Determinants, Comorbidities, and Long-term Prognosis of Stroke

An Epidemiological Approach

Marileen Portegies

Acknowledgements

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Determinanten, comorbiditeiten en langetermijnprognose van beroerte

Een epidemiologische benadering

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aan mijn ouders

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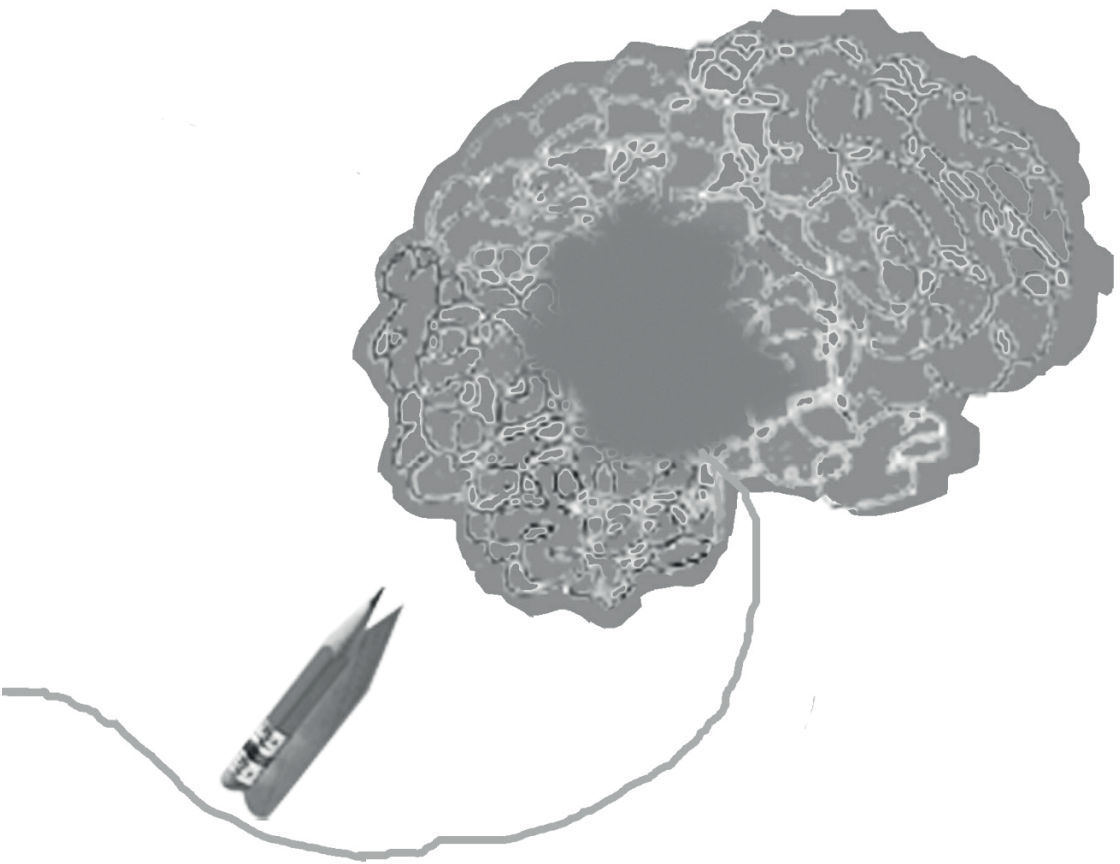
Chapter 1

General introduction

Annually, almost 17 million people suffer a stroke worldwide, making stroke the second leading cause of death.[1,2] Many stroke-survivors remain dependent in their daily living, have cognitive deficits, and need institutional care.[3,4] Stroke therefore poses a tremendous burden on patients, family, caregivers, and society.[1,5] As a consequence of the ageing population this burden is expected to increase further over the next decades.[6] Previous efforts to prevent and treat stroke have been fruitful resulting in reductions in both incidence (12%) and mortality (37%) over the last two decades.[2,7,8] However, stroke remains a serious health problem. This means that prevention and treatment need further improvement. In my thesis I will focus on emerging risk factors and markers of stroke, which may be a first step towards a better prevention.

The key to prevention is answering the question why some people will suffer a stroke whilst others do not. This is challenging, since most strokes are unannounced first-ever events.[7] Suddenly, patients experience weakness, sensory loss, blindness and/or language disorders, as a consequence of an obstructed or ruptured artery in the brain.[9] Despite this rapid start, increasing evidence suggests that pathologic changes leading up to stroke are present years in advance. For instance, consider the most common etiologies of ischemic stroke, specifically cardioembolism, large vessel disease, and small vessel disease.[10] Each of these appears to have a long preclinical phase, characterized by decrease in cardiac function, accumulation of atherosclerotic plaques, or arteriolosclerosis.[11-13] Imaging techniques[12-14] and laboratory tests[15] detecting these preclinical changes earlier in time are emerging. Knowing how such preclinical changes relate to stroke may further unravel the pathophysiology of stroke. This knowledge will aid in the identification of people at high risk of stroke and who may stand to benefit from targeted therapies. This is therefore an essential first step before a better prediction and prevention of stroke can be achieved.

Another approach to reduce the burden of stroke is to prevent its common and invalidating consequences, e.g. recurrent stroke, dementia, and death.[16-19] Due to the implementation of stroke units, intravenous thrombolysis and intra-arterial thrombectomy, and better secondary prevention, an increasing number of people now survives the acute phase of stroke and is at risk of these consequences.[2,8,20-22] Recurrent stroke, post-stroke dementia and post-stroke mortality often have a vascular etiology and seem to benefit from secondary prevention.[23-25] However, as described in the previous paragraph, vascular pathology has a long accumulation time and is a major risk factor for the stroke itself. Part of the prognosis after stroke may therefore already be determined by pre-stroke cardiovascular risk factors. An important consideration here is that most studies on stroke prognosis recruit participants at time of stroke and therefore cannot measure pre-stroke values.[17,26-30] Post-stroke values may not be very representative of pre-stroke values due to reverse causality, i.e. levels of risk factors can change due to the stroke.[31-33] Yet, knowing the influence of pre-stroke factors is highly relevant, since a large influence implies that intensive cardiovascular risk factor management implemented after stroke may start too late.[34]

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Against this background, the aim of my thesis was twofold. First, to detect early risk factors or markers for stroke, mainly related to the cardiovascular system. Second, to detect pre-stroke risk factors of consequences after stroke. A population-based study is required to detect such early markers, since it collects information on risk factors prior to the stroke. Studies in my thesis were therefore embedded within the Rotterdam Study, a population-based study that aims to study the occurrence and determinants of age-related diseases in the general population.[35] The Rotterdam Study started in 1990, with 7,983 participants aged 55 years and over. It was expanded twice; in 2000 a total of 3,011 participants aged 55 years and over were added, and in 2006 another 3,932 participants aged 45 and over joined. All participants resided in Ommoord, a suburb of Rotterdam, the Netherlands.

The outline of this thesis is as follows:

Chapters 2, 3, 4 discuss determinants of stroke. **Chapter 2** focuses on determinants of heart disease, with an emphasis on the relation between subclinical cardiac dysfunction and stroke. Subclinical cardiac dysfunction was measured by echocardiography (**Chapter 2.1**) and N-terminal pro-B-type natriuretic peptide (NT-proBNP), a laboratory value reflecting cardiac ventricular wall stress (**Chapter 2.2**).[36,37]

Chapter 3 includes determinants of large and small vessel disease. **Chapter 3.1** describes the relation between intracranial carotid artery atherosclerosis, a marker of large vessel disease, and the risk of stroke. In **Chapter 3.2**, I examined how vasomotor reactivity, another potential marker of large vessel disease, affects the risk of mortality independent of stroke. **Chapter 3.3** describes the association between microbleeds, a marker of small vessel disease, and the risk of stroke. Hypothesizing that markers may interact in their risk of stroke, I examined in **Chapter 3.4** the interaction between retinal vessel diameter, a marker of small vessel disease, and cerebral blood flow for their association with stroke. In **Chapter 3.5**, I focused on hypertension, which is the most important modifiable vascular risk factor of stroke, as the risk of stroke may be further influenced by long-term trajectories of hypertension.

Some non-cardiovascular disorders also seem to have an impact on the cardiovascular system. This has for instance been suggested for COPD and anxiety.[38,39] **Chapter 4** therefore describes the relation of COPD (**Chapter 4.1**) and anxiety (**Chapter 4.2**) with stroke.

Chapter 5 is dedicated to problems occurring after stroke. In **Chapter 5.1**, I describe the difference in recognition between left- and right-sided strokes. **Chapter 5.2 and 5.3** respectively focus on the effect of pre-stroke cardiovascular risk factors in relation to the risk of mortality and recurrent stroke and dementia after stroke. In **Chapter 5.4**, I explain how high education or cognitive reserve might protect against dementia.

Finally, in **Chapter 6**, I summarize my main findings, discuss the methodological considerations and give suggestions for further research within the field of stroke.

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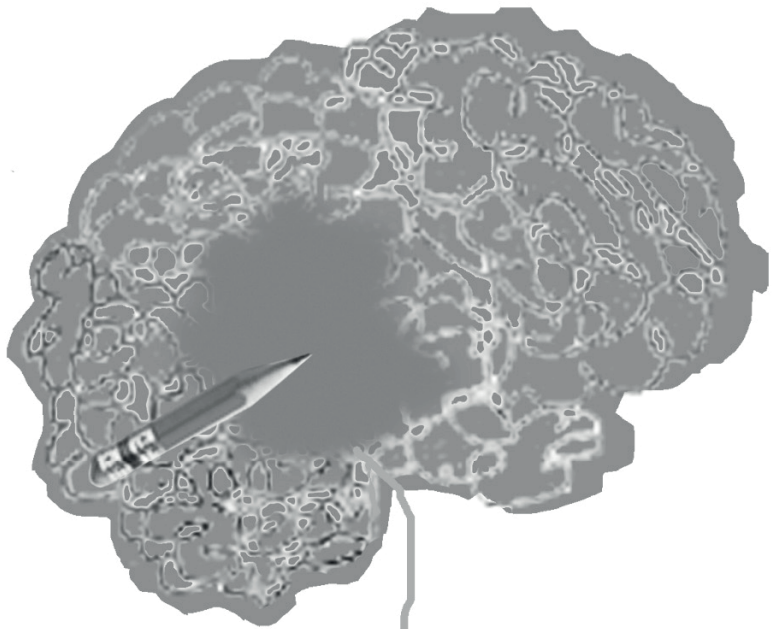
Chapter 2

The heart and stroke

Chapter 2.1

Subclinical cardiac dysfunction increases the risk of stroke and dementia

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Abstract

Background and purpose
Clinical cardiac disease has often been associated with stroke and dementia. However, the association between subclinical cardiac dysfunction and these major neurological diseases is unknown. We investigated the association between cardiac function and the risk of stroke and dementia in elderly free of clinical cardiac disease. Additionally, we investigated the relation between cardiac function and MRI markers of subclinical cerebrovascular disease.

Methods
This study was conducted within the population-based Rotterdam Study. A total of 3,291 participants (60.8% female, age-range 58-98 years) free of coronary heart disease, heart failure, atrial fibrillation, stroke, and dementia, underwent echocardiography in 2002-2005 to measure cardiac function. Follow-up finished in 2012. In 2005-2006, a random subset of 577 stroke-free people without dementia underwent brain MRI on which infarcts and white matter lesion volume were assessed.

Results
During 21,785 person-years of follow-up, 164 people suffered a stroke and during 19,462 person-years of follow-up, 208 people developed dementia. Measures of better diastolic function, such as higher E/A-ratio, were associated with a lower risk of stroke (HR 0.82, 95% CI 0.69; 0.98) and dementia (HR 0.82, 95% CI 0.70; 0.96). Better systolic function, measured as higher fractional shortening, was only associated with a lower risk of stroke (HR 0.84, 95% CI 0.72; 0.98). Better diastolic function was related to a lower prevalence of silent infarcts on MRI, especially lacunar infarcts.

Conclusions
In elderly free of clinical cardiac disease, worse diastolic function is associated with clinical stroke, dementia, and silent infarcts on MRI, whereas worse systolic function is related only to clinical stroke. These findings can form the basis for future research on the utility of cardiac function as potential intervention target for prevention of neurological diseases.

Introduction

Stroke and dementia are major neurological diseases in the elderly.[1,2] Cardiovascular risk factors play a role in the etiology of both stroke and dementia, including Alzheimer disease (AD).[1,3] Additionally, clinical cardiac diseases, such as heart failure,[4,5] atrial fibrillation,[6,7] and coronary heart disease[8,9] have been associated with stroke, dementia, AD, and subclinical cerebrovascular damage, such as silent infarcts[10] and white matter lesions.[11] Cerebrovascular damage accumulates slowly before manifesting as clinical event, and silent brain infarcts and white matter lesions indicate an increased risk of stroke and dementia.[12-14]

On the one hand, shared etiology might explain the link between cardiac and neurological diseases. On the other hand, cardiac disease might be causally related to stroke and dementia via thrombus formation or hypoperfusion.

In the general elderly population, cardiac function is often impaired in the absence of clinical cardiac disease.[15-17] Subclinical cardiac dysfunction has been associated with an increased risk of clinical cardiac events and mortality.[17-19] However, the longitudinal association between subclinical cardiac dysfunction and major neurological outcomes remains unclear. Relating echocardiographic markers of subclinical cardiac dysfunction to stroke and dementia is important for possible preventive strategies.

We investigated whether cardiac function is associated with the risk of stroke and dementia in people without clinical cardiac disease. Additionally, we investigated whether cardiac function is related to MRI markers of subclinical cerebrovascular disease.

Materials and methods

Setting and study population

This study was embedded within the Rotterdam Study, a prospective population-based cohort study among people aged 55 years and older residing in Ommoord, a district of Rotterdam, the Netherlands.[20] The study started in 1990 with 7,983 participants and was extended in 2000 with 3,011 people. Follow-up examinations take place every 3 to 4 years.[20]

For the current study, data on echocardiography were collected in 2002-2005, because echocardiography was only introduced in this round. During this period 3,550 participants of the original cohort attended their fourth examination round and 2,486 participants of the cohort expansion attended their second examination round. Of these participants, 5,395 actually visited the study center and 5,287 underwent echocardiography. Missing echocardiograms were primarily caused by absence of echocardiographers and were random. We excluded participants who had a poor quality echocardiogram (n=233) or missed measurements on the echocardiogram (n=483). Participants with prevalent heart failure, coronary heart disease, atrial fibrillation, stroke, or dementia (n=765), or missing data on these diseases (n=515) were also excluded. Consequently, 3,291 participants were eligible for analysis. Because MRI-scanning was implemented in 2005,[21] only a random subset of 593 non-demented, stroke-free people had available brain MRI, of which 577 had good quality MRI data. For analyses with white matter lesions, cortical infarcts (n=6) were excluded, as tissue loss and

gliosis surrounding cortical infarcts may cause unreliable segmentations. MRI-scanning was performed on average 1.0 (standard deviation (SD) ±0.4) years after echocardiography. The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study and written informed consent was obtained.

Echocardiography

Transthoracic echocardiograms were performed by 4 trained echocardiographers using commercially available systems (AU3 Partner, Esaote Biomedica, Hallbergmoos, Germany, with a 3.5/2.5MHz transducer, n=1,289; or Acuson Cypress, Siemens, Mountain View, USA, with a 3V2c transducer, n=2,002).

To assess diastolic function, transmitral filling velocities were recorded by pulsed wave Doppler in the apical 4-chamber view. The sample volume was placed in the mitral valve orifice near the tips of the leaflets. The early filling velocity occurring with mitral valve opening is the peak E velocity. Peak A velocity is the velocity occurring with contraction of the atrium.[22] Doppler peak E and peak A velocities were averaged over 3 cycles. Doppler peak E velocity was divided by Doppler peak A velocity to calculate E/A-ratio. The time between the peak E wave and the upper deceleration slope extrapolated to the zero baseline is the early mitral valve deceleration time. Left ventricular diastolic function was categorized,[18] using cut-off points as described.[16,22] Diastolic function was classified as normal (E/A-ratio 0.75-1.50 and deceleration time 150-280ms), impaired relaxation (E/A-ratio <0.75 and deceleration time >280ms), or restrictive (E/A-ratio >1.50 and deceleration time <150ms). If only one abnormal criterion was fulfilled, diastolic function was classified as indeterminate instead of normal.[18]

To assess systolic function, we measured left ventricular end-systolic dimension (LVESD) and left ventricular end diastolic dimension (LVEDD) in the parasternal long axis view using M-mode with 2D guidance as described previously.[15,18] Fractional shortening was calculated as (LVEDD-LVESD)/LVEDD*100%. Qualitative global systolic function was assessed from the 2D echocardiogram in 4 categories: normal, fair, moderate, and poor. Inter-reader and intra-reader variability of the echocardiography measurements were good.[18]

Assessment of stroke

At baseline, history of stroke was assessed using home interviews and confirmed by reviewing medical records. Participants were then continuously followed up for stroke through automatic linkage of general practitioners' medical records with the study database. Furthermore, general practitioners' medical records of participants who moved out of the Ommoord district and nursing home physicians' medical records were checked on a regular basis.[23] Of all potential strokes, information from general practitioners and hospital discharge letters were collected and reviewed by research physicians. An experienced neurologist verified the stroke diagnoses. Strokes were subclassified into ischemic or hemorrhagic based on neuroimaging reports. A stroke was classified as unspecified if lacking neuroimaging.[24] Follow-up was complete until January 1st, 2012 for 92.7% of potential person-years.

Assessment of dementia
We used a three-step protocol to screen for dementia at baseline and follow-up examinations. First, participants underwent the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level. Second, screen-positives (MMSE<26 or GMS organic level>0) underwent an examination and informant interview with the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX). Third, participants suspected of dementia underwent further neuropsychological testing if necessary. Additionally, all participants were continuously monitored for dementia linking the study database to digitized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. When clinical neuroimaging was required and available, it was used for decision making on the diagnosis. The final diagnosis was determined by a consensus panel, led by a neurologist, in accordance with international criteria.[25,26] Follow-up for incident dementia was complete until January 1st, 2012, for 97.9% of potential person-years.[27]

Brain MRI
Scans were performed on a 1.5T MRI scanner (General Electric Healthcare, Milwaukee, USA) and reviewed by trained research physicians, who were blinded to clinical data. We used fluid-attenuated inversion recovery (FLAIR), proton density weighted, and T1-weighted sequences to identify infarcts. Focal lesions of ≥3mm and <15mm in size with identical signal characteristics as cerebrospinal fluid, and (when located supratentorially) a hyperintense rim on the FLAIR were classified as lacunar infarcts. Infarcts showing involvement of grey matter were classified as cortical infarcts. Because all people undergoing MRI were free of clinical stroke, all infarcts are silent infarcts. White matter lesions were segmented based on the FLAIR using an automated processing algorithm and voxels were summed to yield total white matter lesion volume in milliliters.[21]

Covariates
Covariates were measured during the same examination round as the echocardiography (2002-2005). Details on assessment of anthropometrics, cardiovascular risk factors (blood pressure, total cholesterol, high-density lipoprotein (HDL) cholesterol, diabetes mellitus, smoking), and medication use have been described.[28] Heart failure, coronary heart disease (defined as myocardial infarction or coronary revascularization procedure), and atrial fibrillation were assessed through active follow-up and adjudicated using standardized definitions.[23] MMSE was performed at the research center. Information on *apolipoprotein E (APOE)*-genotype, coded as one or two ε4 alleles, was obtained using PCR on coded DNA samples.

Statistical analyses
We examined the association of cardiac function with stroke and dementia using Cox proportional hazards models. Follow-up started at the echocardiography date. We censored participants at date of stroke in analyses with stroke and at date of dementia diagnosis in analyses with dementia. Participants were additionally censored at date of death, date of loss to follow-up, or the end of the study period, defined as the last date of follow-up or January 1st,

2012, whichever came first. We conducted a sensitivity analysis censoring for both stroke and dementia concomitantly in every analysis. We used linear and logistic regression models to investigate the associations of cardiac function with silent infarcts and white matter lesions. White matter lesion volume, mitral valve inflow deceleration time, and E/A-ratio were natural log transformed because of skewed distributions to the right. Fractional shortening was square transformed because of a skewed distribution to the left. Continuous echocardiographic variables were entered per SD increase into the models. Qualitative systolic function and diastolic function were entered categorically into the models. Only 10 participants had diastolic dysfunction with a restrictive pattern: therefore we combined diastolic dysfunction with impaired relaxation or a restrictive pattern for the analysis. Only 16 participants had poor left ventricular systolic function, which we therefore combined with moderate systolic function. In sensitivity analyses, we excluded participants (n=66) with moderate or poor systolic function to determine whether associations remained similar in people with a normal left ventricular function. The basic model (model I) was adjusted for age, sex, and type of ultrasonography system. The extended model (model II) was additionally adjusted for cardiovascular risk factors, and for MMSE-score and APOE-ε4 status in dementia analyses only. To further adjust for residual confounding by shared etiology, we repeated the analyses adjusting for cardiovascular risk factors and MMSE-score as assessed at the examination round prior (1997-2001) to our baseline. All analyses with white matter lesion volume were also adjusted for intracranial volume.

We explored potential effect modification by sex, age (stratified at median), blood pressure (stratified at median), and medication use by using interaction terms.

Missing data on covariates (less than 3.6%) were imputed using multiple imputations. Analyses were repeated for ischemic stroke and AD.

Analyses were done using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, USA).

Results

Baseline characteristics are presented in Table 1. Mean age (±SD) was 71.4 (±7.1) years and 60.8% was female. During a mean follow-up of 6.6 (±1.8) years, 164 strokes occurred, of which 117 were ischemic. During a mean follow-up of 5.9 (±1.5) years, 208 people developed dementia, of which 171 were AD.

