk of stroke and cerebral infarction, irrespective of cardiovascular risk factors. Furthermore, we showed that presence of coronary calcifications as detected by electron-beam tomography is related to a history of stroke. The most likely explanation for these results is that calcifications in the vessel wall reflect the extent of atherosclerotic lesions elsewhere. Alternatively, calcifications in the vessel wall may also functionally be related to increased arterial stiffness, leading to an increased pulse pressure and risk of stroke. A third explanation is that aortic and coronary calcifications reflect calcifications in the aortic arch from which emboli can arise. Unfortunately, we did not have data on atherosclerosis in the aortic arch, so we were not able to investigate this further.

When we compared the predictive value of between several measures of atherosclerosis, we found that aortic calcifications were among the best predictors for stroke and were related to stroke, independently of cardiovascular risk factors and intima-media thickness.

Functional changes in the vessel wall

Arterial stiffness

Stiffening of the arterial tree is considered the main cause of an increased pulse pressure. We investigated two measures of arterial stiffness in relation to stroke. The pulse-wave velocity in the aorta is calculated as the ratio between the transit

time of the foot of the pulse wave to travel along the arterial tree and the distance of the arterial segment. Another measure of arterial stiffness is the distensibility, i.e. change in arterial diameter due to change in arterial pressure over the cardiac cycle. We found that aortic stiffness is not clearly related to a previous stroke. However, carotid stiffness was related to a previous stroke. The relation remained after adjustment for carotid plaques. One other but small case control study investigated the relation between pulse-wave velocity and stroke. They reported a relation between pulse-wave velocity and stroke.[23] Recently, it was reported that aortic stiffness as assessed with transoesophageal echocardiography is related to ischemic stroke in an elderly population, independently of thickness of aortic plaques.[24] These results suggest that assessing aortic stiffness, or sclerosis, may add prognostic information in assessing the risk of stroke in the elderly.

A limitation of our studies on coronary calcifications and arterial stiffness is the cross-sectional design. This may have induced misclassification of exposure. More importantly, only survivors of stroke were analyzed. This prevalence-incidence bias may have influenced our results.

Endogenous sex hormones

Sex hormones may play a role in the difference between men and women in the occurrence of atherosclerosis and cardiovascular disease. Estrogen is considered to have a protective effect in women, since the frequency of cardiovascular disease increases after menopause. However, recent clinical trials evaluating the effect of hormone replacement therapy in relation to stroke reported negative results.[25,26] We found no relation between endogenous estrogen levels and stroke risk, neither in men nor in women. A low testosterone level was recently reported as a potential risk factor for cardiovascular disease in men.[27,28] However, prospective studies on the relation between testosterone and stroke were lacking. We showed that a low testosterone level was related to an increased risk of stroke in male non-smokers. Adjustment for atherosclerosis yielded similar results, suggesting that other mechanisms than atherosclerosis may underlie this relation. Levels of testosterone in men gradually decrease with age. One proposed mechanism is that a low testosterone level is related to a bad general health and comorbidity.[29] Other possible pathways are that low testosterone levels are related to oxidative stress, vasoconstriction or arterial

stiffness.[30-32] At present, use of testosterone replacement therapy in men is not yet warranted as a measure to prevent stroke.

Genetic factors

Genetic factors may be related to stroke, most likely by modulating the effects of, or predisposing to risk factors for stroke such as atherosclerosis and hypertension. We evaluated two possible candidate genes in relation to stroke. Mutations in the hemochromatosis gene (HFE), namely C282Y and H63D were related to both stroke and carotid atherosclerosis in persons who smoked or had hypertension. Possible mechanisms underlying these relations are an increased iron level, leading to increased blood viscosity, or increased oxidative stress and subsequent vascular damage.[33,34] For the Angiotensin Converting Enzyme (ACE) gene our results were less clear. The D-allele is hypothesized to influence atherosclerosis and to have other influences on the vascular system, namely by inducing, hypertension, influencing endothelial function or regulation of smooth muscle tone. [35-37] We found that overall, the D-allele was not related to the risk of stroke or cerebral infarction. However, there was a significant interaction between presence of the D-allele and hypertension in relation to cerebral infarction. The results remained similar after adjustment for carotid atherosclerosis. Possibly hypertension has different effects in persons with and without the D-allele.

CLINICAL RELEVANCE

Cardiovascular disease has long time been considered a disease of men. Our study on the incidence of stroke confirmed that incidence rates were higher in men than in women. However, since men have poorer survival, lifetime risks of stroke were similar for men and women. Women have their stroke at a higher age than men. These results illustrate that stroke prevention in the elderly, also in women, is a very important issue, especially since populations are rapidly growing older. In coming decades, stroke will put a larger burden on the health care budget. Treatment possibilities have improved in recent years and implementation of stroke units and stroke services has become widespread. Nevertheless, prevention of stroke is still the best therapy. What is needed for prevention to be effective is identification of modifiable causal risk factors and identification of people at high risk who may benefit from the intervention.

We analyzed different measures of atherosclerosis and we showed that measurement of ankle arm index has no additional value to classical cardiovascular risk factors in the prediction of stroke. Carotid intima-media thickness and aortic calcifications were the best predictors for stroke, independent of cardiovascular risk factors. Adding information on calcification to carotid intima-media thickness may thus be a promising method to select high risk groups. Still, clinical application should be preceded by further research e.g. on cost-effectiveness. Another measure of atherosclerosis related to stroke was carotid plaques. Just counting the number of plaques may provide a useful and easy tool to select persons at high risk for stroke, cerebral infarction and lacunar infarction. However, the prognostic value for stroke was less if compared to intima-media thickness and aortic calcifications. We further showed that coronary calcifications as detected by electron beam tomography may not only predict the risk of myocardial infarction, but also the risk of stroke. Still, this relation first needs to be evaluated prospectively before clinical management of persons with coronary calcifications should be altered.

In several studies we investigated the relation between atherosclerosis and lacunar infarctions. Atherosclerosis is not commonly considered an important risk factor for lacunar infarctions. However, our studies have shown that presence of carotid atherosclerosis increases the risk of lacunar infarctions, irrespective of cardiovascular risk factors like diabetes and hypertension. These results question the traditional distinction between large artery atherosclerosis and small vessel disease as different disease processes. Boiten et al. identified two types of lacunar infarctions. First, singe lacunes related to traditional vascular risk factors and microatheromata and secondly, multiple (silent) lacunar infarctions related to hypertension and leukaraiosis.[15] The latter type is considered to be related to lipohyalinosis or arteriolosclerosis, and has a worse prognosis.[16] In our study, we could not distinguish between the two groups of lacunar infarctions. However, it is conceivable that most of the lacunes in our study were single, related to traditional risk factors and microatheromata. In this light, carotid atherosclerosis as measure of large vessel disease can also act as an indicator of small vessel atheromata. Another possibility is that lacunar infarctions can be caused by micro-emboli, resulting from large artery disease.[18,38] Our results and the recent studies point out that the concept that lacunar infarctions are only related to small vessel disease does not hold.

Hormone therapy in postmenopausal women is a major issue of concern in cardiovascular disease prevention. Recently, evidence has become available to suggest that estrogen replacement therapy in women has no, or even and adverse effect on stroke risk.[25,26,39] Our study showed that endogenous estradiol is not related to stroke in postmenopausal women. However, we cannot generalize these results to exogenous estradiol. Therefore, we cannot infer that estrogen replacement therapy has no effect on stroke in women. In recent years, increasing attention has been payed to testosterone replacement therapy in men. It is considered to have beneficial effects on mood disorders, libido, well being and possibly cardiovascular disease.[40] Adversely, testosterone therapy may also increase the risk of prostate cancer.[40] We found that a low testosterone level in men increases the risk of stroke. These finding needs further confirmation. Further, the precise mechanism underlying this relationship needs to be explored. At present, testosterone suppletion in men in order to prevent stroke can not seriously be considered.

Finally, we showed that the ACE genotype, and mutations in the gene for hemochromatosis (HFE) were related to stroke in certain subgroups. Research on genetic factors in stroke is still in the beginning. As more knowledge on genetic factors in stroke will accumulate, intensified preventive therapy in susceptible persons may become possible.

FUTURE RESEARCH

We examined several measures of atherosclerosis in relation to stroke. Further epidemiological research can help unravel what underlies the relation between atherosclerosis and stroke. Measures like coronary calcifications and arterial stiffness need prospective evaluation in relation to stroke. Furthermore, these measures need comparison with other measures of atherosclerosis in relation to stroke. We identified carotid intima-media thickness and aortic calcifications as strong and independent predictors for stroke. Research is needed to evaluate the cost-effectivity of applying both measures to select high risk persons. A topic related to the latter is the development of risk scores in populations, including measures of atherosclerosis.

Another field of interest is the identification of factors that trigger the occurrence and progression of atherosclerosis. For example, measuring plaque characteristics like echolucency and surface regularity in population-based studies may help the identification of persons at higher risk of embolic stroke. It

is reported that these characteristics are related to unstable plaques and subsequent release of thrombo-emboli.[41-43] The influence of genetic and thrombotic factors on atherosclerosis and stroke also needs further investigation. Another factor that needs further investigation is medication use. The role of aspirin has become more clear in last decades. In a recent clinical trial it was found that use of statins decreases overall mortality and stroke risk in persons with any cardiovascular risk factor.[44] The effect of statins on atherosclerosis, either by reducing cholesterol or by antioxidant or other effects needs further study to explain the preventive mechanism.

We identified a low testosterone level as a risk factor for stroke in men. Further experimental and observational research is needed to investigate whether testosterone is related to vascular disease, or just a measure of bad health. If testosterone turns out to be directly related to the vascular system, then clinical trials on the effect of testosterone-replacement therapy on cardiovascular disease in men at risk for stroke should be conducted.

It is obvious that the field of genetics in stroke is just beginning to develop. We found a weak relation between the ACE D-allele and risk of stroke. The possible underlying mechanism needs further study. Studies on the effect of ACE inhibitors may help in this.

There is a need for an improved identification of stroke subtypes in population-based cohort studies. One approach is a more widespread use of neuro-imaging techniques to assess more subtypes, especially in the elderly who less often get a scan and in whom the burden of stroke is largest. Another approach is the use of more advanced neuro-imaging techniques such as perfusion and diffusion weighted imaging. This may help a more precise subtyping of cerebral infarctions. More detailed information on stroke subtypes and their prognosis may help to understand underlying pathophysiological mechanisms and to optimalize preventive strategies. Moreover, the classification of stroke subtypes may need revision.

Any future stroke investigator should realize that the effects of risk factors can be manifold and that stroke is a heterogeneous disease.