Several measures of diastolic function were associated with the risk of stroke, including mitral valve inflow peak A (hazard ratio (HR) per SD increase 1.27 (95% confidence interval (CI) 1.08; 1.48)), E/A-ratio (HR 0.82 (0.69; 0.98)), and mitral valve inflow deceleration time (HR 1.22 (1.05; 1.41)), independently of cardiovascular risk factors. Results were similar for ischemic stroke. For systolic function, fractional shortening (HR 0.84 (0.72; 0.98)) and moderate or poor qualitative systolic function (HR 2.56 (1.21; 5.43)) were associated with the risk of stroke, independently of cardiovascular risk factors. Results attenuated slightly when we investigated ischemic stroke (Table 2).

Worse diastolic function was also related to an increased risk of dementia through mitral valve inflow peak E (HR 0.85 (0.75; 0.96)), E/A-ratio (HR 0.82 (0.70; 0.96)), mitral valve inflow

deceleration time (HR 1.16 (1.02; 1.33)), and impaired relaxation or restrictive pattern (HR 1.78 (1.08; 2.95)). Results were similar for AD. We did not find any associations between measurements of systolic function and the risk of dementia or AD (Table 3).

Results were similar after censoring for both stroke and dementia concomitantly or after excluding 66 people with moderate or poor left ventricular systolic function (results not shown).

Associations were also similar after adjusting for cardiovascular risk factors and MMSE-score assessed at the examination round prior to our baseline (Supplementary Table I and II).

We did not find a consistent pattern of effect modification by age, sex, blood pressure, or medication use, which was further hampered by smaller sample sizes in the respective strata. The only potential interaction was observed between sex and mitral valve inflow peak E for stroke (HR 0.71 (0.53; 0.94) in men and 1.27 (1.07; 1.51) in women). For dementia, we found potential interactions between sex and mitral valve deceleration time (HR 1.46 (1.16; 1.84) in men and 1.05 (0.89; 1.23) in women) and age and mitral valve deceleration time (HR 1.93 (1.25; 2.99) for <70.4 years and 1.11 (0.96; 1.27) for >70.4 years). However, such interactions were not observed for other parameters related to diastolic function, which might point towards spurious associations.

Of 577 persons with MRI-data, 37 had a silent infarct, of whom 31 a lacunar infarct. Median white matter lesion volume was 3.42 ml (interquartile range 2.17-6.43). Mitral valve inflow deceleration time and E/A-ratio were associated with silent infarcts, especially lacunar infarcts (Table 4). A moderate or poor systolic function was associated with a higher prevalence of silent infarcts, but this group only consisted of 4 participants resulting in wide CIs (Table 4). We found no associations of cardiac function with white matter lesion volume.

Table 1. Baseline characteristics

	At risk N=3,291
Demographics	
Age, years	71.4 (7.1)
Female	2,001 (60.8%)
MMSE score, points	27.6 (2.1)
Cardiovascular risk factors	
Systolic blood pressure, mm Hg	150 (21)
Diastolic blood pressure, mm Hg	80 (11)
Use of blood pressure-lowering medication	1,002 (30.8%)
Heart rate, b/min	69 (10)
Total cholesterol, mmol/L	5.7 (0.9)
HDL-cholesterol, mmol/L	1.5 (0.4)
Lipid-lowering medication	559 (17.2%)
Diabetes mellitus	434 (13.2%)
Smoking	
Past	1,692 (52.6%)
Current	509 (15.8%)
Body mass index	27.4 (3.9)
APOE-ε4 carrier	850 (26.8%)
Measures of diastolic function	
Mitral valve inflow peak E, m/s	0.65 (0.15)
Mitral valve inflow peak A, m/s	0.77 (0.17)
Mitral valve inflow deceleration time, ms[a]	208 (184-240)
E/A-ratio[a]	0.83 (0.71-1.00)
Qualitatively assessed diastolic function	
Normal	2,027 (61.6%)
Indeterminate	1,110 (33.7%)
Impaired relaxation	144 (4.4%)
Restrictive pattern	10 (0.3%)
Measures of systolic function	
Fractional shortening, %[a]	40.4 (35.6-44.0)
Qualitatively assessed systolic function	
Normal	2,102 (63.9%)
Fair	1,123 (34.1%)
Moderate	50 (1.5%)
Poor	16 (0.5%)

Abbreviations: N = number of persons included in study; HDL = high-density lipoprotein; MMSE = Mini-mental state examination; *APOE* = apolipoprotein E.
Data are presented as mean (standard deviations) or counts (percentages).
[a] Median and inter-quartile range because of skewed distribution

Table 2. Cardiac function and the risk of stroke

	Stroke n/N 164/3,291		Ischemic stroke n/N 117/3,291	
	Model I	Model II	Model I	Model II
Diastolic function				
Quantitative diastolic function				
Mitral valve inflow peak E, per SD	1.11 (0.96; 1.29)	1.07 (0.92; 1.25)	1.21 (1.02; 1.43)	1.15 (0.96; 1.38)
Mitral valve inflow peak A, per SD	1.23 (1.06; 1.43)	1.27 (1.08; 1.48)	1.24 (1.04; 1.49)	1.25 (1.04; 1.52)
Mitral valve inflow deceleration time[a], per SD	1.21 (1.04; 1.41)	1.22 (1.05; 1.41)	1.18 (0.99; 1.42)	1.19 (0.99; 1.42)
E/A-ratio[a], per SD	0.88 (0.74; 1.03)	0.82 (0.69; 0.98)	0.95 (0.78; 1.15)	0.88 (0.72; 1.08)
Qualitative diastolic function				
Normal	Reference	Reference	Reference	Reference
Indeterminate	1.20 (0.86; 1.67)	1.27 (0.91; 1.78)	1.22 (0.82; 1.80)	1.32 (0.89; 1.96)
Impaired relaxation or restrictive pattern	1.69 (0.96; 2.99)	1.74 (0.98; 3.08)	1.58 (0.77; 3.25)	1.65 (0.80; 3.40)
Systolic function				
Quantitative systolic function				
Fractional shortening, per SD[a]	0.85 (0.72; 0.99)	0.84 (0.72; 0.98)	0.88 (0.73; 1.06)	0.87 (0.72; 1.05)
Qualitative systolic function				
Normal	Reference	Reference	Reference	Reference
Fair	1.29 (0.93; 1.80)	1.35 (0.97; 1.89)	1.18 (0.80; 1.76)	1.25 (0.84; 1.87)
Moderate or poor	2.21 (1.05; 4.66)	2.56 (1.21; 5.43)	1.89 (0.74; 4.81)	2.27 (0.89; 5.80)

Abbreviations: n = number of cases; N = number of persons at risk; SD = standard deviation.
Values are hazard ratios with 95% confidence intervals.
Model I: Adjusted for age, sex and type ultrasonography system.
Model II: Adjusted for age, sex, type ultrasonography system, body mass index, systolic blood pressure, diastolic blood pressure, blood pressure- lowering medication, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication, diabetes mellitus, current smoking, past smoking, and heart rate.
[a] Natural log or square transformed because of skewed distribution.

Table 3. Cardiac function and the risk of dementia

	Dementia n/N 208/3,291		Alzheimer disease n/N 171/3,291	
	Model I	Model II	Model I	Model II
Diastolic function				
Quantitative diastolic function				
Mitral valve inflow peak E, per SD	0.90 (0.79; 1.04)	0.85 (0.75; 0.96)	0.85 (0.72; 0.99)	0.79 (0.69; 0.91)
Mitral valve inflow peak A, per SD	1.02 (0.89; 1.17)	0.92 (0.81; 1.05)	1.00 (0.86; 1.17)	0.88 (0.76; 1.01)
Mitral valve inflow deceleration time[a], per SD	1.18 (1.03; 1.35)	1.16 (1.02; 1.33)	1.25 (1.08; 1.44)	1.22 (1.06; 1.41)
E/A-ratio[a], per SD	0.86 (0.74; 0.99)	0.82 (0.70; 0.96)	0.83 (0.71; 0.97)	0.78 (0.66; 0.92)
Qualitative diastolic function				
Normal	Reference	Reference	Reference	Reference
Indeterminate	1.24 (0.92; 1.67)	1.41 (1.04; 1.92)	1.19 (0.86; 1.65)	1.41 (1.01; 1.99)
Impaired relaxation or restrictive pattern	1.53 (0.94; 2.52)	1.78 (1.08; 2.95)	1.43 (0.83; 2.48)	1.69 (0.97; 2.96)
Systolic function				
Quantitative systolic function				
Fractional shortening, per SD[a]	0.97 (0.85; 1.11)	0.98 (0.85; 1.13)	0.95 (0.82; 1.11)	0.97 (0.83; 1.13)
Qualitative systolic function				
Normal	Reference	Reference	Reference	Reference
Fair	1.13 (0.85; 1.51)	1.19 (0.88; 1.60)	1.13 (0.82; 1.55)	1.19 (0.86; 1.66)
Moderate or poor	1.25 (0.57; 2.74)	1.21 (0.55; 2.68)	1.10 (0.44; 2.76)	1.08 (0.43; 2.73)

Abbreviations: n = number of cases; N = number of persons at risk; SD = standard deviation.
Values are hazard ratios with 95% confidence intervals.
Model I: Adjusted for age, sex and type ultrasonography system.
Model II: Adjusted for age, sex, type ultrasonography system, body mass index, systolic blood pressure, diastolic blood pressure, blood pressure- lowering medication, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication, diabetes mellitus, current smoking, past smoking, heart rate, MMSE-score and *APOE-ε4* carrier status.
[a] Natural log or square transformed because of skewed distribution.

Table 4. Cardiac function and MRI-markers (N=577)

	Infarcts		White matter lesion volume[a]
	Total OR (95% CI)	Lacunar OR (95% CI)	Mean difference (95% CI)
Diastolic function			
Quantitative diastolic function			
Mitral valve inflow peak E, per SD	0.78 (0.51; 1.20)	0.68 (0.42; 1.09)	0.03 (-0.05; 0.10)
Mitral valve inflow peak A, per SD	1.09 (0.71; 1.69)	1.16 (0.72; 1.88)	0.03 (-0.05; 0.11)
Mitral valve inflow deceleration time[a], per SD	1.56 (1.04; 2.35)	1.70 (1.08; 2.69)	-0.01 (-0.08; 0.06)
E/A-ratio[a], per SD	0.66 (0.41; 1.07)	0.52 (0.30; 0.90)	0.01 (-0.07; 0.09)
Qualitative diastolic function			
Normal	Reference	Reference	Reference
Indeterminate	0.94 (0.38; 2.31)	1.07 (0.40; 2.83)	-0.05 (-0.21; 0.11)
Impaired relaxation or restrictive pattern	4.81 (1.06; 21.84)	4.06 (0.68; 24.13)	-0.01 (-0.43; 0.41)
Systolic function			
Quantitative systolic function			
Fractional shortening, per SD[a]	0.86 (0.54; 1.37)	1.02 (0.61; 1.71)	-0.04 (-0.12; 0.05)
Qualitative systolic function			
Normal	Reference	Reference	Reference
Fair	1.23 (0.51; 2.95)	1.38 (0.54; 3.50)	0.01 (-0.16; 0.17)
Moderate or poor	43.56 (4.13; 460.07)	23.29 (1.22; 445.49)	0.57 (-0.31; 1.45)

Abbreviations: MRI = magnetic resonance imaging; N = number of persons at risk; OR = odds ratio; CI = confidence interval; SD = standard deviation.
Values are odds ratios or differences in volume with 95% confidence intervals.
Adjusted for age, sex, body mass index, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication, diabetes mellitus, current smoking, past smoking, and heart rate. White matter lesion volumes were additionally adjusted for intracranial volume.
[a] Natural log or square transformed because of skewed distribution.

Discussion

In people free of clinical cardiac disease, worse diastolic function was associated with an increased risk of stroke and dementia, whereas worse systolic function was only associated with a higher risk of stroke. Diastolic function was also related to silent infarcts, especially lacunar infarcts, on MRI.

Patients with overt cardiac disease have an increased risk of stroke and dementia. However, the associations between subclinical cardiac disease and stroke and dementia are unclear. As for stroke, other population-based studies have mostly examined the association of cardiac structure and stroke.[19,29,30] However, in patients with heart failure, quality of systolic and diastolic function provides additional information on stroke risk over markers of cardiac structure alone.[31] Little data is available in asymptomatic populations on systolic and diastolic function related to stroke. While diastolic dysfunction was not associated with stroke in the Strong Heart Study, this study population was younger and of different ethnicity than ours.[29] The AGES-Reykjavik study found an association between low E/A ratio and cerebral infarcts on MRI, supporting our observations, but this was a cross-sectional study that did not examine clinical strokes.[32] For systolic function, the Framingham Heart Study found that persons with a lower fractional shortening had a higher risk of cardiovascular disease,[33] which is in line with our observations, although they did not separately examine stroke. Hence, our results provide novel evidence that diastolic and systolic dysfunction are both associated with an increased risk of stroke, even within ranges of normal values.

Regarding dementia, the Rotterdam Study previously found a higher prevalence of dementia in people with atrial fibrillation.[7] Findings from several other population-based studies also point towards an association between cardiac dysfunction and dementia. For instance, the Framingham Heart Study investigated the association between cardiac function and low cognitive performance and MRI markers related to dementia and AD.[34] They found a U-shaped association between left ventricular end systolic function and cognitive performance. Similarly, another study found that diminished cardiac function was related to lower brain volume, an important marker of brain aging.[35] The Cardiovascular Health Study found an association between cardiovascular disease and dementia.[9] However, these studies only investigated people with cardiac disease,[7,9] or were cross-sectional by design.[7,34,35] Our results suggest that diminished diastolic cardiac function is also associated with an increased risk of dementia in people free of cardiac disease. Since results for dementia and AD were similar and even remained stable after censoring for stroke, our study also supports the growing evidence that vascular factors play an important role in the etiology of AD.[36] The question remains why diastolic function, but not systolic function, is related to dementia. Interestingly, we also found diastolic function to be associated with silent infarcts on MRI, which were primarily lacunar infarcts. Cerebral small vessel disease has been suggested to be the underlying link between lacunar infarcts and dementia. Future research should therefore explore whether diastolic function rather than systolic function relates strongest with pathology of the smallest vessels in the brain. Novel imaging techniques, such as arterial spin

labeling for brain perfusion and 7T MRI for visualizing small vessels, may play an important role here.

There are several explanations for the relation between cardiac function and the risk of neurologic disease, which are supported equally by our data. First, cardiac dysfunction can lead to cardioembolism,[37] which in turn causes stroke and contributes to the etiology of dementia.[3,38] Second, impaired cardiac function may lead to cerebral hypoperfusion. In people with cardiac arrhythmias, hypoperfusion leads to watershed infarction.[39] Furthermore, in patients with heart failure, low ejection fraction has been associated with cognitive impairment.[40] However, low cardiac output is closely related to diminished systolic function, which in our study was not associated with dementia. Finally, a non-causal explanation is shared etiology, since impaired cardiac function, stroke, and dementia share risk factors.[3,4] Although our results were independent of cardiovascular risk factors, even when assessed up to 7 years prior to baseline, there might still be residual confounding.

Strengths of this study are the population-based design, the long follow-up period, the systematic collection and adjudication of events, and the standardized assessment of risk factors and echocardiographic parameters. A limitation is that categorization of left ventricular diastolic function was based on E/A-ratio and mitral valve deceleration time, and not on the early diastolic longitudinal velocity of the mitral annulus (E').[16,22] We were thus unable to sub-classify diastolic function in approximately one third of our study population. Neither did we systematically measure valvular diseases, which are well-known substrates for cardioembolisms and can affect cardiac function.[38] Another issue is multiple testing, since we tested several diastolic and systolic measures of cardiac function. However, as the associations we found were not completely independent, adjusting for multiple testing might have led to false negative results. Since echocardiography was performed in the fourth examination of the original cohort and the second examination of the extended cohort, survival bias cannot be ruled out. Finally, most participants of the Rotterdam Study are white and live in a middle income district of Rotterdam, which limits the generalizability of our results.

Our results indicate that in people without clinically overt cardiac disease, impaired diastolic function is associated with the risk of clinical stroke, dementia, and silent infarcts on MRI, whereas impaired systolic function is only associated with the risk of stroke. Future research should determine whether improving cardiac function can prevent stroke and dementia.

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Supplementary information

Supplementary Table I. Characteristics assessed at the examination prior to baseline

	At risk N=3,291
Demographics	
Score on MMSE, points	28.0 (1.7)
Cardiovascular risk factors	
Systolic blood pressure, mm Hg	142 (20)
Diastolic blood pressure, mm Hg	77 (11)
Use of blood pressure-lowering medication	634 (20.1%)
Heart rate, b/min	70 (10)
Total cholesterol, mmol/L	5.9 (0.9)
HDL-cholesterol, mmol/L	1.4 (0.4)
Lipid-lowering medication	314 (9.9%)
Diabetes mellitus	308 (9.7%)
Smoking	
Past	1,535 (47.7%)
Current	639 (19.9%)
Body mass index	26.8 (3.8)
APOE-ε4 carrier	850 (26.8%)

Abbreviations: N = number of persons included in study; HDL = high-density lipoprotein; MMSE = Mini-mental state examination; *APOE =* apolipoprotein E.
Data are presented as mean (standard deviations) or counts (percentages).

Supplementary Table II. Cardiac function and the risk of stroke and dementia, adjusted for potential confounders assessed at the examination prior to baseline

	Stroke n/N 164/3,291	Dementia n/N 208/3,291
Diastolic function		
Quantitative diastolic function		
Mitral valve inflow peak E, per SD	1.09 (0.94; 1.27)	0.89 (0.77; 1.02)
Mitral valve inflow peak A, per SD	1.21 (1.04; 1.41)	1.03 (0.89; 1.18)
Mitral valve inflow deceleration time[a], per SD	1.21 (1.04; 1.41)	1.16 (1.02; 1.32)
E/A-ratio[a], per SD	0.88 (0.74; 1.03)	0.84 (0.72; 0.97)
Qualitative diastolic function		
Normal	Reference	Reference
Indeterminate	1.19 (0.85; 1.66)	1.26 (0.93; 1.70)
Impaired relaxation or restrictive pattern	1.66 (0.94; 2.94)	1.67 (1.02; 2.74)
Systolic function		
Quantitative systolic function		
Fractional shortening, per SD[a]	0.83 (0.71; 0.97)	0.97 (0.84; 1.11)
Qualitative systolic function		
Normal	Reference	Reference
Fair	1.35 (0.96; 1.88)	1.15 (0.86; 1.54)
Moderate or poor	2.62 (1.24; 5.56)	1.26 (0.57; 2.77)

Abbreviations: n = number of cases; N = number of persons at risk; SD = standard deviation.
Values are hazard ratios with 95% confidence intervals.
Adjusted for age, sex, type ultrasonography system, body mass index, systolic blood pressure, diastolic blood pressure, blood pressure- lowering medication, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication, diabetes mellitus, current smoking, past smoking, heart rate, and for MMSE-score and APOE-ε4 carrier status in dementia analyses only.
[a] Natural log or square transformed because of skewed distribution.

Chapter 2.2

N-terminal pro-B-type natriuretic peptide and the risk of stroke and transient ischaemic attack

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Abstract

Background and purpose
Amino-terminal pro-B-type natriuretic peptide (NT-proBNP) is a predictor of heart disease. It has also been related to stroke, but its association with transient ischemic attacks (TIAs) is unclear. Moreover, it is unknown how clinical heart disease influences this relation. Within the prospective population-based Rotterdam Study, we examined the association of NT-proBNP with stroke and TIA, and investigated the role of heart disease on this association.

Methods
NT-proBNP was measured in 1997-2001 in 5611 participants (mean age 68.7 years; 57.7% women) without a history of stroke, TIA, or heart failure. Follow-up for stroke and TIA finished in 2012. Models were adjusted for age and cardiovascular risk factors, and were stratified by sex.

Results
During 22058 person-years 195 men suffered a stroke and 118 a TIA. During 31825 person-years 230 women suffered a stroke and 187 a TIA. Higher NT-proBNP was associated with a higher risk of stroke in men (hazard ratio (HR) per SD increase 1.50, 95% CI 1.29; 1.76) and in women (HR 1.24, 95% CI 1.05 1.46). Associations with TIA were only present in women (HR 1.51, 95% CI 1.26; 1.82), and not in men (HR 1.02, 95% CI 0.83; 1.26). Excluding persons with a history of clinical coronary heart disease, heart failure, or atrial fibrillation, and censoring for clinical heart disease during follow-up did not change the associations.

Conclusions
Higher NT-proBNP is associated with incident stroke in men and women and with incident TIA only in women. These associations are independent of clinical heart disease preceding cerebrovascular disease.

Introduction

An important cornerstone of research on cardiovascular diseases, is to identify subclinical markers that can elucidate etiology or serve as predictive markers. Amino-terminal pro-B-type natriuretic peptide (NT-proBNP) is an emerging marker that is excreted in response to cardiac wall stress,[1,2] and has recently shown to predict heart disease.[3,4] Importantly, NT-proBNP provides information on cardiac overload and dysfunction even in absence of clinical heart failure and therefore could serve as a subclinical marker.[5]

Previous studies have also shown an increased risk of stroke in persons with high NT-proBNP,[3,6-10] but there are still some knowledge gaps. First, it remains unclear how clinical heart disease (e.g. coronary heart disease, heart failure, and atrial fibrillation) affects the reported association between NT-proBNP and stroke. It is conceivable that persons with high NT-proBNP, reflecting subclinical heart disease, first suffer from clinical heart disease during follow-up, which subsequently leads to a stroke.[11-13] Second, if NT-proBNP truly could act as a subclinical marker of stroke, it is important to study its role in the earliest clinical manifestation of cerebrovascular disease, which is often a transient ischemic attack (TIA). Importantly, at time of TIA effective therapy can still be installed to prevent subsequent stroke.[14] Finally, given the differences in occurrence and risk factor profiles of cardiovascular disease between men and women,[15,16] the association between NT-proBNP with cerebrovascular disease merits further investigation for sex differences.

Therefore, we investigated the association of NT-proBNP with stroke and TIA in men and women separately. Furthermore, we studied the role of prevalent and incident heart disease on this association.

Materials and Methods

Setting and study population
This study was embedded within the Rotterdam Study, a prospective population-based cohort study among persons aged 55 years and older residing in Ommoord, a suburb of Rotterdam, the Netherlands.[17] The study started in 1990 with 7983 participants and was extended in 2000 with 3011 persons. Follow-up examinations take place every 3 to 4 years.

For the current study, baseline data was collected between 1997 and 2001. NT-proBNP was measured in 3923 participants of the original cohort and 2566 participants of the second cohort. After excluding 804 participants with a history of stroke, TIA, or heart failure, and 74 participants with NT-proBNP above the age-specific heart failure limit (50-75 years, 108 pmol/L; >75 years, 216 pmol/L),[18] a total of 5611 participants (2374 men and 3237 women) were eligible for analysis. The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study. A written informed consent was obtained from all participants.

NT-proBNP measurement

Blood samples for NT-proBNP assessment were collected in glass tubes containing clot activator and gel for serum separation and stored at -80ºC. NT-proBNP was measured using a commercially available electrochemiluminescence immunoassay (Elecsys proBNP, F Hoffman-La Roche Ltd) on an Elecsys 2010 analyzer.[6] Precision, analytic sensitivity, and stability of the system have been described.[19]

Assessment of cerebrovascular events.

At study entry, history of stroke and TIA was assessed using home interviews and confirmed by reviewing medical records. Subsequently, participants were continuously followed-up for stroke and TIA through digital linkage of general practitioners' files with the study database. Furthermore, nursing home physicians' files and files from general practitioners of participants who moved out of the district were checked on a regular basis.[20] Of all potential strokes and TIAs, hospital discharge letters and information from general practitioner was collected. Research physicians reviewed the information and an experienced neurologist verified the strokes and TIAs.[21,22] Strokes were further classified into ischemic or hemorrhagic based on neuroimaging reports. Subarachnoid hemorrhages were excluded. Infarcts that turned hemorrhagic were classified as ischemic stroke. If neuroimaging was lacking, a stroke was classified as unspecified. TIAs were further classified into focal or mixed. An event was focal if only symptoms attributable to dysfunction of one arterial territory of the brain were reported. An event was classified as mixed if diffuse nonlocalizing cerebral symptoms were reported as well.[22] Purely nonfocal events were not analyzed as they are currently not included in the internationally used definition of TIA.[23] Follow-up was complete until January 1st, 2012 for 95.6% of potential person-years.

Covariates

Details on assessment of anthropometrics, cardiovascular risk factors (blood pressure, total cholesterol, high-density cholesterol, creatinine, diabetes mellitus, and smoking), and use of medication have been described previously.[4] Within the heart disease definition we included: heart failure, coronary heart disease (defined as myocardial infarction or coronary revascularization procedure), and atrial fibrillation. Heart diseases were assessed through active follow-up and adjudicated using standardized definitions similar to the follow-up for stroke and TIA.[20]

Statistical analyses

NT-proBNP was natural log-transformed and entered per standard deviation (SD) increase into Cox proportional hazards models. Hazard ratios (HR) were also calculated for sex-specific tertiles of NT-proBNP. We examined the association of NT-proBNP with any cerebrovascular event (stroke or TIA), and with stroke or TIA separately, censoring follow-up at the date of the other event if that occurred first. Thus, participants were censored at date of stroke, date of TIA, date of death, last date of follow-up, or January 1st, 2012, whichever came first. All models were adjusted for age and were stratified by sex to allow for sex-specific effects. Interaction terms

were tested to identify significant differences between men and women. In multivariate adjusted models, we additionally adjusted for cardiovascular risk factors, and blood pressure-lowering, lipid-lowering, and antithrombotic (including antiplatelet and anticoagulant) drugs. Missing data on covariates (for all covariates 4.6% or less) were imputed based on the other covariates using multiple imputation with 5 imputation sets.

In sensitivity analyses, we investigated associations between NT-proBNP and stroke without censoring for TIA, we stratified for antiplatelet and anticoagulant drug use, and we investigated associations between NT-proBNP and stroke and TIA after additionally excluding participants with a history of atrial fibrillation or coronary heart disease at baseline and censoring for incident atrial fibrillation, coronary heart disease, and heart failure during follow-up. Follow-up for atrial fibrillation and heart failure was available until 2008 and 2010, respectively. Therefore we did a final sensitivity analysis for stroke and TIA with follow-up ending in 2008.

All analyses were done using IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY).

Results

In table 1 the baseline characteristics of the study population are presented. A total of 2374 men and 3237 women was eligible for analysis. Mean age ($\pm$SD) was 67.9 ($\pm$7.5) years in men and 69.2 ($\pm$8.4) years in women. After a mean follow-up of 9.3 ($\pm$3.4) years the first presentation of a cerebrovascular event was stroke in 195 men and TIA in 118 men. After a mean follow-up of 9.8 ($\pm$3.3) years the first presentation of a cerebrovascular event was stroke in 230 women and TIA in 187 women.

In Table 2 associations between NT-proBNP levels and cerebrovascular events are presented. The risk of any cerebrovascular event was similar in men and women, multivariate adjusted HR per SD increase in NT-proBNP in men 1.31 (95% confidence interval (CI) 1.15; 1.48) and in women 1.36 (95% CI 1.20; 1.53). Associations with stroke were stronger in men (HR 1.50, 95% CI 1.29; 1.76) compared to women (HR 1.24, 95% CI 1.05; 1.46), but this difference was not statistically significant (p-value 0.21). Associations were found for both ischemic and hemorrhagic stroke. Associations with TIA were only present in women (HR 1.51, 95% CI 1.26; 1.82) and not in men (HR 1.02, 95% CI 0.83; 1.26), which was a statistically significant interaction (p<0.01) (Table 2). With NT-proBNP levels in tertiles we also found associations with stroke in men and women and with TIA only in women (Table 3, Figure 1). Without censoring for TIA, the effect size for stroke in women was higher (HR 1.36, 95% CI 1.16; 1.59) (Table 4).

Associations with hemorrhagic stroke and mixed TIA attenuated after excluding participants with a history of heart disease and censoring at time of incident heart disease (Table 5). Other associations remained similar. Ending the study follow-up in 2008 did not change the lack of an effect of preceding heart disease on the associations (data not shown).

Associations were slightly weaker in persons that used either anticoagulant or antiplatelet drugs (Supplementary Table I).

Table 1. Baseline characteristics

	Men N=2374	Women N=3237
Age, years	67.9 (7.5)	69.2 (8.4)
Systolic blood pressure, mmHg	144 (21)	142 (21)
Diastolic blood pressure, mmHg	79 (11)	76 (11)
Blood pressure lowering drugs	459 (20.1%)	738 (24.0%)
Total cholesterol, mmol/L	5.6 (1.0)	6.0 (0.9)
High-density lipoprotein cholesterol, mmol/L	1.2 (0.3)	1.5 (0.4)
Lipid-lowering drugs	296 (12.9%)	367 (11.8%)
Anticoagulant drugs	85 (3.7%)	44 (1.4%)
Antiplatelet drugs	373 (16.3%)	333 (10.7%)
Creatinine, μmol/L	88 (16)	70 (13)
Diabetes mellitus	284 (12.0%)	322 (10.0%)
Smoking		
Never	275 (11.7%)	1375 (43.1%)
Former	1530 (64.9%)	1272 (39.9%)
Current	552 (23.4%)	541 (17.0%)
NT-proBNP, pmol/L[a]	7.7 (4.1 – 15.8)	10.5 (5.9 – 18.3)
NT-proBNP, pmol/L[b]		
Tertile 1	0.59 – 5.06	0.59 – 7.30
Tertile 2	5.06 – 12.30	7.30 – 15.38
Tertile 3	12.30 – 210.10	15.38 – 212.20

Data are presented as mean (standard deviations) or counts (percentages).
[a] Median and inter-quartile range.
[b] Range in each tertile

Table 2. NT-proBNP and the risk of cerebrovascular events

	Men N=2374				Women N=3237			
	n	Model I HR (95% CI)		Model II HR (95% CI)	n	Model I HR (95% CI)		Model II HR (95% CI)
Any cerebrovascular event	313	1.37 (1.22; 1.54)		1.31 (1.15; 1.48)	417	1.36 (1.21; 1.53)		1.36 (1.20; 1.53)
Any stroke	195	1.60 (1.39; 1.85)		1.50 (1.29; 1.76)	230	1.28 (1.09; 1.51)		1.24 (1.05; 1.46)
Ischemic stroke	139	1.68 (1.41; 1.99)		1.59 (1.32; 1.92)	143	1.31 (1.07; 1.61)		1.30 (1.05; 1.61)
Hemorrhagic stroke	18	1.86 (1.16; 2.97)		1.70 (1.02; 2.84)	26	1.43 (0.90; 2.30)		1.17 (0.72; 1.90)
Unspecified stroke	38	1.31 (0.95; 1.81)		1.14 (0.80; 1.63)	61	1.15 (0.84; 1.58)		1.11 (0.80; 1.53)
Any TIA	118	1.05 (0.86; 1.27)		1.02 (0.83; 1.26)	187	1.47 (1.23; 1.75)		1.51 (1.26; 1.82)
Focal TIA	101	1.08 (0.88; 1.34)		1.05 (0.83; 1.31)	145	1.48 (1.21; 1.81)		1.55 (1.26; 1.90)
Mixed TIA	17	0.85 (0.50; 1.45)		0.88 (0.52; 1.49)	42	1.42 (0.98; 2.06)		1.41 (0.96; 2.06)

Abbreviations: n = number of events; N = number of persons at risk; SD = standard deviation.
Values are hazard ratios per SD increase in natural log-transformed NT-proBNP level (SD=0.96) with 95% confidence intervals.
Analyses with stroke were censored for incident TIA and analyses with TIA were censored for incident stroke.
Model I: adjusted for age.
Model II: adjusted for age, systolic blood pressure, diastolic blood pressure, blood pressure-lowering drugs, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering drugs, antithrombotic drugs, creatinine, smoking, and diabetes mellitus.
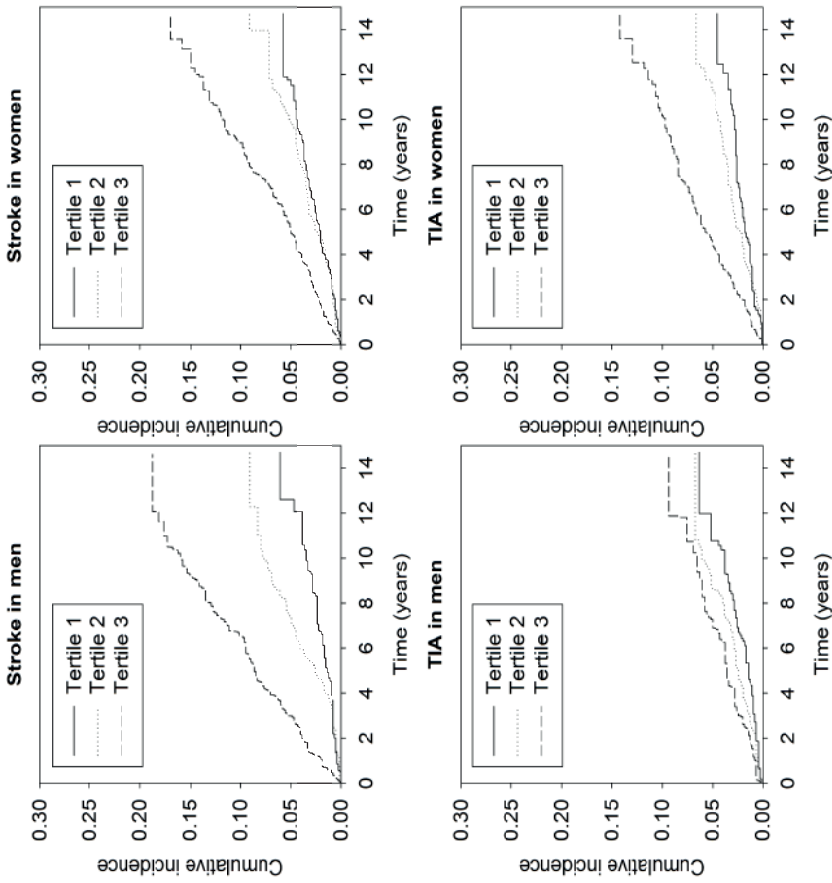
Figure 1. Cumulative incidence of cerebrovascular events in men and women by tertiles of NT-proBNP levels. Stroke was censored for incident TIA. TIA was censored for incident stroke.

Table 3. NT-proBNP in tertiles and the risk of cerebrovascular events

	Men			Women		
	n/N	Model I HR (95% CI)	Model II HR (95% CI)	n/N	Model I HR (95% CI)	Model II HR (95% CI)
Any cerebrovascular event						
Tertile 1	61/790	1 (reference)	1 (reference)	85/1080	1 (reference)	1 (reference)
Tertile 2	97/793	1.35 (0.98; 1.88)	1.29 (0.93; 1.80)	115/1076	1.07 (0.81; 1.42)	1.08 (0.81; 1.43)
Tertile 3	155/791	2.00 (1.44; 2.76)	1.81 (1.29; 2.53)	217/1081	1.78 (1.35; 2.33)	1.77 (1.34; 2.33)
Any stroke						
Tertile 1	29/790	1 (reference)	1 (reference)	50/1080	1 (reference)	1 (reference)
Tertile 2	55/793	1.59 (1.01; 2.51)	1.51 (0.95; 2.39)	63/1076	0.94 (0.65; 1.37)	0.94 (0.64; 1.37)
Tertile 3	111/791	2.91 (1.87; 4.52)	2.57 (1.63; 4.06)	117/1081	1.45 (1.01; 2.08)	1.37 (0.94; 1.99)
Any TIA						
Tertile 1	32/790	1 (reference)	1 (reference)	35/1080	1 (reference)	1 (reference)
Tertile 2	42/793	1.15 (0.72; 1.84)	1.11 (0.69; 1.79)	52/1076	1.26 (0.82; 1.95)	1.28 (0.83; 1.98)
Tertile 3	44/791	1.14 (0.69; 1.89)	1.09 (0.64; 1.83)	100/1081	2.29 (1.51; 3.46)	2.40 (1.57; 3.66)

Abbreviations: n = number of events; N = number of persons at risk.
Values are hazard ratios per sex-specific tertile of NT-proBNP level with 95% confidence intervals.
Analyses with stroke were censored for incident TIA and analyses with TIA were censored for incident stroke.
Model I: adjusted for age.
Model II: adjusted for age, systolic blood pressure, diastolic blood pressure, blood pressure-lowering drugs, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering drugs, antithrombotic drugs, creatinine, smoking, and diabetes mellitus.

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Table 4. NT-proBNP and the risk of stroke, without censoring for TIA

	Men N=2374			Women N=3237		
	n	Model I HR (95% CI)	Model II HR (95% CI)	n/N	Model I HR (95% CI)	Model II HR (95% CI)
Any stroke	208	1.59 (1.38; 1.82)	1.51 (1.30; 1.76)	261	1.41 (1.22; 1.64)	1.36 (1.16; 1.59)
Ischemic stroke	149	1.63 (1.38; 1.92)	1.56 (1.30; 1.86)	162	1.46 (1.20; 1.76)	1.45 (1.19; 1.77)
Hemorrhagic stroke	18	1.85 (1.16; 2.96)	1.71 (1.02; 2.85)	28	1.40 (0.89; 2.21)	1.15 (0.72; 1.83)
Unspecified stroke	41	1.40 (1.02; 1.90)	1.29 (0.92; 1.81)	71	1.31 (0.97; 1.75)	1.22 (0.90; 1.66)

Abbreviations: n = number of events; N = number of persons at risk; SD = standard deviation.
Values are hazard ratios per SD increase in natural log-transformed NT-proBNP level (SD=0.96) with 95% confidence intervals.
Model I: adjusted for age.
Model II: adjusted for age, systolic blood pressure, diastolic blood pressure, blood pressure-lowering drugs, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering drugs, antithrombotic drugs, creatinine, smoking, and diabetes mellitus.

Table 5. NT-proBNP and the risk of cerebrovascular events, censored at the occurrence of incident heart disease

	Men N=1805			Women N=2725		
	n	Model I HR (95% CI)	Model II HR (95% CI)	n	Model I HR (95% CI)	Model II HR (95% CI)
Any cerebrovascular event	198	1.52 (1.29; 1.79)	1.46 (1.22; 1.74)	318	1.38 (1.20; 1.59)	1.37 (1.19; 1.59)
Any stroke	118	1.98 (1.61; 2.43)	1.86 (1.48; 2.34)	174	1.32 (1.09; 1.60)	1.26 (1.03; 1.54)
Ischemic stroke	88	2.09 (1.65; 2.65)	2.02 (1.55; 2.62)	110	1.30 (1.02; 1.66)	1.28 (0.99; 1.64)
Hemorrhagic stroke	7	1.08 (0.44; 2.68)	1.15 (0.42; 3.11)	20	1.33 (0.76; 2.34)	1.17 (0.66; 2.08)
Unspecified stroke	23	1.98 (1.25; 3.13)	1.61 (0.94; 2.75)	44	1.33 (0.91; 1.96)	1.28 (0.86; 1.92)
Any TIA	80	1.01 (0.77; 1.32)	1.01 (0.76; 1.34)	144	1.45 (1.18; 1.79)	1.53 (1.23; 1.90)
Focal TIA	69	1.03 (0.77; 1.37)	1.00 (0.74; 1.35)	112	1.53 (1.21; 1.94)	1.62 (1.27; 2.08)
Mixed TIA	11	0.91 (0.45; 1.86)	1.06 (0.52; 2.18)	32	1.20 (0.77; 1.89)	1.24 (0.77; 1.99)

Abbreviations: n = number of events; N = number of persons at risk; SD = standard deviation.
Values are hazard ratios per SD increase in natural log-transformed NT-proBNP level (SD=0.96) with 95% confidence intervals.
Analyses with stroke were censored for incident TIA and analyses with TIA were censored for incident stroke.
Model I: adjusted for age.
Model II: adjusted for age, systolic blood pressure, diastolic blood pressure, blood pressure-lowering drugs, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering drugs, antithrombotic drugs, creatinine, smoking, and diabetes mellitus.

Discussion

We found that higher levels of NT-proBNP were associated with a higher risk of cerebrovascular events. Associations with stroke were present for both men and women, whereas associations with TIA were only present in women. Associations were independent of clinical heart disease preceding cerebrovascular disease.

Several other population-based studies have shown that an increased NT-proBNP level is related to a higher risk of cerebrovascular events.[3,6-10] However, these studies did not exclude all participants with history of heart disease or account for the occurrence of heart disease during follow-up. One study adjusted for the presence of coronary heart disease, heart failure, and atrial fibrillation and found that the association remained unchanged.[7] This was consistent with our finding that associations were independent of clinical heart disease preceding the cerebrovascular disease, suggesting that a higher NT-proBNP as marker of subclinical heart disease is associated with a higher risk of cerebrovascular events. Subclinical heart disease might cause TIA or ischemic stroke through intracardiac thrombus formation, the most important reason for stroke in persons with overt heart disease.[11-13] Another potential mechanism is that TIA or ischemic stroke in persons with an increased NT-proBNP is caused by paroxysmal atrial fibrillation which is not picked up by diagnostic electrocardiograms.[24] Associations with hemorrhagic stroke might be the consequence of antithrombotic drug use. Furthermore, the associations with any cerebrovascular event might reflect the shared effect of cardiovascular risk factors on NT-proBNP and cerebrovascular events, although after adjusting for cardiovascular risk factors the associations were similar. Still, residual confounding cannot be excluded.

Previous studies found that the effect of NT-proBNP on cerebrovascular disease was similar for men and women.[3,6-10] However, those studies did not examine associations with only TIA or with stroke independent from TIA. We found that a higher NT-proBNP led to a higher risk of TIA in women, whereas in men there was only a higher risk of stroke. This suggests that subclinical heart disease, indicated by a higher NT-proBNP, leads to a cerebrovascular event that at its first presentation is already more severe in men than in women. A reason might be that men have more atherosclerosis[25] and therefore an additional thrombus from the heart possibly has a greater impact. Women might also recover better after a cerebrovascular event caused by subclinical heart disease and therefore suffer a TIA instead of a stroke. Furthermore, this might be an indication of diagnostic bias, a similar event might be reported as a stroke more often in men and in women more often as a TIA. This might happen if men are more likely to have an MRI compared to women, because a short-lasting event is more likely to be classified as a stroke if a relevant acute infarct is seen. Moreover, sex differences might have occurred by chance.

Our findings have several implications for future research. The mechanism by which NT-proBNP relates to ischemic stroke, hemorrhagic stroke, and TIA needs to be elucidated further. As associations with focal TIA were different compared to mixed TIA, differences in etiology of those attacks should also be identified. Furthermore, NT-proBNP needs to be

investigated for its predictive role in stroke, even in persons free of heart disease. In our study NT-proBNP was merely used as a marker for subclinical heart disease, but it might even be a target for therapy, as is shown in the PARADIGM HF trial.[26] Additionally, studies should disentangle the exact role of antithrombotic medication in the associations of NT-proBNP with cerebrovascular events. Finally, future research should confirm whether subclinical heart disease indeed is a more important cause of TIA in women compared to men and whether future strokes in women can be prevented with more attention towards subclinical heart disease after TIA.