REFERENCES

1. Popele van N. Causes and consequences of arterial stiffness. An epidemiological approach. Erasmus Medical Center Rotterdam, 2000.
2. Adams HP, Jr., Woolson RF, Clarke WR, Davis PH, Bendixen BH, Love BB, Wasek PA, Grimsman KJ. Design of the Trial of Org 10172 in Acute Stroke Treatment (TOAST). Control Clin Trials 1997;18(4):358-77.
3. MacKenzie JM. Are all cardio-embolic strokes embolic? An autopsy study of 100 consecutive acute ischaemic strokes. Cerebrovasc Dis 2000;10(4):289-92.
4. Feinleib M, Ingster L, Rosenberg H, Maurer J, Singh G, Kochanek K. Time trends, cohort effects, and geographic patterns in stroke mortality--United States. Ann Epidemiol 1993;3(5):458-65.
5. Wolf PA, RB DA, MA ON, Sytkowski P, Kase CS, Belanger AJ, Kannel WB. Secular trends in stroke incidence and mortality. The Framingham Study [see comments]. Stroke 1992;23(11):1551-5.
6. Broderick JP, Phillips SJ, Whisnant JP, WM OF, Bergstralh EJ. Incidence rates of stroke in the eighties: the end of the decline in stroke? Stroke 1989;20(5):577-82.
7. Howard G. Decline in stroke mortality in North Carolina. Description, predictions, and a possible underlying cause. Ann Epidemiol 1993;3(5):488-92.
8. Tuomilehto J, Sarti C, Torppa J, Salmi K, Puska P. Trends in stroke mortality and incidence in Finland in the 1970s and 1980s. Ann Epidemiol 1993;3(5):519-23.
9. Bonita R. Stroke trends in Australia and New Zealand: mortality, morbidity, and risk factors. Ann Epidemiol 1993;3(5):529-33.
10. Leng GC, Fowkes FG, Lee AJ, Dunbar J, Housley E, Ruckley CV. Use of ankle brachial pressure index to predict cardiovascular events and death: a cohort study. Bmj 1996;313(7070):1440-4.
11. Tsai AW, Folsom AR, Rosamond WD, Jones DW. Ankle-brachial index and 7-year ischemic stroke incidence: the ARIC study. Stroke 2001;32(8):1721-4.
12. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 1997;96(5):1432-7.
13. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK, Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med 1999;340(1):14-22.
14. Chambless LE, Folsom AR, Clegg LX, Sharrett AR, Shahar E, Nieto FJ, Rosamond WD, Evans G. Carotid wall thickness is predictive of incident clinical stroke: the Atherosclerosis Risk in Communities (ARIC) study. Am J Epidemiol 2000;151(5):478-87.
15. Boiten J, Lodder J, Kessels F. Two clinically distinct lacunar infarct entities? A hypothesis. Stroke 1993;24(5):652-6.
16. de Jong G, Kessels F, Lodder J. Two types of lacunar infarcts: further arguments from a study on prognosis. Stroke 2002;33(8):2072-6.
17. Besson G, Clavier I, Hommel M, Perret J. [ssociation infarctus lacunaire et hematome intra-cerebral. Rev Neurol (Paris) 1993;149(1):55-7.
18. Ay H, Oliveira-Filho J, Buonanno FS, Ezzeddine M, Schaefer PW, Rordorf G, Schwamm LH, Gonzalez RG, Koroshetz WJ. Diffusion-weighted imaging identifies a subset of lacunar infarction associated with embolic source. Stroke 1999;30(12):2644-50.
19. Horowitz DR, Tuhrim S, Weinberger JM, Rudolph SH. Mechanisms in lacunar infarction. Stroke 1992;23(3):325-7.

20. Laloux P, Brucher JM. Lacunar infarctions due to cholesterol emboli. Stroke 1991;22(11):1440-4.
21. Serena J, Segura T, Castellanos M, Davalos A. Microembolic signal monitoring in hemispheric acute ischaemic stroke: a prospective study. Cerebrovasc Dis 2000;10(4):278-82.
22. Zhu CZ, Norris JW. Lacunar infarction and carotid stenosis (abstract). Ann Neurol 1991;30(1991):244.
23. Lehmann ED, Hopkins KD, Jones RL, Rudd AG, Gosling RG. Aortic distensibility in patients with cerebrovascular disease. Clin Sci (Colch) 1995;89(3):247-53.
24. Sugioka K, Hozumi T, Sciacca RR, Miyake Y, Titova I, Gaspard G, Sacco RL, Homma S, Di Tullio MR. Impact of aortic stiffness on ischemic stroke in elderly patients. Stroke 2002;33(8):2077-81.
25. Petitti DB, Sidney S, Quesenberry CP, Jr., Bernstein A. Ischemic stroke and use of estrogen and estrogen/progestogen as hormone replacement therapy. Stroke 1998;29(1):23-8.
26. Simon JA, Hsia J, Cauley JA, Richards C, Harris F, Fong J, Barrett-Connor E, Hulley SB. Postmenopausal hormone therapy and risk of stroke: The Heart and Estrogen-progestin Replacement Study (HERS). Circulation 2001;103(5):638-42.
27. English KM, Mandour O, Steeds RP, Diver MJ, Jones TH, Channer KS. Men with coronary artery disease have lower levels of androgens than men with normal coronary angiograms. Eur Heart J 2000;21(11):890-4.
28. Jeppesen LL, Jorgensen HS, Nakayama H, Raaschou HO, Olsen TS, Winther K. Decreased serum testosterone in men with acute ischemic stroke. Arterioscler Thromb Vasc Biol 1996;16(6):749-54.
29. van den Beld AW, de Jong FH, Grobbee DE, Pols HA, Lamberts SW. Measures of bioavailable serum testosterone and estradiol and their relationships with muscle strength, bone density, and body composition in elderly men. J Clin Endocrinol Metab 2000;85(9):3276-82.
30. Webb CM, McNeill JG, Hayward CS, de Zeigler D, Collins P. Effects of testosterone on coronary vasomotor regulation in men with coronary heart disease. Circulation 1999;100(16):1690-6.
31. Hanke H, Lenz C, Hess B, Spindler KD, Weidemann W. Effect of testosterone on plaque development and androgen receptor expression in the arterial vessel wall. Circulation 2001;103(10):1382-5.
32. Ong PJ, Patrizi G, Chong WC, Webb CM, Hayward CS, Collins P. Testosterone enhances flow-mediated brachial artery reactivity in men with coronary artery disease. Am J Cardiol 2000;85(2):269-72.
33. Datz C, Haas T, Rinner H, Sandhofer F, Patsch W, Paulweber B. Heterozygosity for the C282Y mutation in the hemochromatosis gene is associated with increased serum iron, transferrin saturation, and hemoglobin in young women: a protective role against iron deficiency? Clin Chem 1998;44(12):2429-32.
34. Whitfield JB, Cullen LM, Jazwinska EC, Powell LW, Heath AC, Zhu G, Duffy DL, Martin NG. Effects of HFE C282Y and H63D polymorphisms and polygenic background on iron stores in a large community sample of twins. Am J Hum Genet 2000;66(4):1246-58.
35. Zee RY, Ridker PM, Stampfer MJ, Hennekens CH, Lindpaintner K. Prospective evaluation of the angiotensin-converting enzyme insertion/deletion polymorphism and the risk of stroke. Circulation 1999;99(3):340-3.