Strengths of this study are the population-based setting, the large study population and the thorough collection of events. A limitation is that follow-up for heart failure and atrial fibrillation was incomplete. Events in the last years of follow-up could therefore not be censored. However, also with shorter follow-up until 2008 censoring at time of incident heart disease did not attenuate the results. Another limitation is that we did not systematically measure valvular heart disease as potential confounding factor.[12,27]

In conclusion, our results show that higher levels of NT-proBNP are associated with a higher risk of cerebrovascular events. For stroke this risk was present in men and women, for TIA only in women. These associations were independent of prevalent and incident heart disease during follow-up, suggesting that a high NT-proBNP as marker of subclinical heart disease leads to an increased risk of stroke in men and women and of TIA in women.

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2

Supplementary information

Supplementary Table I. NT-proBNP and the risk of any cerebrovascular event, stratified for antithrombotic drug use

	Men		Women	
	n/N	HR (95% CI)	n/N	HR (95% CI)
Use of anticoagulant drugs	15/85	1.30 (0.81; 2.08)	12/44	1.33 (0.68; 2.61)
Use of antiplatelet drugs	57/373	1.26 (0.95; 1.68)	63/333	1.17 (0.86; 1.58)
No use of any antithrombotic drug	223/1841	1.43 (1.24; 1.66)	319/2730	1.39 (1.21; 1.60)

Abbreviations: n = number of events; N = number of persons at risk; SD = standard deviation
Values are hazard ratios per SD increase in natural log-transformed NT-proBNP level (SD=0.96) with 95% confidence intervals.
Analyses with stroke were censored for incident TIA and analyses with TIA were censored for incident stroke.
Adjusted for age and other therapy use.
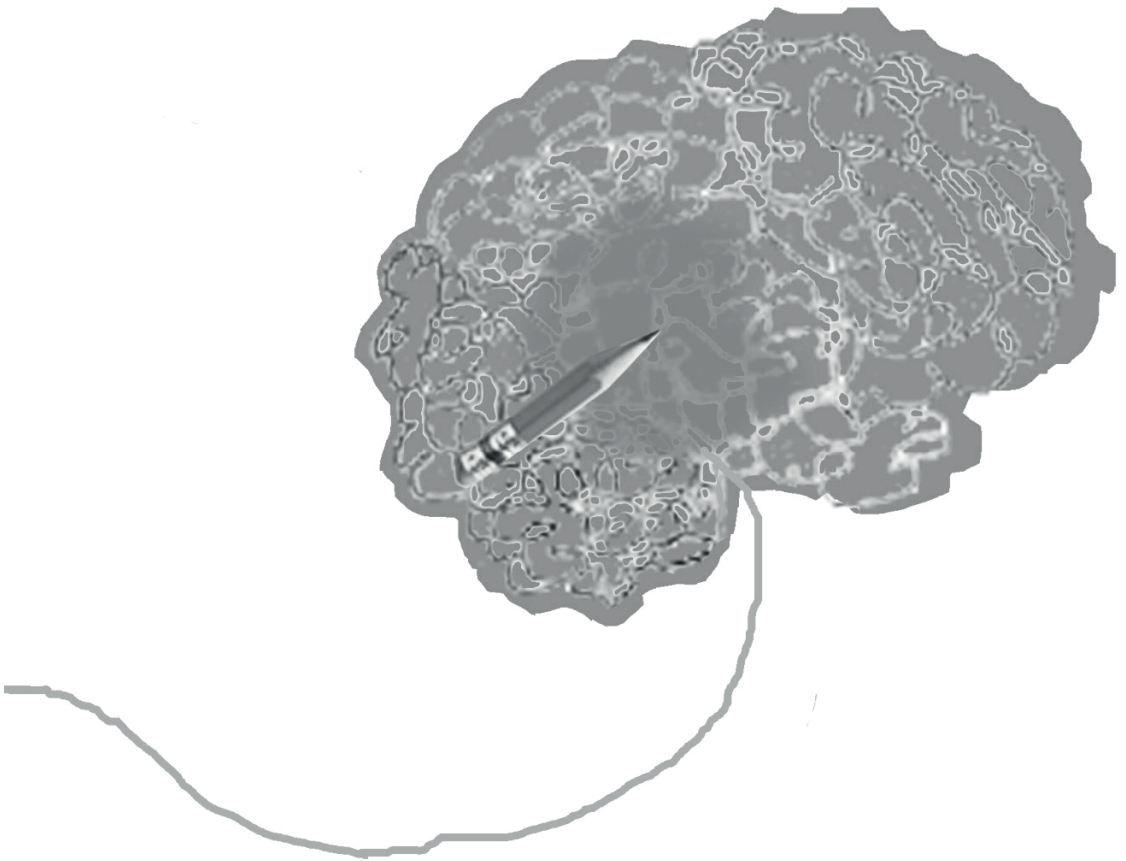
Chapter 3

Large and small vessel disease markers and stroke

Chapter 3.1

Intracranial carotid artery atherosclerosis and the risk of stroke in whites

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Abstract

Background and purpose
Intracranial atherosclerosis represents a relatively unexplored, but potentially important cause of stroke in whites. We investigated the relationship between intracranial carotid artery calcification (ICAC), as marker of intracranial atherosclerosis, and the risk of stroke in a white population.

Methods
Between 2003 and 2006, 2323 stroke-free persons from the general population (mean age: 69.5 years) underwent computed tomography (CT) to quantify ICAC volume. All participants were continuously followed for the occurrence of stroke until January 1, 2012. We constructed Cox regression models to relate ICAC with the risk of stroke, adjusting for age and sex. Additionally, we adjusted for cardiovascular risk factors, ultrasound carotid plaque score, and CT-assessed calcification volume in the coronary arteries, aortic arch and carotid bifurcation. Finally, we calculated the proportion of stroke attributable to ICAC, using the population attributable risk (PAR).

Results
During 14,055 person years of follow-up, 91 participants had a stroke, of which 74 were ischemic. Larger ICAC volume was related to a higher risk of stroke, independent of cardiovascular risk factors, ultrasound carotid plaque score, and calcification in other vessels [fully adjusted hazard ratio (HR) per SD increase in ICAC volume: 1.43 (95% CI 1.04; 1.96)]. ICAC contributed to 75% of all strokes, whilst for aortic arch and extracranial carotid artery calcification this was only 45% and 25% respectively.

Conclusions
Our findings establish intracranial atherosclerosis as major risk factor for stroke in the general white population and suggest that its contribution to the proportion of all strokes may be greater than that of large-artery atherosclerosis in more proximal located vessel beds.

Introduction

Stroke is the most frequent neurological disease, and the second most important cause of global mortality.[1-3] The lifetime risk of stroke is estimated to be at least 1 in 6 and in the coming decades its burden is expected to increase even further.[4,5]

Of all strokes, approximately 80% to 90% are of ischemic origin.[2] Ischemic stroke has a multifactorial etiology involving various overlapping and interacting pathways, such as vascular disease, inflammation, hemostasis, metabolism, and genetic factors.[2] Among these, vascular disease is by far the most important and has been the target of many preventive and therapeutic interventions for stroke.[1,2] When studying vascular disease, it is important to consider that its burden may vary substantially across different vessel beds.[6,7] As such, several locations of vascular disease are considered relevant in stroke etiology. First, cardiac diseases, such as coronary heart disease and atrial fibrillation, increase the risk of stroke.[1,2,8] Second, large-artery atherosclerosis is recognized as a major risk factor of stroke.[2,9,10] Thirdly, strokes may occur after occlusion of the small penetrating intracerebral arteries, in so-called cerebral small vessel disease.[1,2,9] It is important to note that these do not represent mutually exclusive categories, but that in most patients vascular pathology is present in multiple sites.

Within this etiologic framework, large-artery atherosclerosis is considered to include all vessels from the aortic arch to the major cerebral arteries. Atherosclerosis of the intracranial vasculature is globally considered the most important risk factor for stroke.[11] However, current numbers are solely driven by populations from Asian and African origin, which make up the largest proportion of strokes worldwide.[11-14] In contrast, there are no data on the burden of stroke attributable to intracranial atherosclerosis in white populations. Yet surprisingly, most clinical trials targeting intracranial atherosclerosis include a majority of whites in their samples.[15,16] There is now an emerging awareness that robust data from white populations on the role of intracranial atherosclerosis in ischemic stroke are urgently needed.[11,17-19] These can then serve as basis for designing future intervention studies.

Recently, using intracranial carotid artery calcification (ICAC) as proxy, we demonstrated a prevalence of intracranial atherosclerosis exceeding 80% in a general white population.[20] In this report, we set out to investigate in a white population the longitudinal association of ICAC with incident stroke over a six year follow-up period. We were specifically interested in estimating the total burden of stroke attributable to ICAC in whites.

Methods

Setting
This study is based on the Rotterdam Study, a prospective, population-based study investigating determinants of chronic diseases in the elderly.[21] The original cohort consisted of 7983 participants aged 55 years or older and was extended in 2000-2001 with 3011 persons. At study entry and every 3 to 4 years, all participants are re-examined in a dedicated research center. The Rotterdam Study represents a homogeneous middle-class population, largely of white descent (>96%).

For the current study, we used the visit between 2003 and 2006 as baseline, because only in this period we invited participants who visited the research center to undergo non-enhanced computed tomography (CT) of the intracranial carotid arteries.[7] We scanned 2524 participants (response rate 78%). The follow-up for stroke takes place continuously and was complete until January 1, 2012.

This study was approved by the institutional review board of Erasmus Medical Center, Rotterdam, the Netherlands. All participants gave informed consent.

Assessment of intracranial atherosclerosis

We used a 16-slice (n = 785) or 64-slice (n = 1739) multidetector CT-scanner (Somatom Sensation 16 or 64, Siemens, Forcheim, Germany) to perform non-enhanced CT-scanning. Using a cardiac scan and a scan that reached from the aortic arch to the intracranial vasculature (1 cm above the sella turcica), we scanned the following vessel beds: the coronary arteries, the aortic arch, the extracranial carotid arteries, and the intracranial carotid arteries. Detailed information regarding imaging parameters of both scans is provided elsewhere.[7]

As proxy for intracranial atherosclerosis, we measured ICAC bilaterally in the intracranial internal carotid artery from its horizontal petrous segment to its top (until the circle of Willis) (Figure 1). For quantification of ICAC, we used a semi-automated scoring method which is described in detail elsewhere.[20] Briefly, we manually drew regions of interest around calcification in the course of the intracranial internal carotid arteries in consecutive CT-slices. Next, we calculated calcification volumes by multiplying the number of pixels above 130 Hounsfield Units with the pixel-size and the increment.
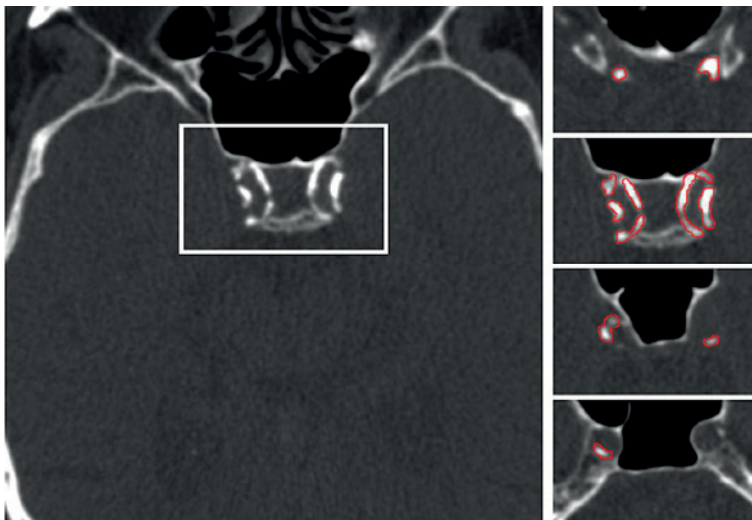
Figure 1. Example of calcification of the intracranial internal carotid artery.
The left image shows an axial CT slice in which the white rectangle marks the region of the internal carotid arteries. The four images on the right represent the course of the intracranial carotid artery from the petrous bone (lower image) to the top (upper image) with calcifications (in red).

Calcification volumes in the coronary arteries, aortic arch and extracranial internal carotid arteries were quantified using dedicated commercially available software (Syngo CalciumScoring, Siemens, Germany).[7] All calcification volumes are expressed in mm³. Correlations between calcification across the four vessel beds ranged from 0.5 to 0.6.[7,22]

Follow-up for Stroke

The definition of stroke was based on the WHO criteria including a syndrome of rapidly developing symptoms of focal or global cerebral dysfunction lasting 24 hours or longer or leading to death, with apparent vascular cause.[23,24] We assessed prevalent stroke at baseline during interview and verified these data with medical records.[23] After enrolment, we continuously monitored participants for incident stroke through linkage of the study database with files from general practitioners. Nursing home physicians' files and files from general practitioners of participants who moved out of the district were also checked.[23] Additional information was obtained from hospital records. Potential strokes were reviewed by research physicians and verified by an experienced stroke neurologist. We categorized strokes into ischemic or haemorrhagic on the basis of neuroimaging-reports. If neuroimaging was unavailable, the stroke was classified as unspecified.[23] Subarachnoid haemorrhages due to ruptured aneurysms were not considered stroke events.[23] Follow-up for incident stroke was complete until January 1, 2012.

Other measurements in the Rotterdam Study

We obtained information on cardiovascular risk factors by interview, physical examination and blood sampling.[21] Obesity was defined as a body-mass index (BMI) $\geq$ 30 kg/m². Hypertension was defined as a systolic blood pressure $\geq$ 140mmHg and/or a diastolic blood pressure $\geq$ 90 mmHg and/or use of blood pressure-lowering medication.[25] Diabetes was defined as fasting serum glucose levels $\geq$ 7.0 mmol/l and/or use of antidiabetic therapy.[26] Hypercholesterolemia was defined as a serum total cholesterol $\geq$ 6.2 mmol/l and/or use of lipid-lowering medication.[27] We defined low high-density lipoprotein (HDL)-cholesterol as HDL-cholesterol < 1.0 mmol/l.[27] Smoking was categorized into never or ever smoked.

Besides these cardiovascular risk factors, we also assessed other imaging markers of atherosclerosis. These were atherosclerotic calcification volumes in the coronary arteries, aortic arch and extracranial carotid arteries (as detailed above), and ultrasound carotid plaquescore. Using ultrasound, we visualized the common carotid artery, carotid artery bifurcation, and internal carotid artery and examined both left and right to assess a weighted plaque score ranging from 0 to 6.[28]

Population for analysis

We restricted our population to persons from white descent, as assessed by self report (n = 2452, from the 2524 that underwent CT). Due to image artefacts, 27 from the 2452 CT-examinations were not gradable for ICAC, leaving a total of 2425 participants with complete data on ICAC volume. Persons with prevalent stroke (n = 98) and persons that did not

participate in the stroke follow-up (n = 4) were excluded, leaving 2323 participants at risk for stroke in the population for analysis.

Statistical analysis

Due to the non-normal distribution of ICAC volume, we performed a natural log-transformation and added 1.0 mm^3 to the non-transformed values to deal with calcium volumes of zero [Ln(ICAC + 1.0 mm^3)]. We calculated hazard ratios (HR) for stroke per standard deviation (SD) increase in ICAC volume, using Cox regression models. The proportional hazards assumption was met. In model 1, we adjusted for age, sex, and scanner type. In model 2, we additionally adjusted for the following cardiovascular risk factors: obesity, hypertension, diabetes mellitus, hypercholesterolemia, low HDL-cholesterol, and smoking. In model 3, we additionally adjusted for the ultrasound carotid plaquescore and CT-assessed calcification volumes in the coronary arteries, aortic arch, and extracranial carotid artery.

Next, we dichotomized ICAC into presence versus absence of ICAC and related that to the risk of stroke. In contrast to established categories for absolute coronary calcification scores, i.e. Agatston score,[29] there are no such categories for ICAC. Therefore, we categorized calcification into present versus absent, because this is most readily identified in a clinical situation. We used the same three Cox-regression models as above, with the only difference that in model 3, calcification in other vessel beds was also dichotomized into present/absent. Stroke-free survival in the absence and presence of ICAC was estimated and compared using the Kaplan-Meier method and log rank test.

Finally, we calculated the population attributable risk (PAR) for stroke of calcification in each of the four vessel beds using the following formula:[30]

$$PAR = PD\left(\frac{RR-1}{RR}\right)x100\%$$

In this formula, *RR* represents the relative risk, i.e. HR, which we calculated for the presence of calcification in each vessel bed. *PD* is the proportion of cases exposed to the risk factor (the presence of calcification). PAR provides a measure between 0 and 100%, which can be interpreted as the fraction of strokes that is due to calcification.[30] IBM SPSS Statistics version 20 (International Business Machines Corporation, Armonk, New York) was used for statistical analyses.

Results

Table 1 shows the baseline characteristics of the study population. Mean age was 69.5 years and 52.2% of the participants were female. During 14,055 person years of follow-up (mean 6.1 years), 91 participants had a stroke, of which 74 were ischemic, 10 were haemorrhagic, and 7 unspecified.

Table 1. Baseline characteristics of study participants

	Sample size N=2323
Women	52.2%
Age, years	69.5 (6.7)
Obesity	23.7%
Body Mass Index, kg/m^2	27.7 (4.0)
Hypertension	73.9%
Systolic blood pressure, mmHg	146.7 (20.1)
Diastolic blood pressure, mmHg	80.2 (10.8)
Use of blood pressure-lowering medication	39.3%
Diabetes	10.7%
Serum glucose, mmol/L	5.7 (1.2)
Use of antidiabetic medication	6.0%
Hypercholesterolemia	48.9%
Serum total cholesterol, mmol/L	5.7 (1.0)
Use of lipid-lowering medication	23.2%
Low HDL-cholesterol	10.6%
Serum HDL-cholesterol, mmol/L	1.4 (0.4)
Past or current smokers	67.5%
Presence of intracranial carotid artery calcification	81.4%
Intracranial carotid artery calcification volume[a], mm^3	41.1 (6.2 – 135.1)

Abbreviations: HDL = high-density lipoprotein.
Values are means (standard deviation) for continuous variables or percentages for dichotomous variables.
[a]Median (interquartile range)

Table 2 shows the associations between ICAC and the risk of stroke. We found that larger ICAC volumes were associated with a higher risk of stroke. These results were similar for ischemic stroke. Additional adjustment for cardiovascular risk factors did not change any of these results [HR per SD increase in ICAC volume: 1.52 (95% CI 1.17; 1.98) for stroke, and 1.53 (95% CI 1.14; 2.04) for ischemic stroke]. Also after additional adjustment for ultrasound carotid plaquescore and calcification volumes in the other vessel beds, we found that larger ICAC volumes remained significantly associated with a higher risk of stroke [HR per SD increase in ICAC volume: 1.43 (95% CI 1.04; 1.96)] The effect size for the association with ischemic stroke was similar, although statistically non-significant (Table 2, model 3, upper panel).

We found that the presence of ICAC was associated with a higher risk of any stroke and ischemic stroke, even after adjustment for cardiovascular risk factors [HR for presence versus absence of ICAC: 4.15 (95% CI 1.51; 11.42) for stroke, and 3.43 (95% CI 1.24; 9.51) for ischemic stroke]. Additional adjustment for ultrasound carotid plaquescore and the presence of calcification in the other vessel beds also did not change these associations (Table 2, model 3, lower panel). Kaplan-Meier curves revealed that the stroke-free survival in persons with ICAC was significantly shorter than persons without ICAC (Figure 2).

Table 3 shows the proportion of strokes that is attributable to calcification in each of the four vessel beds. We found that ICAC played a role in up to 75% of all strokes, whilst for aortic arch calcification and calcification in the extracranial carotid artery this was 45% and 25%,

respectively. For coronary artery calcification we did not calculate the PAR for stroke, because the HR was below one.

Table 2. Intracranial carotid artery calcification and the risk of stroke

	Any stroke	Ischemic stroke
	n/N = 91/2323	n/N = 74/2323
ICAC Characteristic	HR (95% CI)	HR (95% CI)
Continuous, per 1-SD increase		
Model 1	1.53 (1.18; 1.97)	1.51 (1.14; 2.01)
Model 2	1.52 (1.17; 1.98)	1.53 (1.14; 2.04)
Model 3	1.43 (1.04; 1.96)	1.39 (0.98; 1.99)
Dichotomous, presence vs absence		
Model 1	4.25 (1.55; 11.66)	3.49 (1.27; 9.64)
Model 2	4.15 (1.51; 11.42)	3.43 (1.24; 9.51)
Model 3	4.64 (1.44; 14.95)	3.52 (1.08; 11.47)

Abbreviations: n = number of cases; N = number of persons at risk; HR = hazard ratio; CI = confidence interval; ICAC = intracranial carotid artery calcification; SD = standard deviation.
Model 1: Adjusted for age, sex, and scanner type.
Model 2: Adjusted for age, sex, scanner type, obesity, hypertension, diabetes mellitus, hypercholesterolemia, low HDL-cholesterol, and smoking.
Model 3: Adjusted for age, sex, scanner type, obesity, hypertension, diabetes mellitus, hypercholesterolemia, low HDL-cholesterol, smoking, ultrasound carotid plaque score, and calcification in the coronary arteries, the aortic arch and the carotid bifurcation (as appropriate; volume for continuous analyses, presence for dichotomous analyses).
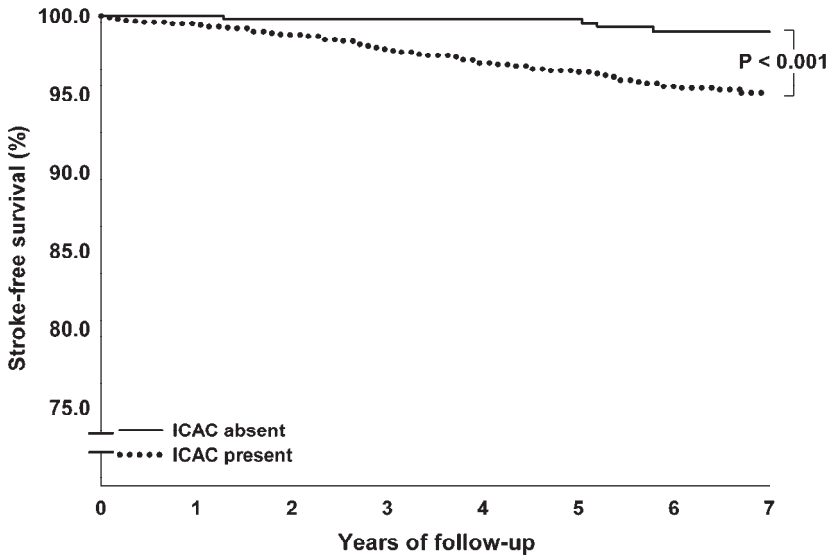
Figure 2. Kaplan-Meier survival plot for stroke-free survival in persons with and without intracranial carotid artery calcification.
Abbreviations: ICAC, intracranial carotid artery calcification; CT, computed tomography
Kaplan-Meier survival curve for the stroke-free survival of persons with and without ICAC. The difference between the two groups was statistically significant (P < 0.001 by the log-rank test).
ICAC absent: number of persons at risk = 431, number of cases = 4.
ICAC present: number of persons at risk = 1892, number of cases = 87.

Table 3. Population attributable risks for stroke per vessel bed

	Proportion of stroke-cases exposed to calcification	Any stroke HR (95% CI)	PAR
Presence of calcification in:			
Coronary arteries	0.87	0.80 (0.40;1.57)	N.A.[a]
Aortic arch	0.98	1.84 (0.44;7.77)	45%
Extracranial carotid arteries	0.86	1.41 (0.71;2.83)	25%
Intracranial carotid arteries	0.96	4.64 (1.44;14.95)	75%

Abbreviations: HR, hazard ratio; CI, confidence interval; PAR, population attributable risk.
Adjusted for age, sex, scanner type, obesity, hypertension, diabetes mellitus, hypercholesterolemia, low HDL-cholesterol, smoking, presence of calcification in the other vessel beds, and for ultrasound carotid plaquescore.
[a] The PAR for coronary artery calcification was not calculated because the HR is below one.

Discussion

In this large population-based cohort study among middle-aged and elderly white persons we found that both presence and severity of ICAC were associated with a higher risk of stroke. This was independent of both conventional cardiovascular risk factors and atherosclerosis in other extracranial vessel beds. We also found that ICAC contributed to 75% of all strokes, whereas for aortic arch calcification and extracranial carotid artery calcification this was only 45% and 25%.

With the current study we demonstrated that intracranial atherosclerosis is a major risk factor for stroke in a white population. Thus far, evidence for a role of intracranial atherosclerosis in the etiology of stroke mainly comes from research in populations from Asian and African descent.[14,17,18] In these populations, intracranial atherosclerosis is an established major risk factor for ischemic stroke, accounting for up to 50% of all strokes.[14,17]

We acknowledge that the total burden of intracranial atherosclerosis comprises atherosclerotic disease in more arteries than only the intracranial carotid arteries that we measured.[14,18] However, atherosclerotic plaques in other cerebral arteries are typically non-calcified.[31,32] Visualisation of non-calcified atherosclerotic disease demands administration of intravenous contrast material which was not possible in our population-based setting. Yet, we note that autopsy studies have shown that specifically for the intracranial vasculature there is a strong correlation between the severity and extent of atherosclerosis across cerebral arteries.[33,34] Therefore it is likely that if there is more ICAC, there are probably also more atherosclerotic changes in the distal cerebral vessels. In this light ICAC would be a marker of total intracranial atherosclerosis. On the other hand, there may also be a causal role of intracranial carotid artery calcification in stroke. Highly calcified vessels may in some instances have a stenotic lumen leading to hemodynamic disturbances.[35,36] However, this association is complex due to remodeling (i.e. compensatory enlargement) of arteries.[35,37] Also, large calcification volumes reflect large plaques, which may be a source of emboli. To further unravel these mechanisms and the putative causal role of intracranial carotid artery calcification, future studies should focus on classifying strokes systemically according to vascular territories.

Using PAR, we estimated that intracranial atherosclerosis contributes to the occurrence of 75% of all strokes. The PAR for stroke of intracranial atherosclerosis was notably larger than that of atherosclerosis in the aortic arch or carotid bifurcation. Theoretically, a PAR of 75% indicates that the incidence of stroke may be reduced by 75% if intracranial atherosclerosis could be completely eradicated. However, a few considerations should be noted for better interpretation of a PAR. First, this figure of 75% does not mean that only 25% of strokes remain to be explained by other causes than intracranial atherosclerosis.[30] In fact, the sum of PARs for all possible risk factors of stroke exceeds 100%, reflecting interaction between risk factors. This also signifies that other unknown causes may still contribute considerably to the development of stroke. Nevertheless, the high PAR illustrates the large potential gain in public health that could be achieved by further developing therapeutic and preventive strategies aimed at reducing the amount of intracranial atherosclerosis. Possibly, earlier and more aggressive treatment of modifiable risk factors for intracranial atherosclerosis could stave off its formation and may thereby contribute to the primary prevention of stroke. A beneficial effect of

aggressive medical management on the occurrence of stroke has already been demonstrated in patients with symptomatic intracranial atherosclerotic stenosis.[15] Second, although we adjusted for conventional cardiovascular risk factors, as well as for calcification in other vessel beds, the HR that we used may still be subject to residual confounding. If so, a more unbiased HR would have yielded an attenuation of the PAR. Still, the PAR of ICAC was highest among all studied vessel beds.

In the current literature, a major focus is on coronary artery calcification as an emerging determinant for various cardiovascular events. Some studies have shown coronary artery calcification to even improve the risk prediction of stroke,[8] but this remains debatable.[38,39] In our dataset we found no association between coronary artery calcification and stroke when taking into account calcification in all vessel beds. In a post-hoc analysis, we found that coronary artery calcification was associated with stroke in a crude age and sex-adjusted model only. This further corroborates that ICAC is a more important determinant of stroke than coronary artery calcification. Future studies should thus also investigate the predictive value of ICAC for stroke.

Strengths of our study include the population-based setting and the longitudinal design. Moreover, we used an accurate image-based method to quantify ICAC. We tried to minimize the number of persons that were lost to follow-up by using thorough stroke monitoring procedures. These allowed us to identify nearly all stroke events, including fatal strokes and strokes in participants living in nursing homes, who were not referred to a hospital. There are also several potential limitations that should be taken into account. First, by using non-enhanced CT we were only able to measure calcification and not the complete atherosclerotic plaque. Although strong evidence from autopsy studies shows that CT-based calcification is a sensitive and reliable marker of the total underlying atherosclerotic burden,[40,41] this might have led to misclassification in certain instances. Second, it was not possible to describe any additional plaque characteristics, for example shape, stenosis, or ulceration, which may all be of importance with regard to future events. Although a high correlation between CT-measured calcification and stenosis has been found in the carotid siphon,[36,42] advances in plaque imaging with other imaging techniques, e.g. MRI, may aid to overcome this issue in the future.

In conclusion, our findings suggest that intracranial atherosclerosis is a major risk factor for stroke in the general white population. Moreover its contribution to the proportion of all strokes may be greater than that of large-artery atherosclerosis in other vessel beds.

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Chapter 3.2

Cerebral vasomotor reactivity and risk of mortality

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Abstract

Background and Purpose
Accumulating vascular pathology in cerebral arteries leads to impaired cerebral vasomotor reactivity. In turn, impaired cerebral vasomotor reactivity is a risk factor for stroke in clinical populations. It remains unclear whether impaired cerebral vasomotor reactivity also reflects more systemic vascular damage. We investigated whether cerebral vasomotor reactivity is associated with the risk of mortality, focusing particularly on cardiovascular mortality independent from stroke.

Methods
Between 1997-1999, 1695 participants from the Rotterdam Study underwent cerebral vasomotor reactivity measurements using transcranial Doppler. Follow-up was complete until January 1, 2011. We assessed the associations between cerebral vasomotor reactivity and mortality using Cox proportional hazards models, adjusting for age, sex, and blood pressure changes and subsequently for cardiovascular risk factors. We additionally censored for incident stroke.

Results
During 17,004 person-years 557 participants died, of whom 181 due to a cardiovascular cause. In the fully adjusted model, the hazard ratio per SD decrease in vasomotor reactivity was 1.10 (95% CI 1.01; 1.19) for all-cause mortality, 1.09 (95% CI 0.94; 1.26) for cardiovascular mortality, and 1.10 (95% CI 0.99; 1.21) for non-cardiovascular mortality. These associations remained unchanged after censoring for incident stroke.

Conclusions
We found that lower cerebral vasomotor reactivity is associated with an increased risk of death. Incident stroke does not affect this association, suggesting that a lower cerebral vasomotor reactivity reflects a generally impaired vascular system.

Introduction

Vascular diseases are the main cause of mortality worldwide and lead to considerable societal burden, both in terms of care and cost. The World Health Organization estimates that by 2030, more than 23 million people will die yearly from vascular diseases.[1] Despite the acute clinical presentation, an important feature of vascular diseases is the long preclinical phase, during which various pathologies interact leading to accumulating vascular damage. These pathologies include atherosclerosis, arterial stiffening, inflammation, and endothelial damage.[2-6] In the brain, this pathologic process ultimately manifests itself as either ischemic or hemorrhagic stroke.[7]

   A cornerstone of preventive research has been to identify markers that reflect such preclinical vascular pathology and thus may predict shorter survival. For cerebrovascular damage, diminished vasomotor reactivity has been identified in recent years as a prognostic marker.[8] Cerebral vasomotor reactivity reflects the ability of the cerebral arterioles to dilate in the event of hypercapnia to improve cerebral blood flow.[9,10] Clinically, cerebral vasomotor reactivity can be measured using transcranial Doppler. In persons with vascular damage at the level of the arterioles, or less hemodynamic reserve as a consequence of vascular disease in larger vessels, this ability is significantly diminished.[9,11] Most studies investigating vasomotor reactivity were in clinical populations of patients with carotid artery stenosis. In these studies impaired vasomotor reactivity was associated with an increased risk of stroke and TIA.[8,12-17] However, its role in the general community-dwelling population is less clear. Vasomotor reactivity has been measured within the population-based Rotterdam Study, but no association between vasomotor reactivity and stroke was found.[18, 19]

   Still, the question remains whether impaired cerebral vasomotor reactivity associates with poorer survival in a general elderly population. Specifically, it is unknown whether any such associations are driven by stroke, or whether cerebral vasomotor reactivity actually reflects more systemic vascular damage. Therefore, we investigated the association of cerebral vasomotor reactivity with all-cause mortality and cardiovascular mortality in a community-dwelling elderly population. Furthermore, we studied whether any associations were independent of incident stroke.

Materials and Methods

Setting
This study is part of the Rotterdam Study, a prospective population-based cohort study that started in 1990 among inhabitants of 55 years and older residing in Ommoord, a suburb of Rotterdam, the Netherlands. Of the 10,215 eligible inhabitants, 7983 agreed to participate in the baseline examinations. Up until 2013, there have been 4 follow-up examinations. Details of the study have been described elsewhere.[20] The medical ethics committee at Erasmus University of Rotterdam approved the study and written informed consent was obtained from all participants. For the current study, the second follow-up examination from 1997-1999 was

used as baseline, because transcranial Doppler measurements were performed only at that visit.

Transcranial Doppler assessment

At the examination in 1997-1999, participants underwent transcranial Doppler ultrasonography (Multi-Dop X-4; DWL, Sipplingen, Germany). Vasomotor reactivity was measured as follows:[18] the cerebral blood flow velocity was measured at the middle cerebral artery continuously. End diastolic, peak systolic, and mean cerebral blood flow velocities were recorded automatically. Mean blood flow velocity was calculated automatically as
(1/3 * (peak systolic flow velocity + 2 * end diastolic flow velocity)).[19]

Blood pressure was measured automatically (Dynamap, Datascope, The Netherlands) before and during the transcranial Doppler recordings. The participants first breathed room air through an anesthetic mask, tightly fit over mouth and nose, until a steady expiratory end tidal CO_2 was obtained. One way valves were placed in the tubes for inspiration and expiration. End tidal CO_2 pressure (kPa), measured in the exhaled air, was recorded continuously with a CO_2 analyzer (Multinex; Datascope, Hoevelaken, the Netherlands). End expiratory CO_2 was assumed to reflect arterial CO2.21 Participants then inhaled a mixture of 5% carbon dioxide in 95% oxygen for 2 minutes. Vasomotor reactivity was defined as the percentage increase in mean cerebral blood flow velocity during inspiration of 5% CO_2, divided by the absolute increase in end tidal CO_2 in the same time period (%/kPa). TCD-8 DWL special software (VMR- CO2) was used. All transcranial Doppler data were stored on hard disk for offline analysis.

Assessment of mortality

Deaths were continuously reported through automatic linkage of general practitioner files. In addition, municipal records were checked bimonthly for information on vital status. Information about cause and circumstances of death was obtained from general practitioner and hospital records. Research physicians reviewed all available information and coded the events according to the International Classification of Diseases, 10th edition (ICD-10). If the cause of death was coded as I20-I25, I46, I50, I61, I63, I64, I66, I68-70, or R96, the cause of death was labeled as cardiovascular. A consensus panel, led by a physician with expertise in cardiovascular disease, adjudicated the final cause of death according to the ICD-10 codes using standardized definitions, as described in detail previously.[22] The follow-up was complete until January 1, 2011, for 97.1% of potential person-years.

Assessment of stroke

At study entry, history of stroke was assessed using home interviews and confirmed by reviewing medical records. Once participants enter the Rotterdam Study, they are continuously followed-up for stroke through automatic linkage of general practitioner files with the study database. Also, nursing home physicians' files and files from general practitioners of participants that moved out of the district were checked on a regular basis. Of the potential strokes, additional hospital and general practitioner information was collected. Research physicians reviewed the stroke information and an experienced neurologist adjudicated the

strokes using standardized definitions, as described in detail previously.[23] The follow-up was complete until January 1, 2011, for 97.3% of potential person-years.

Other measurements

Covariates were measured during the same examination round as transcranial Doppler measurements were performed (1997-1999). Smoking status and medication use were assessed using a home interview. Smoking was classified into current smoking, former smoking, or never smoking. Diabetes mellitus was defined as having a fasting glucose level of 7.0 mmol/L or higher or using blood glucose-lowering medication. Total cholesterol and HDL-cholesterol levels were acquired by an automated enzymatic procedure. Blood pressure was measured at the research center twice in the sitting position on the right arm with a random-zero sphygmomanometer. The average of the two measurements was used in the analyses. Blood pressure was also measured prior to and during the vasomotor reactivity measurements. We used the difference between these two measurements in the analyses since changes in blood pressure caused by CO_2 inhalation can influence vasomotor reactivity measurements.[24] Prevalent vascular disease (myocardial infarction, CABG, PCI, heart failure, and peripheral arterial disease) was, except for peripheral arterial disease, assessed through active follow-up and adjudicated using standardized definitions, as described in detail previously.[22] Peripheral arterial disease was assessed using the ankle-brachial index. Ankle-brachial index was assessed by computing the ratio of systolic blood pressure at the right and left ankle to the systolic blood pressure at the right arm. The lowest value was used in the analyses. Values of ankle-brachial index greater than 1.4 were excluded because high ankle-brachial index might represent a different underlying pathology. Peripheral arterial disease was defined as an ankle-brachial index of 0.9 or less.[25] To measure carotid intima-media thickness (cIMT), ultrasonography of the left and right carotid arteries was performed with a 7.5-MHz linear array transducer (ATL UltraMark IV; Advanced Technology Laboratories, Bethel, Washington). The maximal cIMT, summarized as the mean of the maximal measurements from the near and far walls of both the left and right sides, was used for analysis.[25,26]

Study population

Of the 5990 participants that were alive in 1997-1999, 4797 persons participated in the examination used as baseline for this study. Of these, 4215 visited the study center. Due to lack of technical support, vasomotor reactivity measurements started later in the examination round (from July 1, 1997) and could only be offered to 2732 random participants. After excluding participants with prevalent stroke at time of transcranial Doppler assessment (n=100), 2632 participants were eligible for transcranial Doppler assessment. Of these, 937 participants were excluded because of window failure on both sides (n=656), restlessness, anxiety, and discomfort (n=56), or missing data for other reasons (n=225). This left 1695 participants eligible for the analysis of this study. Persons with a prevalent stroke (i.e. stroke before vasomotor reactivity measurement) have a higher probability of vascular damage of the middle cerebral artery where vasomotor reactivity was measured. Moreover, these persons are both at a higher risk of a recurrent stroke and at a higher risk of mortality compared to persons

without prevalent stroke. Including such persons with prevalent stroke into our analysis, even if they had a remote stroke, would therefore bias our results.

Statistical analyses

We investigated the associations of vasomotor reactivity with all-cause mortality, cardiovascular mortality, non-cardiovascular mortality, and stroke using Cox proportional hazards models. We used these models since we investigated time-to-event data. The underlying time-scale in these models was the follow-up time in years, which was complete until January 1, 2011. Participants were censored within this follow-up period at date of death, date of loss to follow-up, or January 1, 2011, whichever date came first. The proportional hazards assumption was met.

Because of a right skewed distribution of vasomotor reactivity, we first performed a natural logarithmic transformation to obtain a roughly normal distribution of the data. Logarithmic transformed vasomotor reactivity was entered continuously per standard deviation (SD) decrease into the models, because a decrease reflects an impaired reactivity. We presented the results per standard deviation merely for a uniform representation of the data, this presentation was also used in the previous paper of Bos MJ et al.[19] Furthermore, we studied vasomotor reactivity in quartiles taking the upper quartile as reference. All models were adjusted for age, sex, and blood pressure changes during vasomotor reactivity measurement. We adjusted subsequently for current smoking, former smoking, use of blood pressure-lowering medication, systolic blood pressure, diastolic blood pressure, diabetes mellitus, total cholesterol, HDL-cholesterol, use of statins, history of vascular disease, and carotid intima-media thickness for being potential confounders. Missing data on covariates (8.4% or less) were imputed based on sex and age using linear regression models.

To investigate whether vasomotor reactivity within ranges of normal was associated with mortality, we repeated the analysis after excluding participants with a vasomotor reactivity lower than 10%/kPa.[15]

To assess the role of stroke on these associations, we related vasomotor reactivity to stroke as well as to mortality after censoring for stroke. We performed an additional analysis to investigate whether diminished vasomotor reactivity acts as intermediate between various cardiovascular risk factors and risk of mortality. We associated the various cardiovascular risk factors with mortality and investigated whether adjustment for vasomotor reactivity affected these associations.

All analyses were done using the IBM SPSS statistics version 20.0 (IBM Corp, Armonk, NY, USA).

Results

Table 1 shows baseline characteristics of the study population. Non-participants were older and more often women compared to participants. Also, they smoked less, had a higher HDL-cholesterol, a larger carotid intima-media thickness, had more often prevalent vascular disease, and used more often blood pressure-lowering medication and statins than participants.

The average follow-up duration for total mortality was 10.0 years, during which a total of 557 of the 1695 participants died, of whom 181 due to a cardiovascular cause and 376 due to a non-cardiovascular cause. The most important cardiovascular causes of death were stroke (N=41), heart failure (N=39), cardiac arrest (N=29), other sudden death with unknown cause (N=24), and acute myocardial infarction (N=26). The most important non-cardiovascular causes of death were cancer (N=170, especially lung (N=45), colon (N=22), pancreas (N=15), and breast cancer (N=10)), and dementia (N=40). Of the non-participants, 457 of the 1037 participants (44.1%) died. This difference was statistically significant compared to the number of deaths in the study population.

A total of 168 participants suffered from a stroke of which 92 participants died, either due to the stroke or other causes. After censoring for stroke, a total of 465 participants died, 131 due to a cardiovascular cause and 334 due to a non-cardiovascular cause. The most important cardiovascular causes of death in this last group were heart failure (N=36), cardiac arrest (N=29), other sudden death with unknown cause (N=21), and acute myocardial infarction (N=24).

Table 2 shows the hazard ratios of all-cause mortality. A lower vasomotor reactivity was associated with a higher risk of all-cause mortality (hazard ratio (HR) per SD decrease in vasomotor reactivity 1.12; 95% confidence interval (CI) 1.03; 1.21). These associations remained unchanged after additional adjustments (HR 1.10, 95% CI 1.01; 1.19). Also, persons in the lowest two quartiles had an increased risk of death compared to the upper quartile (Table 2). Figure 1 shows the corresponding Kaplan-Meier curves for these associations.