36. Butler R, Morris AD, Burchell B, Struthers AD. DD angiotensin-converting enzyme gene polymorphism is associated with endothelial dysfunction in normal humans. Hypertension 1999;33(5):1164-8.
37. Carluccio M, Soccio M, De Caterina R. Aspects of gene polymorphisms in cardiovascular disease: the renin- angiotensin system. Eur J Clin Invest 2001;31(6):476-88.
38. Gerraty RP, Parsons MW, Barber PA, Darby DG, Desmond PM, Tress BM, Davis SM. Examining the lacunar hypothesis with diffusion and perfusion magnetic resonance imaging. Stroke 2002;33(8):2019-24.
39. Viscoli CM, Brass LM, Kernan WN, Sarrel PM, Suissa S, Horwitz RI. A clinical trial of estrogen-replacement therapy after ischemic stroke. N Engl J Med 2001;345(17):1243-9.
40. Tenover JL. Experience with testosterone replacement in the elderly. Mayo Clin Proc 2000;75 Suppl:S77-81; discussion S82.
41. Cohen A, Tzourio C, Bertrand B, Chauvel C, Bousser MG, Amarenco P. Aortic plaque morphology and vascular events: a follow-up study in patients with ischemic stroke. FAPS Investigators. French Study of Aortic Plaques in Stroke. Circulation 1997;96(11):3838-41.
42. Iribarren C, Sidney S, Sternfeld B, Browner WS. Calcification of the aortic arch: risk factors and association with coronary heart disease, stroke, and peripheral vascular disease. Jama 2000;283(21):2810-5.
43. Mitusch R, Doherty C, Wucherpfennig H, Memmesheimer C, Tepe C, Stierle U, Kessler C, Sheikhzadeh A. Vascular events during follow-up in patients with aortic arch atherosclerosis. Stroke 1997;28(1):36-9.
44. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet 2002;360(9326):7-22.

7
Summary/Samenvatting

7.1 | Summary

This thesis describes the relation between atherosclerosis, hormones and genetic factors in relation to the risk of stroke. The results are based on the Rotterdam Study, a large population based cohort study among 7,983 persons aged 55 years or older. A total of 7,721 persons were free from previous stroke. The cohort was followed for morbidity, including stroke. Until January 1, 1999, 432 persons suffered from a first stroke.

Chapter 2 describes the incidence and survival of stroke. The incidence rate increased with age and was higher in men than in women over the entire age range. The incidence rate varied from 1.7 per 1000 personyears in men aged 55 to 59 to 69.8 per 1000 personyears in men aged 90 years or over. Corresponding rates for women were 1.2 and 33.1 per 1000 personyears, respectively. Although incidence rates were higher in men than in women, lifetime risks were similar (21% for persons aged 55 years). The explanation is that the longer life expectancy in women counterbalances the higher incidence rates of stroke in men. Because of this, women have their stroke on average at a higher age than men. We observed no differences in survival between men and women.

Chapter 3 shows the relation between several measures of atherosclerosis and risk of stroke. **Chapter 3.1** reviews the knowledge regarding subclinical markers of atherosclerosis and risk of stroke. In **chapter 3.2** the relation between an increased wall thickness in the carotid artery and subtypes of stroke is described. We found that an increased wall thickness did not only increase the risk of stroke, but also the risk of intracerebral hemorrhage and lacunar infarction, irrespective of cardiovascular risk factors. The relevance of carotid plaques in persons without a previous stroke or TIA was not yet clear. **Chapter 3.3** describes the prospective study on the relation between plaques, measured at six locations in the carotid artery, and stroke and subtypes of cerebral infarction. We found that plaques increased the risk of stroke, irrespective of location in the carotid artery. Further, plaques were related to infarctions in the anterior, but not the posterior circulation. There was a strong relation between carotid plaques and risk of lacunar infarction. We concluded that carotid plaques are sources of emboli as well as markers of generalized atherosclerosis. Presence of calcifications in coronary arteries as assessed by electron beam tomography is another measure of atherosclerosis that we investigated in **chapter 3.4**. The amount of calcification was related to a history of stroke. Other cardiovascular risk factors did not explain this relation. **Chapter 3.5** shows the results of our study on the relation between arterial stiffness, a functional measure of

atherosclerosis, and risk of stroke. Stiffness in the aorta was not clearly related to a history of stroke, whereas persons with severe carotid stiffness were 12 times more likely to to have had a stroke. Finally we compared different measures of atherosclerosis in relation to stroke, namely carotid plaques and intima-media thickness, ankle-arm index and calcifications in the aorta (**chapter 3.6**). We found that carotid intima-media thickness and aortic calcifications were strongest related to the risk of stroke. Cardiovascular risk factors largely explained the relation that we found between ankle-arm index and stroke. The relations with other measures of atherosclerosis remained after adjustment for cardiovascular risk factors. Aortic calcifications and intima-media thickness were independently of each other related to the risk of stroke. They may be used to identify persons at high risk of stroke who may benefit from intervention.

Chapter 4 deals with the relation between endogenous sex hormones (testosterone and estrogen) and risk of stroke in men and postmenopausal women. Estradiol was not related to the risk of stroke, neither in men nor in women. The effect of estrogen replacement in postmenopausal women on cardiovascular disease is a hot topic. Our results are in line with recent clinical trials that failed to report a beneficial effect from estrogen replacement therapy on the risk of stroke. A decreased testosterone level was related to an increased risk of stroke in men, in particular in those who did not smoke. The relation remained after adjustment for comorbidity. It is still too early to conclude that men with decreased testosterone levels may benefit from testosterone replacement therapy. The precise underlying mechanism needs to be explained.

In **chapter 5**, the relation between two candidate genes and stroke is described. In **chapter 5.1** we report that there is no clear relation between the D-allele in the angiotensin converting enzyme (ACE) gene polymorphism and risk of stroke. **Chapter 5.2** describes the relation between mutations in the hemochromatosis gene and stroke. Two mutations (C282 and H63D) were related to the risk of stroke and increased carotid intima-media thickness in persons who smoked or had hypertension.

Finally, in **chapter 6** we discuss our findings in the light of other studies. We comment on methodological issues regarding atherosclerosis and stroke subtypes. We further discuss the results and comment on mechanisms underlying the relation between atherosclerosis and stroke. Also, we discuss the results regarding sex hormones and genetic factors in relation to stroke. Subsequently we comment on the clinical relevance of our findings. We state

that in ageing societies, prevention of stroke is of great importance in men as well as in women. Further, the combination of information on wall thickness and arterial calcifications could be used to identify persons at high risk of stroke. From our results on sex hormones we cannot conclude that estrogen replacement therapy in women has no effect in on the risk of stroke. The mechanism underlying the relation between testosterone and stroke in men needs clarification before we can conclude that testosterone replacement therapy in men can have beneficial effects on the risk of stroke. The results on genetic factors suggest that information on genetic factors may be used to intensify preventive therapy in persons with a specific genetic make-up.

Finally we do recommendations for future research on the role of atherosclerosis, hormones and genetic factors in relation to stroke. A few measures of atherosclerosis need prospective evaluation in relation to stroke. Further, we do suggestions for research that may help the identification of persons at risk for stroke and who may benefit most from preventive therapy. We further discuss how the assessment of stroke subtypes can be improved in population-based studies. Further epidemiological research can help the prevention of stroke.

7.2 | Samenvatting

Dit proefschrift beschrijft de relatie tussen aderverkalking, hormonen en erfelijke factoren en het risico op beroerte. De resultaten zijn gebaseerd op het Erasmus Rotterdam Gezondheid en Ouderen (ERGO) onderzoek. Dit is een bevolkingsonderzoek onder 7983 personen van 55 jaar of ouder dat in 1990 is gestart in de Rotterdamse wijk Ommoord. Onder de deelnemers hadden 7721 personen geen beroerte in de voorgeschiedenis. Het cohort werd gevolgd en het optreden van verschillende ziekten, inclusief beroerte, werd vastgelegd. In de periode tot januari 1999 kregen 432 personen voor het eerst een beroerte.

Hoofdstuk 2 beschrijft de incidentie van beroerte, ofwel het aantal nieuwe ziektegevallen dat zich in de loop van een tijdperiode voordoet. Het incidentiecijfer stijgt met de leeftijd en is op alle leeftijden hoger voor mannen dan voor vrouwen. De incidentie varieert van 1,7 voor mannen in de leeftijd van 55 tot 59 jaar tot 69,8 per 1000 persoonsjaren voor mannen ouder dan 95 jaar. De corresponderende incidentiecijfers voor vrouwen zijn 1,2 en respectievelijk 33,1 per 1000 persoonsjaren. Alhoewel beroerte vaker voorkomt bij mannen dan bij vrouwen, vonden we dat de kans om gedurende de rest van het leven een beroerte te krijgen voor mannen en vrouwen even groot is (21% voor personen van 55 jaar). Dit wordt verklaard doordat een langere levensverwachting voor vrouwen de lagere incidentiecijfers ten opzichte van mannen compenseert. Ten opzichte mannen krijgen vrouwen op hogere leeftijd een beroerte.