Table 1. Baseline characteristics

	Participants N=1695	Non-participants N=1037
Age, mean (SD), years	70.7 (6.3)	73.2 (6.8)[b]
Follow-up time mortality, mean (SD), years	10.0 (3.0)	9.5 (3.3)[b]
Female, No. (%)	785 (46.3)	760 (73.3)[b]
Systolic blood pressure, mean (SD), mmHg	142.7 (20.8)	145.1 (21.4)
Diastolic blood pressure, mean (SD), mmHg	75.9 (11.1)	75.2 (11.0)
Blood pressure-lowering medication, No. (%)	384 (23.2)	293 (29.2)[b]
Diabetes mellitus, No. (%)	158 (9.5)	111 (10.9)
Former smoking, No. (%)	990 (58.8)	447 (43.5)[b]
Current smoking, No. (%)	311 (18.5)	176 (17.1)
Total cholesterol, mean (SD), mmol/L	5.81 (0.99)	5.85 (1.01)
HDL-cholesterol, mean (SD), mmol/L	1.38 (0.38)	1.42 (0.39)[b]
Statins, No. (%)	216 (12.9)	154 (15.1)[b]
History of vascular disease, No. (%)	368 (23.3)	256 (27.1)[b]
Carotid intima-media thickness, mean (SD), mm	1.06 (0.18)	1.09 (0.20)[b]
Difference in systolic blood pressure before and during measurement, mean (SD), mmHg	14.9 (13.8)	NA
Difference in diastolic blood pressure before and during measurement, mean (SD), mmHg	5.7 (7.0)	NA
Vasomotor reactivity, median (IQR)[a], %/kPa	39.3 (28.1 – 54.0)	NA

Abbreviations: HDL = high-density lipoprotein; NA = not available.
Data are presented as mean (standard deviations) or No. (%) unless otherwise specified. Percentages are calculated without missing data. For all reported variables, missing numbers occurred in 8.4% or less of all participants.
[a] Median (interquartile range) presented because of skewed distribution.
[b] Significantly different (p<0.05) between participants and non-participants, after sex and age adjustment – if applicable.

Associations between vasomotor reactivity and mortality were stronger for cardiovascular mortality (HR per SD decrease 1.15, 95% CI 1.00; 1.32), whilst the associations for non-cardiovascular mortality were weaker (HR 1.10, 95% CI 1.00; 1.21) (Table 3). After additional adjustments, the association with cardiovascular mortality attenuated slightly and became statistically non-significant (HR 1.09, 95% CI 0.94; 1.26). Again, persons in the lowest two quartiles had an increased risk of death compared to the upper quartile (Table 2). However, we could not observe a clear dose-response relation between lower vasomotor reactivity and higher risk of cardiovascular mortality since quartile 2 showed a stronger association than quartile 1.

After excluding participants with an impaired vasomotor reactivity (<10%/kPa), there were only minor changes in effect size (data not shown).

Table 2. Vasomotor reactivity and the risk of all-cause mortality

		All-cause mortality	
		Model I	Model II
Vasomotor reactivity[a]	n/N	HR (95% CI)	HR (95% CI)
Quartile 1	182/423	1.40 (1.10; 1.80)	1.30 (1.01; 1.67)
Quartile 2	154/424	1.36 (1.06; 1.74)	1.33 (1.03; 1.71)
Quartile 3	117/424	1.04 (0.79; 1.35)	1.07 (0.82; 1.40)
Quartile 4	104/424	1 (reference)	1 (reference)
Per SD decrease	*557/1695*	*1.12 (1.03; 1.21)*	*1.10 (1.01; 1.19)*

Abbreviations: n = number of deaths; N = number of persons at risk; HR = hazard ratio; CI = confidence interval; SD = standard deviation; HDL = high-density lipoprotein.
Values are hazard ratios with 95% confidence intervals.
The range of vasomotor reactivity for each quartile was as follows: quartile 1: 0–28.1 %/kPa, quartile 2: 28.1-39.3 %/kPa, quartile 3: 39.3-54.0 %/kPa, quartile 4: 54.0-229.3 %/kPa.
Model I: Adjusted for age, sex and difference in blood pressure before and during vasomotor reactivity measurement.
Model II: Adjusted for age, sex, current smoking, former smoking, blood pressure-lowering medication, systolic blood pressure, diastolic blood pressure, diabetes mellitus, total cholesterol, HDL-cholesterol, statins, history of vascular disease, carotid intima-media thickness, and difference in blood pressure before and during vasomotor reactivity measurement.
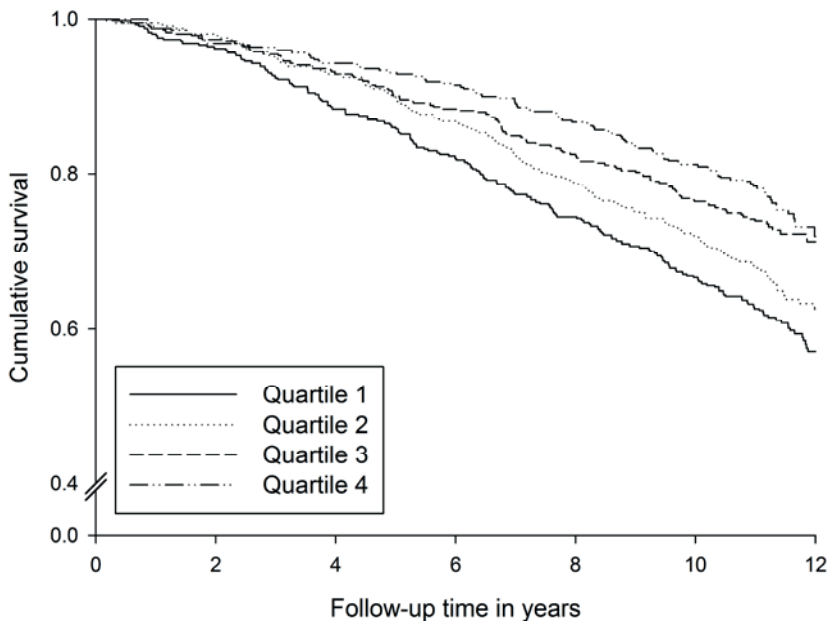[a] Vasomotor reactivity was natural log transformed.

Figure 1. Kaplan-Meier curve for crude survival per quartile of vasomotor reactivity
Quartile 1 represents the lowest vasomotor reactivity, quartile 4 the highest. The range of vasomotor reactivity for each quartile was as follows: quartile 1: 0–28.1 %/kPa, quartile 2: 28.1-39.3 %/kPa, quartile 3: 39.3-54.0 %/kPa, quartile 4: 54.0-229.3 %/kPa.

Table 3. Vasomotor reactivity and the risk of cardiovascular and non-cardiovascular mortality

Vasomotor reactivity[a]	Cardiovascular mortality			Non-cardiovascular mortality		
	n/N	Model I HR (95% CI)	Model II HR (95% CI)	n/N	Model I HR (95% CI)	Model II HR (95% CI)
Quartile 1	56/423	1.62 (1.01; 2.59)	1.36 (0.84; 2.18)	126/423	1.33 (0.99; 1.78)	1.28 (0.95; 1.72)
Quartile 2	59/424	1.99 (1.26; 3.15)	1.93 (1.21; 3.05)	95/424	1.14 (0.84; 1.54)	1.11 (0.82; 1.51)
Quartile 3	39/424	1.30 (0.79; 2.13)	1.37 (0.84; 2.25)	78/424	0.94 (0.69; 1.30)	0.97 (0.70; 1.33)
Quartile 4	27/424	1 (reference)	1 (reference)	77/424	1 (reference)	1 (reference)
Per SD decrease	181/1695	1.15 (1.00; 1.32)	1.09 (0.94; 1.26)	376/1695	1.10 (1.00; 1.21)	1.10 (0.99; 1.21)

Abbreviations: n = number of deaths; N = number of persons at risk; HR = hazard ratio; CI = confidence interval; SD = standard deviation; HDL = high-density lipoprotein. Values are hazard ratios with 95% confidence intervals.

The range of vasomotor reactivity for each quartile was as follows: quartile 1: 0–28.1 %/kPa, quartile 2: 28.1–39.3 %/kPa, quartile 3: 39.3–54.0 %/kPa, quartile 4: 54.0–229.3 %/kPa.

Model I: Adjusted for age, sex and difference in blood pressure before and during vasomotor reactivity measurement.

Model II: Adjusted for age, sex, current smoking, former smoking, blood pressure-lowering medication, systolic blood pressure, diastolic blood pressure, diabetes mellitus, total cholesterol, HDL–cholesterol, statins, history of vascular disease, carotid intima-media thickness, and difference in blood pressure before and during vasomotor reactivity measurement.

[a] Vasomotor reactivity was natural log transformed.

Table 4. Vasomotor reactivity and the risk of stroke

| Vasomotor reactivity[a] | | Stroke | |
| | | Model I | Model II |
	n/N	HR (95% CI)	HR (95% CI)
Quartile 1	47/423	1.11 (0.72; 1.71)	1.10 (0.71; 1.70)
Quartile 2	46/424	1.15 (0.75; 1.77)	1.15 (0.74; 1.77)
Quartile 3	37/424	0.90 (0.57; 1.43)	0.92 (0.58; 1.45)
Quartile 4	38/424	1 (reference)	1 (reference)
Per SD decrease	*168/1695*	*1.06 (0.91; 1.23)*	*1.06 (0.91; 1.23)*

Abbreviations: n = number of strokes; N = number of persons at risk; HR = hazard ratio; CI = confidence interval; SD = standard deviation; HDL = high-density lipoprotein.
Values are hazard ratios with 95% confidence intervals.
The range of vasomotor reactivity for each quartile was as follows: quartile 1: 0–28.1 %/kPa, quartile 2: 28.1-39.3 %/kPa, quartile 3: 39.3-54.0 %/kPa, quartile 4: 54.0-229.3 %/kPa.
Model I: Adjusted for age, sex and difference in blood pressure before and during vasomotor reactivity measurement.
Model II: Adjusted for age, sex, current smoking, former smoking, blood pressure-lowering medication, systolic blood pressure, diastolic blood pressure, diabetes mellitus, total cholesterol, HDL-cholesterol, statins, history of vascular disease, carotid intima-media thickness, and difference in blood pressure before and during vasomotor reactivity measurement.
[a] Vasomotor reactivity was natural log transformed.

Table 5. Vasomotor reactivity and the risk of mortality, after censoring for incident stroke

| Vasomotor reactivity[a] | All-cause mortality | | Cardiovascular mortality | |
	n/N	HR (95% CI)	n/N	HR (95% CI)
Quartile 1	150/423	1.49 (1.13; 1.96)	41/423	1.76 (1.01; 3.07)
Quartile 2	128/424	1.39 (1.05; 1.83)	43/424	2.07 (1.20; 3.56)
Quartile 3	103/424	1.13 (0.85; 1.51)	28/424	1.32 (0.74; 2.38)
Quartile 4	84/424	1 (reference)	19/424	1 (reference)
Per SD decrease	*465/1695*	*1.12 (1.03; 1.22)*	*131/1695*	*1.20 (1.03; 1.40)*

Abbreviations: n = number of deaths; N = number of persons at risk; HR = hazard ratio; CI = confidence interval; SD = standard deviation.
Values are hazard ratios with 95% confidence intervals.
The range of vasomotor reactivity for each quartile was as follows: quartile 1: 0–28.1 %/kPa, quartile 2: 28.1-39.3 %/kPa, quartile 3: 39.3-54.0 %/kPa, quartile 4: 54.0-229.3 %/kPa.
[a] Vasomotor reactivity was natural log transformed.
Models are adjusted for age, sex and difference in blood pressure before and during vasomotor reactivity measurement.

Interestingly, vasomotor reactivity was not associated with the risk of stroke (Table 4). Instead, the associations with both all-cause mortality and cardiovascular mortality remained significant after censoring for incident stroke (Table 5).

Our additional analyses showed no evidence for vasomotor reactivity being an intermediate between various cardiovascular risk factors and risk of mortality (Supplementary Table l).

Discussion

We found that persons with a lower cerebral vasomotor reactivity have an increased risk of mortality. These associations were independent from cardiovascular risk factors and from incident stroke.

Strengths of this study are the population-based design; the thorough collection of events; and the long follow-up for both mortality and stroke. A potential limitation is selection bias because transcranial Doppler measurements failed in a large group of participants. The percentage of participants who died was significantly higher among the excluded participants, which indeed might point towards selection bias. Transcranial Doppler measurements failed mainly because of window failure, which occurred more often in women and in older participants.[19] Given that vasomotor reactivity decreases with age[27], coupled with higher risk of cardiovascular disease with age, this could have led to a dilution of the effect. Another consideration is potential survivor effect as transcranial Doppler measurements were only performed at the second follow-up examination of the Rotterdam Study. It is possible that unhealthy persons may have died during the intermediate time period. This would have resulted in an underestimation of the effect. A final remark is that we did not assess extracranial carotid stenosis. We did measure carotid intima-media thickness (cIMT), but did not measure the lumen of the extracranial carotid artery and were not able to assess extracranial carotid stenosis. Given that extracranial carotid artery stenosis is prevalent in the general population and influences both cerebral vasomotor reactivity measurements and the risk of stroke, extracranial carotid artery stenosis could have influences our results.[8,28]

We found that a lower vasomotor reactivity was associated with higher risk of mortality, especially cardiovascular mortality. These results suggest that a low vasomotor reactivity is a marker of accumulating vascular damage. We did find a dose-response relation between vasomotor reactivity and all-cause mortality, however this relation was not found for cardiovascular mortality. Moreover, no clear cut-off between normal and abnormal vasomotor reactivity values can be obtained from these analyses, as the cut-off points for the quartiles are based on this study population and cannot be generalized to other study populations. Vasomotor reactivity is measured in the cerebral vessels and previous studies have shown that in patients with carotid stenosis, those with a lower vasomotor reactivity have an increased risk of stroke.[8,12-17] Consequently, our findings with all-cause and cardiovascular mortality could be explained by stroke and stroke-related deaths. However, we did not find vasomotor reactivity to be associated with stroke. This is consistent with what was found before within the same study population, with shorter follow-up.[19] Furthermore, the association of lower vasomotor reactivity with both all-cause and cardiovascular mortality was independent from incident stroke. The main causes of cardiovascular death after censoring for strokes were heart failure, cardiac arrest, sudden death with unknown cause, and myocardial infarction. This supports the hypothesis that loss of cerebral vasomotor reactivity is a reflection of a more systemic dysfunction of the vascular system rather than only cerebrovascular damage. Further evidence comes from previous studies that have reported a link between peripheral artery endothelial dysfunction and cerebrovascular reactivity.[29,30] Still, we note that some studies did not find an

association between flow-mediated vasodilatation (FMD) in the brachial artery, which is an indirect measure of peripheral endothelial dysfunction, and cerebrovascular reactivity.[31,32] Inconsistencies across studies might be explained by methodological differences and differences in study population.

Although associations with cardiovascular mortality attenuated after adjustment for cardiovascular risk factors and became statistically non-significant, an effect size in excess of 1.3 remained for each of the lowest three quartiles compared to the upper quartile. This suggests that part of the effect of vasomotor reactivity is independent from cardiovascular risk factors. Also, it is questionable whether such an adjustment is a correction for potential confounders or actually an over-adjustment for possible intermediates of the causal chain. Nevertheless, some remarks can be made on the results after these adjustments. First, prior studies have shown that hypertension, smoking, and dyslipidemia disrupt the vascular homeostasis. This might cause endothelial dysfunction, which eventually contributes to cardiovascular disease.[3,33] Endothelial dysfunction leads to a lower excretion of dilatory factors, such as nitric oxide, and could therefore also lead to a lower vasomotor reactivity.[2] Second, it is possible that participants with unrecognized risk factors did not receive preventative treatment and therefore were more at risk for a cardiovascular event than those in whom cardiovascular risk factors were present and thus treated. This would lead to a minimal effect of adjusting for such risk factors. Third, we adjusted for baseline measurements of cardiovascular risk factors, which might be less representative for life-long exposure. A final consideration is that vasomotor reactivity reflects a different mechanism of vascular damage, not explained by cardiovascular risk factors, but by risk factors that we did not measure, such as genetic factors.[34,35] Conversely, we found that the associations of cardiovascular risk factors with mortality remained unchanged after adjusting for vasomotor reactivity. It is likely that these factors exert their effect through many different mediators, among which vasomotor reactivity.

Since vascular disease is the leading cause of mortality worldwide, there is an urgent need for preventive options. As such, markers of early vascular damage are of much interest. Our study on vasomotor reactivity and mortality is set in a community-dwelling population. Unlike in a clinical setting, our participants are relatively healthy and the amount of available in-depth data is limited. We therefore do not have additional data to clarify the potential cause of diminished vasomotor reactivity. The association between a lower vasomotor reactivity and higher risk of cardiovascular mortality thus merits further investigation. Specifically, unravelling the underlying mechanism and the possible contribution to identification of high-risk individuals is worthy of future research. Also, it would be of interest to investigate the association of vasomotor reactivity and white matter lesions measured on Magnetic Resonance Imaging (MRI).

In conclusion, our results indicate that loss of cerebral vasomotor reactivity is associated with an increased risk of mortality, especially cardiovascular mortality, independent of stroke. This suggests that impaired cerebral vasomotor reactivity reflects a systemically impaired vascular system.

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3

Supplementary information

Supplementary Table I. Associations between cardiovascular risk factors and all-cause mortality, before and after adjustment for vasomotor reactivity

	All-cause mortality n/N 557/1695	
	Model I HR (95% CI)	Model II HR (95% CI)
Age	1.12 (1.10; 1.13)	1.12 (1.10; 1.13)
Sex	0.76 (0.61; 0.94)	0.75 (0.60; 0.93)
Systolic blood pressure	1.00 (0.99; 1.00)	1.00 (0.99; 1.00)
Diastolic blood pressure	1.00 (0.99; 1.01)	1.00 (0.99; 1.01)
Blood pressure-lowering medication	1.17 (0.96; 1.43)	1.16 (0.95; 1.42)
Diabetes mellitus	1.20 (0.93; 1.54)	1.18 (0.92; 1.52)
Former smoking	1.16 (0.94; 1.43)	1.17 (0.95; 1.45)
Current smoking	2.08 (1.65; 2.63)	2.09 (1.66; 2.64)
Total cholesterol	0.93 (0.85; 1.02)	0.93 (0.84; 1.01)
HDL-cholesterol	0.86 (0.67; 1.11)	0.87 (0.68; 1.12)
Statins	0.72 (0.55; 0.95)	0.72 (0.55; 0.95)
History of vascular disease	1.55 (1.27; 1.88)	1.52 (1.25; 1.85)
Carotid intima-media thickness	1.84 (1.13; 2.99)	1.81 (1.11; 2.95)

Abbreviations: n = number of deaths; N = number of persons at risk; HR = hazard ratio; CI = confidence interval; HDL = high-density lipoprotein.

Values are hazard ratios with 95% confidence intervals.

Model I: Adjusted for age, sex, current smoking, former smoking, blood pressure-lowering medication, systolic blood pressure, diastolic blood pressure, diabetes mellitus, total cholesterol, HDL-cholesterol, use of statins, history of vascular disease, and carotid intima-media thickness.

Model II: Adjusted for age, sex, current smoking, former smoking, blood pressure-lowering medication, systolic blood pressure, diastolic blood pressure, diabetes mellitus, total cholesterol, HDL-cholesterol, use of statins, history of vascular disease, carotid intima-media thickness, and vasomotor reactivity (natural log transformed).

Chapter 3.3

Cerebral microbleeds are associated with an increased risk of stroke

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Abstract

Background and purpose
Cerebral microbleeds are highly prevalent in people with clinically manifest cerebrovascular disease, and have been shown to increase the risk of stroke recurrence. Microbleeds are also frequently found in healthy elderly, a population in which the clinical implication of microbleeds is unknown.

Methods
In the population-based Rotterdam Study, presence, number, and location of microbleeds were assessed at baseline on brain MRI of 4,759 participants aged ≥45 years. Participants were followed for incident stroke throughout the study period (2005 until 2013). We used Cox proportional hazards to investigate if people with microbleeds were at increased risk of stroke compared to those without microbleeds, adjusting for demographic, genetic, and cardiovascular risk, and cerebrovascular imaging markers.

Results
Microbleed prevalence was 18.7% (median count 1 [1-111]). During mean follow-up of 4.9 years (SD 1.6) 93 strokes occurred (72 ischemic, 11 hemorrhagic, and 10 unspecified). Microbleed presence was associated with an increased risk of all strokes (HR 1.93, 95% CI 1.25; 2.99). The risk increased with greater microbleed count. Compared to those without microbleeds, participants with microbleeds in locations suggestive of CAA (lobar with or without cerebellar microbleeds) were at increased risk of intracerebral hemorrhage (HR 5.27, 95% CI 1.38; 20.23). Microbleeds at other locations were associated with an increased risk of both ischemic stroke and intracerebral hemorrhage.

Conclusions
Microbleeds on MRI are associated with an increased risk of stroke in the general population. Our results strengthen the notion that microbleeds mark progression of cerebrovascular pathology and represent a precursor of stroke.

Introduction

Stroke is the second leading cause of death worldwide and the third most common cause of disability-adjusted life-years.[1,2] Although stroke has an acute onset, there is abundant evidence for a long subclinical period in which cerebrovascular pathology accumulates and can be visualized on non-invasive brain imaging. Markers of subclinical cerebrovascular pathology that are known to increase the risk of ischemic stroke are white matter lesions and lacunes.[3] In the last decade, punctuate hemorrhagic lesions in the brain parenchyma, so called cerebral microbleeds, have emerged as another manifestation of subclinical cerebrovascular pathology.[4] In contrast to white matter lesions and lacunes, microbleeds are thought to reflect the presence of both ischemic and hemorrhagic brain vasculopathy.[5,6] In addition, evidence suggests that the location of cerebral microbleeds provides more information on the type of underlying vasculopathy, i.e. cerebral amyloid angiopathy (CAA) in the presence of lobar microbleeds and hypertensive arteriopathy when deep or mixed microbleeds are seen.[4,7,8]

There is a growing need to clarify whether microbleed presence indicates an increased risk of stroke, as this may provide new insights in the link between subclinical cerebrovascular pathology and stroke. So far, it is known that in patients with a history of stroke microbleed presence increases the risk of recurrent stroke, either hemorrhagic or ischemic.[9-18] Whether microbleeds are associated with an increased risk of stroke in community-dwelling elderly without a history of stroke remains unclear. Only two prospective studies investigated the association of microbleeds with subsequent stroke in people without a history of cerebrovascular events.[19,20] Both studies found that microbleed presence was associated with an increased risk of stroke, although important limitations were small sample size, absence of microbleed subgroup analysis, and lack of generalizability due to an exclusive focus on Asian populations.[19,20]

In the population-based Rotterdam Study, we investigated whether cerebral microbleeds at various locations are associated with an increased risk of recurrent and first-ever ischemic stroke and intracerebral hemorrhage.