Hoofdstuk 3 laat de relatie tussen verschillende maten van aderverkalking en beroerte zien. **Hoofdstuk 3.1** geeft een overzicht van de kennis omtrent subklinische maten van aderverkalking in relatie tot beroerte. In **hoofdstuk 3.2** wordt het onderzoek naar de relatie tussen wanddikte in de halsslagader en subtypen van beroerte beschreven. Een dikkere vaatwand verhoogt het risico op beroerte, maar ook het risico op een hersenbloeding en lacunair infarct, onafhankelijk van andere risicofactoren voor hart- en vaatziekten. De relevantie van plaques in de halsslagader bij personen zonder beroerte of TIA in de voorgeschiedenis was nog onduidelijk. **Hoofdstuk 3.3** beschrijft het prospectieve onderzoek naar de relatie tussen plaques in de halsslagader op 6 verschillende locaties en het risico op beroerte en subtypen van herseninfarct. We vonden dat relatie tussen plaques en beroerte onafhankelijk is van de locatie van de plaque in de halsslagader. Plaques zijn verder gerelateerd aan infarcten in de voorste, en niet in de achterste hersencirculatie. Verder vonden we een sterk verband tussen plaques en

lacunaire infarcten. We concludeerden dat plaques zowel bronnen van embolieën zijn als een maat van gegeneraliseerde aderverkalking. **Hoofdstuk 3.4** gaat in op de relatie tussen aanwezigheid van kalk in de kransslagaderen, gemeten met behulp van electron-beam tomografie, en beroerte. We vonden dat de hoeveelheid kalk in de kransslagaderen gerelateerd is aan beroerte in de voorgeschiedenis. Het verband werd niet verklaard door andere risicofactoren voor hart- en vaatziekten. **Hoofdstuk 3.5** laat resultaten zien van de studie over de relatie tussen vaatwandstijfheid, een functionele maat van aderverkalking, en beroerte. Stijfheid in de grote lichaamsslagader was niet duidelijk gerelateerd aan beroerte, maar voor personen met ernstige vaatwand stijfheid in de halsslagader was de kans wel groter om een beroerte te hebben doorgemaakt. Tenslotte vergeleken we verschillende maten van aderverkalking in relatie tot het risico op beroerte, namelijk vaatwanddikte en plaques in de halsslagader, enkel-arm index en kalk in de grote lichaamsslagader (**hoofdstuk 3.6**). We vonden dat wanddikte in de halsslagader en aanwezigheid van kalk in de grote lichaamsslagader het sterkst waren gerelateerd aan de kans op beroerte, onafhankelijk van elkaar. Rekening houdend met andere risicofactoren voor hart- en vaatziekten verdween de relatie tussen enkel-arm index en beroerte. De overige maten bleven gerelateerd aan het risico op beroerte. Informatie over deze maten kan mogelijk worden gebruikt om personen met een verhoogd risico op beroerte op te sporen en te behandelen.

Hoofdstuk 4 gaat over de relatie tussen geslachtshormonen die het lichaam zelf aanmaakt (testosteron en oestrogeen) en beroerte bij mannen en vrouwen na de overgang. Noch bij mannen noch bij vrouwen vonden we een relatie tussen oestrogenen en risico op beroerte. Er is veel discussie over het effect van oestrogeen pillen bij vrouwen na de overgang. Onze resultaten sluiten deels aan bij resultaten van klinische trials die geen gunstig effect hebben aangetoond van oestrogeen pillen op het risico op beroerte bij vrouwen. Een lager testosteron gehalte was gerelateerd aan een hoger risico op beroerte en herseninfarct bij mannen, in het bijzonder bij de niet-rokers. Rekening houdend met maten van algemene gezondheid bleef de relatie bestaan. Het is nog te vroeg om te concluderen dat oudere mannen met een verlaagd testosteron gehalte baat kunnen hebben bij testosteron pillen. Het precieze mechanisme van de relatie moet nog verder worden onderzocht.

De relatie tussen twee kandidaat-genen en beroerte wordt beschreven in **hoofdstuk 5**. In **hoofdstuk 5.1** beschrijven we dat er geen duidelijke relatie

bestaat tussen de aanwezigheid van het D-allel in het angiotensine-converting enzym (ACE) polymorfisme en risico op beroerte. **Hoofdstuk 5.2** beschrijft de relatie tussen mutaties in het gen voor hemochromatose en risico op beroerte. Twee mutaties (C282Y en H63D) waren gerelateerd aan beroerte en wanddikte in personen die rookten of een verhoogde bloeddruk hadden.

Tenslotte bespreken we in **hoofdstuk 6** de bevindingen in het kader van andere studies. We maken een aantal methodologische kanttekeningen bij het bestuderen van aderverkalking en subtypen van beroerte. Verder bespreken we de resultaten waarbij we ingaan op het mechanisme wat ten grondslag ligt aan de relatie tussen maten van aderverkalking en beroerte. Tevens bespreken we de resultaten met betrekking tot geslachtshormonen en erfelijke factoren in relatie tot beroerte. Daarna bespreken we de klinische relevantie van de resultaten. Daarbij komt naar voren dat preventie van beroerte in een vergrijzende samenleving van groot belang is, zowel voor mannen als voor vrouwen. Verder zou informatie over verdikking van de vaatwand, gecombineerd met gegevens over verkalking gebruikt kunnen worden om personen met een verhoogd risico op te sporen. Met betrekking tot geslachtshormonen kunnen we op grond van onze studie niet concluderen dat oestrogeen suppletie bij vrouwen geen effect heeft op het risico op beroerte. Voordat we kunnen concluderen dat testosteron suppletie bij mannen een gunstig effect heeft op het risico op beroerte moet eerste het onderliggende mechanisme van de relatie tussen testosteron en hart- en vaatziekten worden onderzocht. De uitkomst van onze studies naar kandidaat genen suggereert dat informatie over erfelijke factoren in de toekomst mogelijk kan worden gebruikt om bij bepaalde personen intensievere preventie toe te passen.

Tot slot geven we aanbevelingen voor verder onderzoek naar de rol van aderverkalking, hormonen en erfelijke factoren in relatie tot beroerte. Een aantal maten van aderverkalking moet nog prospectief worden onderzocht in relatie tot beroerte. Vervolgens bespreken we hoe de diagnose van beroerte en typering kunnen worden verbeterd in bevolkingsonderzoeken. Verder epidemiologisch onderzoek naar oorzaken van beroerte kan bijdragen aan preventie van beroerte.

Dankwoord

Er zijn veel mensen die ik graag wil bedanken voor hun bijdrage aan de totstandkoming van dit proefschrift. Op de eerste plaats dank ik de deelnemers aan het ERGO onderzoek en alle meewerkende huisartsen, zonder wie dit bevolkingsonderzoek niet zou kunnen worden uitgevoerd.

Mijn co-promotor Monique Breteler, dank dat jij me mede hebt aangenomen op dit project. Ik heb veel geleerd van jouw kritische kijk op analyses, stijl en inhoud van manuscripten. De vrijheid die je me in het begin gaf is goed geweest voor mijn zelfstandigheid. Wanneer ik je echt nodig had, was je er. Ik heb het als erg prettig ervaren dat we naast het werk ook over andere onderwerpen met elkaar van gedachten konden wisselen. Mijn eerste promotor Bert Hofman, dank voor de energieke manier waarop je mensen, waaronder mij, enthousiast maakt voor de epidemiologie. Bedankt dat je deur zowel letterlijk als figuurlijk altijd voor mij openstond. Mijn tweede promotor Rick Grobbee; dat de afstand tussen Rotterdam en Utrecht betrekkelijk is, blijkt uit het feit dat ons contact in de loop van de tijd intensiever is geworden. Ik heb dat als plezierig ervaren. Veel dank voor de kritische en stimulerende commentaren op mijn werk. Dan een bijzonder woord van dank voor Prof. Koudstaal, zonder wie het onderzoek naar beroerte in ERGO niet zo'n vlucht had kunnen nemen. U was als een promotor voor mij. Zeer veel dank voor de tomeloze energie waarmee u beroerten hebt gecodeerd en hebt meegedacht, vooral over de classificatie van subtypen van beroerte. Verder veel dank aan Dr. Michiel Bots, van wie ik wat stroke betreft het estafettestokje overnam. Beste Michiel, ik heb veel van je geleerd en ik kijk terug op een prettige samenwerking.

Mijn neuro-epi collega's Annemieke, Ewoud, Frank Erik, Frank Jan, Henning, Jan Cees, Marianne, Mirjam, Muriël, Niels, Patricia, Sandra, Sanjay, Sarah en Tom bedank ik voor de gezellige tijd op de hoogbouw. Zoltan Voko, thank you for your contribution to the stroke research and for your friendship.

Dat beroerte een cerebro*vasculaire* ziekte is, brengt met zich mee dat er veel samenwerking was met de cardiovasculaire onderzoeksgroep. Daarbij wil ik allereerst Jacqueline Witteman bedanken voor haar gedegen commentaar op papers en goede ideeën voor analyses. Mijn kamergenoot en medeonderzoeker op het NWO-project Antonio Iglesias; dank voor je bijdrage aan de carotis-metingen. Ik ben blij met de eerlijkheid waarmee we dingen konden bespreken. Verder wil ik Nicole van Popele, Rozemarijn Vliegenthart en Liesbeth Hak

danken voor de samenwerking bij een aantal artikelen. Liesbeth, jouw hartverwarmende mailtjes hebben mij laatste periode erg gesteund. Verder Annette van den Elzen, heerlijk dat we oeverloos over onze kinderen konden kletsen. Rogier Nijhuis, veel dank voor je uitleg over de AIC analyse. Ik heb je zien trouwen!

Verder dank ik Prof. Cock van Duijn voor de mogelijkheid om samen te werken met Omer Njajou en Fakhredin Sayed. Omer and Fakhredin, thank you for your contribution to the analyses and interpretation regarding genetic factors and stroke. Prof. Pols, Prof. de Jong en Dr. Mirjam Geerlings dank ik voor de bijdrage aan de interpretatie van analyses betreffende geslachtshormonen.

Mijn kamergenoten Antonio Iglesias, Henning Tiemeier, Marjolein van der Klift en Arjan Bergink dank ik voor de vele uren waarin we lief en leed hebben gedeeld. Het is een enorme steun voor me geweest dat we in het zelfde schuitje zaten met de daarbij behorende ups en downs. Henning, ik kijk met veel plezier terug op onze discussies die niet alleen over onderzoek, maar ook over allerdaagse beslommeringen konden gaan. Ik heb veel gehad aan je morele steun. Succes met het verder opzetten van je eigen onderzoekslijn. Tot mijn kamergenoten behoorden ook Toos Stehman, Inge Haumersen, Pauli van Eldik en Louise van Kleeff. Allen zeer bedankt voor de bijdrage aan de carotis metingen en voor de gezellige uurtjes op de hoogbouw. Dick Slof, dank voor je praktische steun.

Alle medewerkers van het ERGO-centrum bedank ik voor hun inzet voor het onderzoek. Anneke Korving, bijzonder is de manier waarop je iedereen thuis kunt laten voelen op het centrum, maar ook geen blad voor de mond neemt. Korte tijd werkten we intensief samen en daar kijk ik met veel plezier op terug. Jolande, Anneke, Lida, Elly, Lilian en niet te vergeten Deidre: dank voor jullie bijdrage aan de follow-up van o.a. beroerte. Ingrid van Vuuren, dank voor je belangstelling en inzet voor de codering van beroerte. Ik mis de gezamenlijke koffies. Frank van Rooij bedankt voor de samenwerking op meerdere terreinen. Nano Suwarno, Eric Neeleman en Rene Vermeeren dank ik voor de onmisbare ondersteuning op gebied van automatisering. Marga en Marti, bedankt voor jullie inzet op het secretariaat. Theo Stijnen, Lidia, Maria en Bettina dank ik voor de statistische ondersteuning.