Methods

Study population
The Rotterdam Study is an ongoing prospective population-based cohort that studies occurrence, determinants and consequences of diseases in an aging population.[21] The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. The cohort originated in 1990, was expanded twice in 2000 and 2006, and now comprises 14,926 participants aged ≥45 years, predominantly of Caucasian origin. All participants gave written informed consent. In 2005, brain MRI, including microbleed assessment, was implemented within the core protocol of the study.[22] Between January 1st 2005 and January 1st 2013, 5,074 out of 5,735 eligible participants (88.5%) underwent brain scanning. Participants with scans that were incomplete (N=72) or of insufficient quality for

microbleed rating (N=57) were excluded. Also, we excluded participants (n=186) in whom follow-up for incident stroke ended before date of MRI due to the absence of automatic linkages between the general practitioners office and our study database, leaving a total of 4,759 participants for analyses.

Brain MRI and microbleeds
A 1.5-Tesla MRI scanner (GE Healthcare, Milwaukee, WI) was used to obtain T1-weighted, T2-weighted, fluid-attenuated inversion recovery (FLAIR), and T2*-weighted sequences.[22] Cerebral microbleeds were recognized as focal, small, round to ovoid areas of signal loss on T2*-weighted images (gradient-recalled echo with repetition time= 45ms, echo time= 31, matrix size= 320x244, flip angle= 13, field-of-view= 25x17.5cm2, parallel imaging acceleration factor = 2, 3D acquisition with 96 slices encoded with a slice thickness of 1.6mm zero padded to 192 slices of 0.8mm, acquisition time 5min 55sec).[23] Their presence, number, and location were scored by 1 of 5 trained research physicians using a protocol that is in place since initiation of the study in 2005 with good intraobserver (k=0.87) and interobserver agreement (k=0.85). Signal voids caused by sulcal vessels, calcifications, and signal averaging from bone were considered mimics of microbleeds.[4] Raters were blinded to all clinical data. Microbleeds were classified based on their location in the brain into groups most befitting their presumed etiology. Microbleeds at locations where CAA is highly prevalent (strictly lobar or lobar with cerebellar locations) were termed "CAA related" microbleeds, and microbleeds at other locations (deep gray matter, deep white matter, and brainstem with or without lobar microbleeds; cerebellar with or without deep or brainstem microbleeds) were termed "non-CAA related" microbleeds.

Aside from microbleeds we assessed the presence of infarcts and the load of white matter lesions. Lacunes were defined as focal lesions between ≥3 and <15mm seen on FLAIR, T1-weighted, and T2-weighted sequences. If lacunes were ≥15mm they were defined as subcortical infarcts, and if cortical gray matter was affected they were classified as cortical infarcts. Brain tissue was segmented into gray matter, white matter, and cerebrospinal fluid using automated post-processing tools that included conventional k-nearest-neighbor brain tissue classifier extended with white matter lesion segmentation.[24] Intracranial volume was defined as the sum of gray matter, white matter, and white matter lesion volume, and cerebrospinal fluid.

Stroke assessment and follow-up
Upon study entry, history of stroke was assessed using home interviews and by reviewing medical records. Subsequently, participants were continuously followed-up for occurrence of stroke through automated linkage of general practitioners' medical records with the study database.[25] For participants who moved outside the study district or lived in nursing homes, medical records were regularly checked by contacting their treating physicians. Research physicians reviewed all potential strokes using hospital discharge letters, information from general practitioners and from nursing home physicians. An experienced vascular neurologist verified the stroke diagnoses. In accordance with World Health Organization (WHO) criteria,

stroke was defined as a syndrome of rapidly emerging clinical signs of focal or global disturbance of cerebral function. Symptoms should last ≥24 hours or cause death, with no apparent cause other than of vascular origin. Strokes were classified into ischemic strokes or spontaneous intracerebral hemorrhages based on neuroimaging reports (computed tomography). In the absence of neuroimaging, strokes were classified as unspecified.

Follow-up started on the date that participants came for brain MRI. Participants were followed until date of (fatal or non-fatal) stroke occurrence, date of death, date of last contact in case of loss to follow up, or January 1st 2013 (end of the study period), whichever came first. Follow-up was complete for 23,356 (94.9%) of potential person-years.

Assessments of covariates
Participants' demographics, genetic and cardiovascular risk factors were assessed during the center visit preceding the brain MRI, using structured interviews, physical, and laboratory examinations. We defined various potential cardiovascular confounders. Blood pressure measurements were averaged over two readings using a random zero sphygmomanometer. Hypertension was defined as a blood pressure of >140 systolic or >90 diastolic, or the use of blood pressure-lowering medication. Serum total and high-density lipoprotein (HDL) cholesterol were measured using an automated enzymatic procedure. Smoking behavior was defined as smoked 'ever' versus 'never'. Participants were considered diabetic when fasting blood glucose levels were ≥7.0 mmol/L or when they used glucose-lowering medication. Blood pressure-lowering and lipid-lowering medication use was assessed in home interviews. Pharmacy records were used to determine the use of antithrombotic medication (ATC code B01AA04 acenocoumarol, B01AA07 phenprocoumon, B01AB heparins, and B01AC platelet aggregation inhibitors) between baseline MRI and stroke event, death or January 1st 2013, whichever came first. Finally, *APOE* genotyping was done on coded genomic DNA samples.

Statistical analysis
Student's *t*-test and Fisher's exact test were used to compare characteristics of study participants by microbleed status. Cox proportional hazards models were fitted to obtain the estimated hazard ratios (HR) and 95% confidence intervals for the association of microbleeds with stroke (all fatal and non-fatal strokes, fatal and non-fatal ischemic strokes, and fatal and non-fatal intracerebral hemorrhages). We first investigated the risk of recurrent and first-ever stroke combined. Afterwards we excluded participants with a history of stroke at baseline (n=8) and separately investigated the risk of first-ever stroke. Analyses were repeated for location of microbleeds ("CAA related" and "non-CAA related" locations versus no microbleed), and for microbleed count to investigate a potential dose-response effect (predefined categories of 1, 2-4, and >4 microbleeds versus no microbleeds).[26-28] We constructed 4 models to adjust for potential confounding. In order not to overfit the statistical models we used logistic regression to compute propensity scores for cardiovascular risk. In these, microbleed status (yes versus no) was considered the dependent variable and the following cardiovascular risk factors were considered independent covariates: hypertension, total and HDL cholesterol, smoking status, diabetes mellitus status, use of lipid-lowering and antithrombotic medication. The estimated

propensity score was the derived predicted value of this equation. Missing covariate data (≤7.0%) were imputed based on sex, age, and cardiovascular risk factors using regression models. The first model was adjusted for age-squared, sex, and Rotterdam Study subcohort. The second model was additionally adjusted for *APOE* ɛ4 carriership. The third model was adjusted for age-squared, sex, Rotterdam Study subcohort and the propensity score described above. Finally, the fourth model equaled model 1 with additional adjustments for lacunes, white matter lesions, and total intracranial volume. An additional 77 participants were excluded in the analysis of model 4 because the white matter lesion segmentation of these MRI scans was considered unreliable. The proportionality assumption was tested using Schoenfeld residuals.

Kaplan Meier incident stroke-free survival curves were constructed for groups with microbleed count 0,1, 2-4, >4, and compared using the log-rank test. In a sensitivity analysis, we repeated the analysis of model 1 after stratification by antithrombotic drug use (ever versus never), and applied formal interaction tests to determine significant differences in subgroups.

Finally, we examined all research and hospital scans of incident intracerebral hemorrhage cases, and visually correlated the locations of baseline microbleeds and incident hemorrhages, since it was hypothesized that symptomatic hemorrhages would occur in close proximity of microbleeds.

Analyses were done using IBM SPSS statistics for windows, version 21.0 (IBM Corp., Armonk, NY), using an alpha-value of 0.05.

Results

Characteristics of the study population are presented in Table 1. Of the total 4,759 participants 55.3% were women and the average age at baseline was 63.8 years (SD 10.9). Microbleed prevalence was 18.7% (median microbleed count 1 [range 1-111]). Older age, hypertension, lower total cholesterol, smoking, use of lipid-lowering and antithrombotic medication, and ischemic vascular lesions on brain MRI were all significantly associated with microbleed presence. At baseline, 8 (0.2%) participants had a history of stroke (six ischemic and two unspecified). Over a mean follow up of 4.9 years (SD 1.6), 93 (2.0%) participants suffered a stroke, of whom 72 ischemic strokes (3.1 per 1,000 person-years), 11 intracerebral hemorrhages (0.5 per 1,000 person-years), and 10 unspecified strokes (0.4 per 1,000 person-years).

Table 1. Characteristics of the study population

	Total population (N=4,759)	Microbleeds absent (N=3,867)	Microbleeds present (N=892)	P-value
Age, years	63.8 (10.9)	62.4 (10.5)	69.7 (10.9)[a]	<0.001
Women	2,631 (55.3)	2,155 (55.7)	476 (53.4)	0.204
Hypertension	2,914 (61.2)	2,267 (58.6)	647 (72.5)[a]	<0.001
Total cholesterol, mmol/L	5.5 (1.0)	5.6 (1.0)	5.4 (1.1)[a]	0.001
High-density lipoprotein cholesterol, mmol/L	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)	0.720
Ever smoker	3,315 (69.7)	2,665 (68.9)	650 (72.9)[a]	0.021
Diabetes mellitus	410 (8.6)	322 (8.3)	88 (9.9)	0.145
Lipid-lowering medication use	1,118 (23.5)	860 (22.2)	258 (28.9)[a]	<0.001
Antithrombotic medication use	1,274 (26.8)	891 (23.0)	383 (42.9)[a]	<0.001
Apolipoprotein E ε4 carriership	1,435 (30.2)	1,137 (29.4)	298 (33.4)[a]	0.021
White matter lesion volume[b]	2.9 (1.6-6.1)	2.7 (1.5-5.1)	5.0 (2.5-11.7)[a]	<0.001
Lacunes on MRI	310 (6.5)	194 (5.0)	116 (13.0)[a]	<0.001
Subcortical infarcts on MRI	8 (0.2)	4 (0.1)	4 (0.4)[a]	0.045
Cortical infarcts on MRI	111 (2.3)	85 (2.2)	26 (2.9)	0.218

Values presented mean (SD) for continuous variables and number (%) for categorical variables.
[a]P-value <0.05, indicates significant difference in people with and without microbleeds.
[b]Presented as median (IQR) and assessed in 4,687 participants.

People with microbleeds at baseline were at increased risk of developing new strokes compared with those without microbleeds (age-squared and sex adjusted HR 1.93, 95% CI 1.25; 2.99) (Table 2). Risk was highest for participants with microbleeds in brain locations not typically affected by CAA (HR 3.35, 95% CI 1.94; 5.78). For subtypes of strokes, we found that microbleeds in locations not typically affected by CAA associated with an increased risk of both ischemic stroke (HR 3.05, 95% CI 1.65; 5.63) and intracerebral hemorrhage (HR 5.92, 95% CI 1.07; 32.86), whereas microbleeds in locations suggestive of CAA were only associated with an increased risk of intracerebral hemorrhage (HR 5.27, 95% CI 1.38; 20.23). Additional adjustments for APOE ε4 carriership, cardiovascular risk, and imaging markers of vascular brain disease weakened these associations, but significance remained (Table 2). Microbleed presence was also associated with an increased risk of first-ever stroke, in a similar differential pattern for the various microbleed locations as described above (Table 3). Especially the presence of multiple microbleeds, irrespective of their location, was associated with an increased risk of first-ever stroke (Figure 1). Microbleeds did not associate differently with stroke risk after stratifying on antithrombotic drug use (Supplementary Table I).

Six participants with microbleeds at baseline developed a first-ever intracerebral hemorrhage during follow-up. All of them presented with multiple microbleeds (median count 8.5, range 2-17), and three had used antithrombotic agents (either platelet inhibitors or oral anticoagulants) during follow-up. The symptomatic hemorrhages were located respectively in lobar (thrice), thalamic (twice), and cerebellar region (once). The location of the symptomatic hemorrhage of five participants correlated exactly with the anatomical location of one of the microbleeds on their prior research scan (Figure 2).

Table 2. Cerebral microbleeds and the risk of any stroke

	All strokes			Ischemic stroke			Intracerebral hemorrhage		
	n/N	HR (95% CI)	P-value	n/N	HR (95% CI)	P-value	n/N	HR (95% CI)	P-value
Model 1									
No microbleeds	59/3,867	1.00 (Reference)		49/3,867	1.00 (Reference)		5/3,867	1.00 (Reference)	
Any microbleeds	34/892	1.93 (1.25; 2.99)	0.004	23/892	1.52 (0.91; 2.53)	0.124	6/892	5.64 (1.66; 19.13)	0.006
Non-CAA related microbleeds	18/259	3.35 (1.94; 5.78)	<0.001	14/259	3.05 (1.65; 5.63)	<0.001	2/259	5.92 (1.07; 32.86)	0.046
CAA related microbleeds	16/633	1.30 (0.74; 2.28)	0.383	9/633	0.84 (0.41; 1.74)	0.629	4/633	5.27 (1.38; 20.23)	0.017
Model 2									
No microbleeds	59/3,867	1.00 (Reference)		49/3,867	1.00 (Reference)		5/3,867	1.00 (Reference)	
Any microbleeds	34/892	1.87 (1.20; 2.90)	0.007	23/892	1.49 (0.89; 2.49)	0.145	6/892	5.34 (1.56; 18.32)	0.009
Non-CAA related microbleeds	18/259	3.28 (1.89; 5.68)	<0.001	14/259	2.97 (1.61; 5.51)	0.001	2/259	5.97 (1.07; 33.39)	0.046
CAA related microbleeds	16/633	1.24 (0.70; 2.19)	0.477	9/633	0.81 (0.39; 1.68)	0.560	4/633	5.04 (1.30; 19.47)	0.021
Model 3									
No microbleeds	59/3,867	1.00 (Reference)		49/3,867	1.00 (Reference)		5/3,867	1.00 (Reference)	
Any microbleeds	34/892	1.79 (1.16; 2.78)	0.010	23/892	1.40 (0.84; 2.34)	0.213	6/892	5.41 (1.58; 18.46)	0.008
Non-CAA related microbleeds	18/259	2.92 (1.69; 5.04)	<0.001	14/259	2.60 (1.41; 4.80)	0.003	2/259	5.77 (1.01; 32.80)	0.051
CAA related microbleeds	16/633	1.24 (0.71; 2.19)	0.469	9/633	0.80 (0.39; 1.65)	0.535	4/633	5.25 (1.37; 20.17)	0.017
Model 4									
No microbleeds	55/3,812	1.00 (Reference)		45/3,812	1.00 (Reference)		5/3,812	1.00 (Reference)	
Any microbleeds	34/875	1.68 (1.07; 2.65)	0.025	23/875	1.28 (0.75; 2.17)	0.371	6/875	4.64 (1.33; 16.19)	0.017
Non-CAA related microbleeds	18/254	2.34 (1.31; 4.19)	0.004	14/254	2.06 (1.07; 3.95)	0.031	2/254	3.38 (0.53; 21.54)	0.196
CAA related microbleeds	16/621	1.28 (0.72; 2.28)	0.399	9/621	0.82 (0.39; 1.70)	0.587	4/621	4.88 (1.27; 18.83)	0.023

Abbreviations: n/N = number of people with stroke per exposure category/ total number of participants within the exposure category.

Values represent estimated hazard ratios with 95% confidence interval for any incident stroke (recurrent or first-ever) in participants with microbleeds compared to those without microbleeds. "CAA related" microbleeds included strictly lobar or lobar with cerebellar microbleeds. "Non-CAA related" microbleeds included microbleeds in all other brain locations.

Model 1: adjusted for age-squared, sex, and Rotterdam Study subcohort.

Model 2: as model 1, additionally adjusted for *APOE ε4* carriership.

Model 3: as model 1, additionally adjusted for propensity score.[a]

Model 4: as model 1, additionally adjusted for lacunes, white matter lesion volume, and intracranial volume.

[a]Propensity score included: hypertension, total and high-density lipoprotein cholesterol, smoking, diabetes mellitus, lipid-lowering medication, and antithrombotic medication use.

Table 3. Cerebral microbleeds and the risk of first-ever stroke

	All strokes			Ischemic stroke			Intracerebral hemorrhage		
	n/N	HR (95% CI)	P-value	n/N	HR (95% CI)	P-value	n/N	HR (95% CI)	P-value
Model 1									
No microbleeds	56/3,864	1.00 (Reference)		47/3,864	1.00 (Reference)		5/3,864	1.00 (Reference)	
Any microbleeds	29/887	1.71 (1.08; 2.73)	0.026	19/887	1.28 (0.74; 2.22)	0.395	6/887	5.68 (1.68; 19.27)	0.006
Non-CAA related microbleeds	15/256	2.89 (1.61; 5.20)	<0.001	11/256	2.45 (1.25; 4.81)	0.011	2/256	5.98 (1.08; 33.16)	0.044
CAA related microbleeds	14/631	1.20 (0.66; 2.18)	0.570	8/631	0.78 (0.36; 1.67)	0.777	4/631	5.32 (1.39; 20.37)	0.016

Abbreviations: n/N = number of people with stroke per exposure category/ total number of participants within exposure category.
Values represent estimated age-squared, sex, and Rotterdam Study subcohort adjusted hazard ratios with 95% confidence interval for incident first-ever stroke in participants with microbleeds compared to those without microbleeds.
"CAA related" microbleeds included strictly lobar or lobar with cerebellar microbleeds. "Non-CAA related" microbleeds included microbleeds in all other brain locations.

3

Figure 1. Kaplan Meier stroke-free survival curves

Panel A of Figure 1 shows the crude cumulative stroke-free survival curves for participants without cerebral microbleeds and with increasing microbleed count over a maximum follow-up of 7 years. P-values for the stroke-free survival curves with 1,2-4, >4 microbleeds compared to no microbleeds were respectively: 0.708; <0.001, <0.001.

Panel B of Figure 1 shows the crude cumulative stroke-free survival curves after additional stratification on cerebral microbleed location. The left graph represents the stroke-free survival curves for participants without cerebral microbleeds and with increasing count of non-CAA related microbleeds over a maximum follow-up of 7 years. Events per strata/ total number per strata were: no microbleeds: 56 events/3988; 1 microbleed: 2 events/132; 2-4 microbleeds: 4 events/89; >4 microbleeds: 9 events/68. P-values for the stroke-free survival curves with 1,2-4,>4 non-CAA related microbleeds compared to no microbleeds were respectively: 0.750; 0.030; <0.001. The right graph represents the stroke-free survival curves for participants without cerebral microbleeds and with increasing count of CAA related microbleeds over a maximum follow-up of 7 years. Events per strata/ total number per strata were: no microbleeds: 56 events/3988; 1 microbleed: 4 events/445; 2-4 microbleeds: 7 events/167; >4 microbleeds: 3 events/56. P-values for the stroke-free survival curves with 1,2-4,>4 CAA related microbleeds compared to no microbleeds were respectively: 0.547; 0.001; 0.001. "CAA related" microbleeds included strictly lobar or lobar with cerebellar microbleeds. "Non-CAA related" microbleeds included microbleeds in all other brain locations.