Susan Vos, alhoewel jouw mobiliteit beperkt was door je vergevorderde zwangerschap was je bereid de cover te ontwerpen. Dankjewel voor het fraaie resultaat.

Ik ben erg blij dat mijn paranimfen Sarah Vermeer en Lucian Wolthuis mij bij zullen bijstaan tijdens de promotie. Sarah, zonder jou zou mijn tijd op de hoogbouw er heel anders hebben uitgezien. Naast verschillen ontdekten we dat we behalve ons onderzoeksonderwerp ook andere overeenkomsten hadden en dat smeedde een hechte band tussen ons. Jouw werklust is voor mij aanstekelijk geweest. Dank voor je steun op vele fronten. Dat professioneel onze wegen gaan scheiden zal zeker niet inhouden dat we elkaar uit het oog verliezen. Lucian, door je heldere en nuchtere kijk op het leven en ons vak ben je altijd een goed klankbord voor mij geweest. Dat we op veel punten op dezelfde manier tegen dingen aankijken komt ook tot uiting doordat we uiteindelijk voor hetzelfde vak kiezen. Ik hoop dat ik in de komende tijd weer ruimte heb om je vaker op te zoeken.

Nanette, Akkelies, Hester, Marco en Erna; bedankt voor jullie vriendschap en voor de interesse in alles wat niets met mijn werk te maken heeft.

Lieve pa en ma, als geen ander hebben jullie moeten leren leven met de gevolgen van vaat- en hersenschade. Ik bewonder jullie veerkracht en steun aan jullie kinderen, wars van verwachtingspatronen. Dank voor de vrijheid om mijn eigen weg te gaan. Mijn broer Geert-Jan, dank voor je oprechtheid en interesse. Jaap en Vera, dank voor jullie belangstelling en praktische steun.

Tenslotte de personen die mij het meest dierbaar zijn. Lieve Nienke, je bent nog te klein om te realiseren wat er allemaal gebeurt. Je bent een kanjer, ik hou van je en kijk er naar uit om de tijd die ik met jou gemist heb in te halen. Geert Jan, zonder jouw steun had ik dit niet kunnen bolwerken. Jij bent vanaf het schrijven van mijn sollicitatiebrief tot en met de lay-out van dit proefschrift mijn grootste supporter en criticus geweest. Zeker de laatste tijd is voor jou niet makkelijk geweest. Ik hou van je en verlang er naar om samen meer te genieten van de mooie dingen in het leven. De cirkel is rond.

CURRICULUM VITAE

Monika Hollander was born on March 31, 1972 in Groningen, The Netherlands. In 1990 she graduated at the 'Augustinus College' in Groningen (VWO). For one year she studied Sociology and English at the Rijks Universiteit Groningen. In 1991 she started her medical study at the Rijks Universiteit Groningen. During this period she participated in research on chronic obstructive pulmonary disease at the Department of Pediatrics at the Academical Hospital Groningen. In 1998 she started the work described in this thesis at the Department of Epidemiology and Biostatistics (Prof.dr. A. Hofman), in close collaboration with the Department of Neurology, Erasmus MC Rotterdam (Prof.dr. P.J. Koudstaal). During that period she obtained a Master of Science degree in Clinical Epidemiology from the Netherlands Institute of Health Sciences. From June 2002 to January 2003 she worked at the department of Neurology at the Erasmus MC Rotterdam (Prof.dr. P.A.E. Sillevis Smitt). She plans to start a training as general practitioner.

LIST OF PUBLICATIONS

M Hollander, PJ Koudstaal, ML Bots, DE Grobbee, A Hofman, MMB Breteler. Incidence, risk and case fatality of stroke in the elderly; the Rotterdam Study. (J Neurol Neurosurg Psychiatry 2003, accepted).

M Hollander, MMB Breteler. Markers of subclinical vascular disease and stroke. In: Gorelick PB, Alter M, eds. Stroke Prevention. London, United Kingdom, Parthenon Publishing, 2002, page 105-114.

M Hollander, ML Bots, A Iglesias del Sol, PJ Koudstaal, JCM Witteman, DE Grobbee, A Hofman, MMB Breteler. Carotid plaques increase the risk of stroke and subtypes of cerebral infarction in asymptomatic elderly; the Rotterdam Study. Circulation 2002;105:2872-7.

R Vliegenthart, M Hollander, MMB Breteler, DAM van der Kuip, A Hofman, M Oudkerk, JCM Witteman. Stroke is associated with coronary calcifications as detected by electron-beam CT. The Rotterdam Coronary Calcification Study. Stroke 2002;33:462-465.

OT Njajou, M Hollander, PJ Koudstaal, A Hofman, JCM Witteman, MMB Breteler, CM van Duijn. Mutations in the hemochromatosis gene (HFE) and stroke. Stroke 2002;33:2363-6.

A Iglesias del Sol, KG Moons, M Hollander, A Hofman, PJ Koudstaal, DE Grobbee, MMB Breteler, JCM Witteman, ML Bots. Is carotid intima-media thickness useful in cardiovascular disease risk assessment? The Rotterdam Study. Stroke 2001;32:1532-8.