Panel A: Stratified on cerebral microbleed count

Panel B: Stratified on both cerebral microbleed count and location
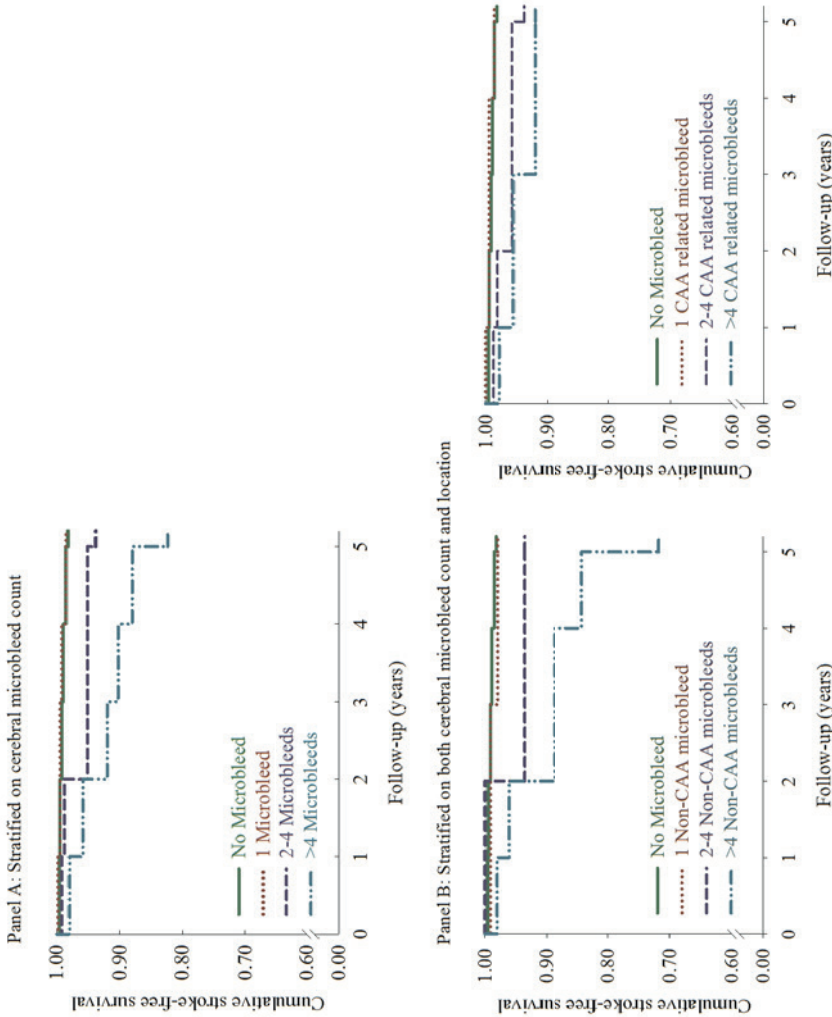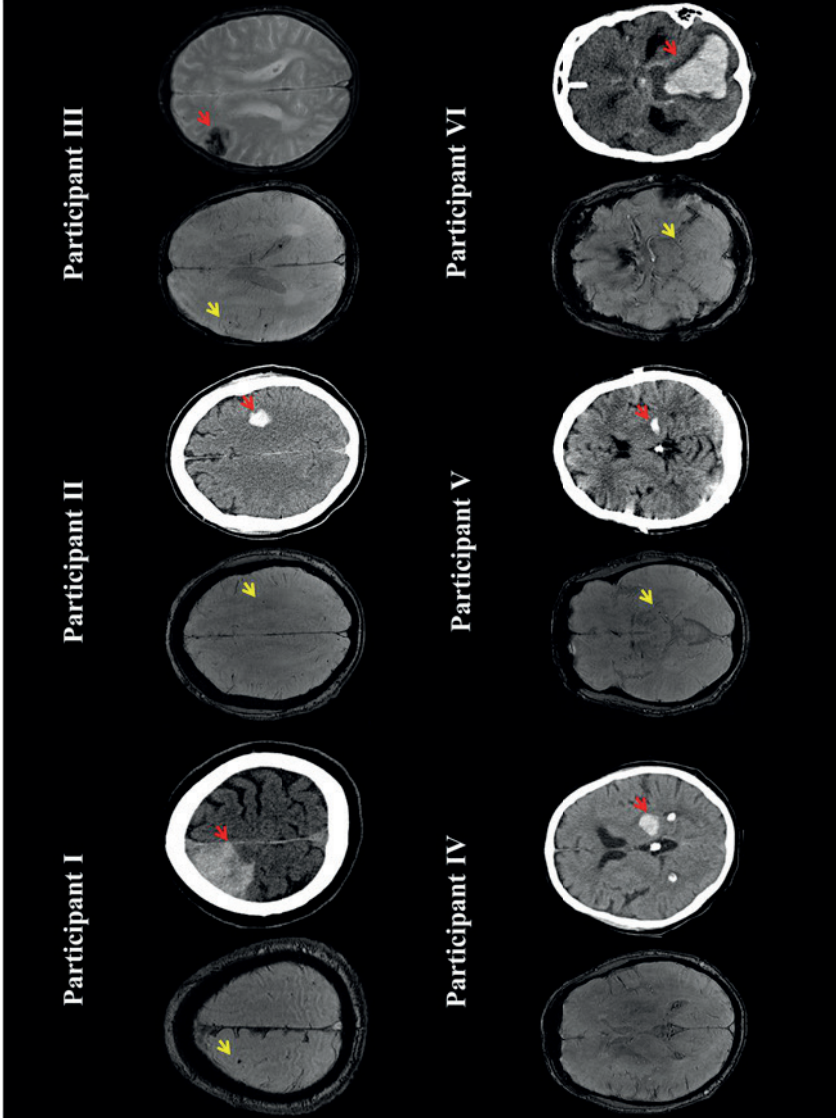
Figure 2. Co-localization of baseline cerebral microbleeds and subsequent intracerebral hemorrhages
For each participant: left image represents baseline research brain MR image co-registered to the hospital CT or MR image (right) which shows an acute intracerebral hemorrhage (red arrow). The yellow arrow represents a microbleed on T2*-weighted MRI that preceded the intracerebral hemorrhage.

Discussion

In the population-based Rotterdam Study we investigated whether microbleed presence on MRI is a determinant of future stroke. We found that the presence of microbleeds, and especially multiple microbleeds, was associated with an increased risk of stroke, including first-ever stroke. Cerebral microbleeds located in regions not typically affected by CAA were associated with an increased risk of both ischemic stroke and intracerebral hemorrhage, whereas microbleeds located in regions where CAA is known to be highly prevalent seemed to particularly associate with an increased risk of intracerebral hemorrhage.

In a population of community-dwelling middle-aged people and elderly we found that microbleeds on MRI associated with an increased risk of both ischemic stroke and intracerebral hemorrhage. The latter has been demonstrated previously in patients with lobar intracerebral hemorrhages due to CAA. These patients were at increased risk of recurrent stroke when microbleeds were present on MRI.[9,17] Evidence that microbleeds also associate with ischemic or occlusive brain disease was found in a recent meta-analysis of clinical studies.[29] They showed that Caucasian patients with microbleeds who were admitted for acute ischemic stroke or transient neurological attack had a 3.87 times (95% CI 0.91; 16.4) higher odds of spontaneous intracerebral hemorrhage, and 2.23 times (95% CI 1.29; 3.85) higher odds of ischemic stroke compared to patients without microbleeds. We now showed that these results can be extrapolated to Caucasian community-dwelling persons without a history of stroke.

We found that the risk of stroke subtype differed according to the location of microbleeds in the brain, and thus possibly differs with underlying vasculopathy. Our results suggest that microbleeds, regardless of their presumed underlying pathology (either CAA or hypertensive arteriopathy) strongly associate with intracerebral hemorrhage. Only microbleeds in non-lobar brain regions (suspected of hypertensive arteriopathy) were related to ischemic stroke. It may be that in the general population CAA-related microbleeds do not relate to overt ischemic stroke or that our sample of ischemic stroke cases was too small to detect a significant association. The latter may be more accurate given prior findings from other studies, which suggest that CAA related microbleeds associate with silent and overt ischemic brain lesions.[5,29]

We also observed that intracerebral hemorrhages were predisposed to occur in the same anatomical location as pre-existing microbleeds. Although we previously showed that microbleeds indicate the presence of more widespread small vessel disease,[28] this finding implies that microbleeds may be of value in pinpointing focal areas in the brain with more active vasculopathy. It should be noted, however, that the number of ICH cases was small and hampered our ability to conduct relevant statistical analysis. Therefore, the overlap in anatomic location between microbleeds and ICH may also be due to chance.

Only two previous studies, both in Asian populations, investigated the association of microbleeds with stroke in elderly without a history of cerebrovascular events.[19,20] One study found an increased risk of ischemic stroke in participants with any microbleeds compared with no microbleeds.[19] The other study found that deep or mixed (i.e., both in deep and lobar brain regions) microbleeds was associated with an increased risk of ischemic stroke and deep intracerebral hemorrhage.[20] Methodological limitations of both studies included, small number

of stroke cases, limited or no correction for potential confounders, and absence of subgroup analysis for microbleed location. Compared to our study, both studies had an overrepresentation of intracerebral hemorrhage cases compared with ischemic stroke cases, which could be explained by ethnic differences in study population as Asians on average have a two-times higher risk of intracerebral hemorrhages compared with Caucasians.[29,30] In addition, blood vessels in Asian intracerebral hemorrhage patients may more often be affected by fibrohyalinosis rather than b-amyloid deposition, reflecting differences in cardiovascular risk for Asians and Caucasians.[31]

In line with another clinical study,[9] our results suggest a dose-response effect, with higher risk of stroke in people with multiple microbleeds on MRI. We should, however, consider that small vessel pathology progresses gradually and cut-off points are not easily chosen, especially since microbleed detection – and the perceived number – strongly depends on technical imaging parameters.[32] Also, the presence of microbleeds (including just a single microbleed) has been associated with an increased risk of new bleeds, and to relate to more diffuse brain damage, i.e., to white matter.[28,33] Although microbleed burden may thus be used as a severity measure, we note that their presence in itself seems to indicate diffuse vascular brain disease which is progressive in nature.

The use of antithrombotic drugs in people with microbleeds on MRI remains a topic of debate, and is fueled by studies like ours that show that microbleed presence is associated with an increased risk of both ischemic stroke and intracerebral hemorrhage. Our current results suggest that stroke risk associated with microbleeds was not affected by antithrombotic drugs use. However, we were unable to investigate this association separately for intracerebral hemorrhage due to the small number of cases. Results from ongoing clinical trials and cost-benefit studies should settle whether the benefits of ischemic stroke prevention by use of antithrombotic drugs outweigh the risk of intracerebral hemorrhage in people with microbleeds.

Strengths of our study include the population-based character, the longitudinal design, and a systematic stroke detection protocol that allows for thorough collection of fatal and non-fatal stroke events, both in-hospital and outside. Some potential limitations have to be mentioned before discussing the implication of our findings. First, participants who were eligible and underwent brain MRI scanning were on average younger and had lower cardiovascular risk than those who were eligible for MRI but did not participate.[33] Also, excluding participants with incomplete follow-up for incident stroke may have been a source of bias, as these participants might have been less healthy on average. This potentially induced selection bias in our study and may have led to an underestimation of associations. Second, in accordance with the incidence of intracerebral hemorrhage in the general population the number of intracerebral hemorrhage cases during follow-up in our study was small, and hampered our ability to conduct in depth statistics. Third, microbleeds were rated by trained research physicians. Since microbleed detection depends on rater expertise, our results may not translate directly to routine clinical practice. Fourth, the categorization into "CAA related" and "non-CAA related" microbleeds, though in line with current research and clinical practice, is artificial and does not accurately reflect the multifactorial nature of microbleeds, in whatever

location. Therefore, this categorization will inherently have led to misclassification between categories. Nevertheless, it is important to note that the most robust results were found for any versus no microbleeds. In addition, the prevalence of CAA-related microbleeds may have been diluted because we included relatively young participants in our study. Fifth, residual confounding due to unmeasured confounders, e.g., atrial fibrillation, may have affected our results to some extent."

In conclusion, in the general population, microbleed presence on MRI was associated with an increased risk of stroke, both ischemic and hemorrhagic. The risk differs for the subtypes of stroke depending on the location of the cerebral microbleeds. Those with the largest microbleed burden are at highest risk of stroke. Microbleeds may thus present a subclinical precursor of stroke. Future studies should investigate whether microbleed presence contributes to stroke risk on an individual level.

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Supplementary information

Supplementary Table I. Cerebral microbleeds and the risk of first-ever stroke stratified on use of antithrombotic drugs

| | Non-users of antithrombotic drugs (N=3,327) | | Users of antithrombotic drugs (N=1,424) | |
| | All strokes | | All strokes | |
	n/N	HR (95% CI)	n/N	HR (95% CI)
No microbleeds	30/2,818	1.00 (Reference)	26/1,046	1.00 (Reference)
Any microbleeds	12/509	1.69 (0.84; 3.39)	17/387	1.64 (0.88; 3.07)
Non-CAA microbleeds	5/125	2.42 (0.91; 6.47)	10/131	2.95 (1.41; 6.19)
CAA related microbleeds	7/384	1.41 (0.60; 3.28)	7/247	1.02 (0.44; 2.39)

Values represent estimated age-squared, sex, and Rotterdam Study subcohort adjusted hazard ratios with 95% confidence interval for incident first-ever stroke in participants with microbleeds compared to those without microbleeds, stratified by antithrombotic drug use (ATC code B01A). Antithrombotic drug use was assessed between baseline MRI and stroke event, death or January 1st 2013. Complete case analysis.
"CAA related" microbleeds included strictly lobar or lobar with cerebellar microbleeds. "Non-CAA related" microbleeds included microbleeds in all other brain locations.
Abbreviation: n/N= number of people with stroke per exposure category/ total number of participants within exposure category.
Formal interaction test for all strokes: any microbleeds P-value=0.779, non-CAA related P-value=0.946, CAA related P-value=0.518.

3

Chapter 3.4

The interaction between total brain perfusion and retinal vessels for the risk of stroke

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Abstract

Background and purpose
A stroke is often attributed to one causal mechanism. However, pathological mechanisms may interact. One hypothesis is that people with small vessel disease, which can be visualized using retinal diameters, may be more vulnerable to a decrease in brain perfusion. Within the Rotterdam Study, we examined whether total brain perfusion and retinal vessel interact for their risk of stroke or TIA.

Methods
Data on total brain perfusion and retinal vessel diameter was collected in 2004-2008 in 3000 participants (mean age 58.8 years, 56.4% women) without history of a stroke or TIA. Follow-up finished in 2014. Models were adjusted for age, sex, cardiovascular risk factors, and the other vessel diameter. Effect modification was tested using an interaction term of total brain perfusion and vessel diameter and by stratification in tertiles.

Results
During 19,007 person-years, 29 people suffered a stroke and 48 a TIA. We observed a significant interaction between retinal venular diameter and brain perfusion. Stratified analyses showed that venular diameter was only associated with stroke or TIA in people with the lowest tertile of total brain perfusion (hazard ratio (HR) 1.71, 95% confidence interval (CI) 1.17; 2.59). Total brain perfusion was only associated with stroke in people with the largest tertile of venular diameter (HR 1.69, 95% CI 1.11; 2.58) or arteriolar diameter (HR 1.74, 95% CI 1.10; 2.68).

Conclusions
Our results suggest that the risk of stroke and TIA is only increased in people with a combination of impaired brain perfusion and small vessel disease.

Introduction

Annualy, 17 million people suffer a first-ever stroke worldwide.[1] About 80% of these strokes is ischemic and the consequence of insufficient blood flow to the brain. Reasons for that may be hypoperfusion or an occlusion, which can have its origin anywhere in the vascular system, from the heart to the brain.[2,3] For each stroke, usually one location is indicated as cause.[2] Yet, people often have vascular disease at multiple locations, for instance in the large and small vessels.[4-6] Since people with vascular disease at multiple locations seem to have a higher risk of stroke,[5] it may be that assigning one cause is insufficient. Several pathological mechanisms may interact.

Two mechanisms that have the potential to interact are a diminished brain perfusion and small vessel disease. Individually, a low cerebral blood flow has been related to stroke in people with severe intracranial atherosclerotic disease.[7] Small vessel disease, as visualized through the retinal vessels, has been related to stroke in the general population.[8,9] It has been hypothesized that if these markers are present together, their effect is amplified. Specifically, a previous study showed that a diminished cerebral blood flow had a stronger association with cognitive decline in combination with white matter lesions, suggesting that people with small vessel disease may be more vulnerable to changes in cerebral perfusion.[10] This could also mean that people with small vessel disease are more likely to get a stroke if cerebral perfusion is low compared to people without small vessel disease.

Against this background, our aim was to examine whether brain perfusion and retinal vessel diameter interact in their relation with stroke or TIA. First, we measured their individual effect in our general population. Then, we measured possible effect modification.

Methods

Setting and study population

This study was conducted within the Rotterdam Study, a prospective population-based study that aims to investigate occurrence and determinants of invalidating diseases in the elderly. Details regarding the objectives and design of the study have been reported previously.[11,12] The study started in 1990 amongst 7983 participants (Rotterdam Study I (RS-I)) and was extended twice: in 2000 with 3011 persons (RS-II) and in 2006 with 3932 persons (RS-III). The study now consists of 14,926 participants aged 45 years and older. Data on both cerebral perfusion and retinal vessels were collected in the second visit of RS-II (2004-2005) and the first visit of RS-III (2006-2008). In these periods, 6438 participants participated, of whom 4599 were invited for an MRI scan and 4161 actually underwent MRI scanning. Participants with an incomplete MRI (n=429), no fundus color photography for the assessment of retinal vessels (n=538), no informed consent for collection of follow-up data (n=21), prevalent stroke or TIA (n=132), silent cortical infarcts (n=34), incomplete follow-up (n=6), and an outlier (n=1) were excluded (Figure 1). Eventually, 3000 participants were eligible for analysis.

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study). All participants

provided written informed consent to participate in the study and to obtain information from their treating physicians.
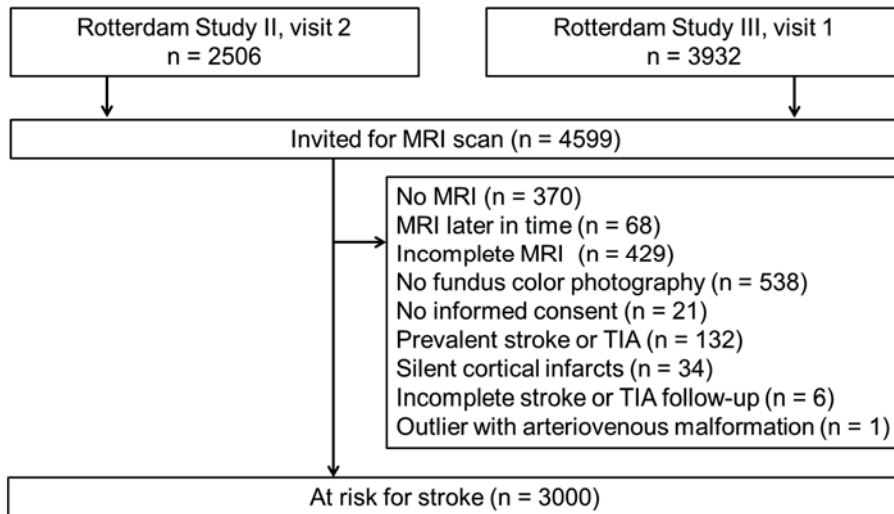

Figure 1. Study population

Brain MRI and brain perfusion

Magnetic resonance imaging was performed on a 1.5T MRI scanner (GE Healthcare, Milwaukee, WI, USA), using an 8-channel head coil. Flow measurement was performed using 2D phase-contrast imaging, as described previously.[13] Additionally, three high-resolution axial MRI sequences were performed, namely a T1-weighted sequence, a proton-density-weighted sequence, and a fluid attenuated inversion recovery sequence.[13]

Cerebral blood flow was calculated from the phase-contrast images using interactive data language-based custom software (Cinetool version 4, General Electric Healthcare, Milwaukee, WI, USA). Regions of interest were drawn manually around both carotids and the basilar artery at a level just under the skull base. Flow rates were calculated using the velocity and cross-sectional area of the vessels. To calculate total cerebral blood flow (tCBF), flow rates for the carotid arteries and the basilar artery were summed and expressed in mL/min. Total brain perfusion (in mL/min per 100mL) was calculated by dividing tCBF by each individual's brain volume (mL) and multiplying the obtained result by 100.[13]

Two independent experienced technicians drew all manual regions of interest and subsequently performed the flow measurements (interrater correlations (n=533) >0.94 for all vessels).[13]

Retinal vessel measurements

Details regarding retinal vessel measurements have been described previously.[8,14] Participants underwent a full eye examination including simultaneous stereoscopic fundus color photography of the optic disc (20º field, Topcon Optical Company, Tokyo, Japan) after

pharmacologic mydriasis. For each participant, the image with the best quality (left or right eye) was analyzed with the Retinal Vessel Measurement System (Retinal Analysis, Optimate, WI; Department of Ophthalmology & Visual Science, University of Wisconsin-Madison).[15] For each participant, one summary measure was calculated for the arteriolar diameters (in μm) and one for the venular diameters, corrected for magnification changes attributable to refractive errors of the eye. In a random subsample of 100 participants in RS-I, we found no differences between the right and left eyes for the arteriolar and venular calibers. Measurements were performed by 2 trained raters, blinded to the clinical characteristics and outcomes of the participants.

Pearson's correlation coefficients for interrater agreement varied 0.87 for arteriolar caliber and 0.91 for venular caliber in RS-II and 0.85 for arteriolar caliber and 0.87 for venular caliber in RS-III. Intrarater agreement ranged from 0.65-0.87.[16,17]

Assessment of stroke and TIA

History of stroke and TIA was assessed during the home interview at baseline and confirmed by reviewing medical records.[18,19] Subsequently, participants were continuously followed for occurrence of stroke and TIA through automatic linkage of general practitioners' medical records with the study database. Additionally, general practitioners' medical records of participants who moved out of the district and nursing home physicians' medical records were checked on a regular basis. For all potential strokes and TIAs, information from general practitioners and hospital discharge letters were collected and reviewed by research physicians. An experienced vascular neurologist verified the diagnoses.[18,19] Strokes were defined according to the World Health Organization Criteria[20] and subclassified into ischemic or hemorrhagic using neuroimaging reports. A stroke was classified as unspecified if neuroimaging was lacking.[18] Follow-up was complete until January 1, 2014, for 97.2% of potential person-years.

Assessment of covariates

Covariates were assessed during the same examination round as the fundus photography, with the use of structured interviews, physical examinations, and blood sampling.[21] Medication use and smoking status were assessed by interview. Smoking was categorized into current, former, or never smoking. Blood pressure was measured twice on the right arm with a random zero sphygmomanometer. The average of the two measurements was used. Total cholesterol and high-density lipoprotein cholesterol were acquired by an automated enzymatic procedure. Diabetes mellitus was defined as having a fasting glucose level of ≥7 mmol/L, a non-fasting glucose level of ≥11 mmol/L, or the use of antidiabetic medication. Body mass index was calculated as weight divided by length squared. Assessment of significant carotid stenosis (>50%) was performed using 5-MHz pulsed Doppler ultrasonography through interpretation of velocity profiles according to standard criteria.[22]

Statistical analysis

We analyzed the association of total brain perfusion and of retinal vessel diameter with ischemic stroke and TIA using Cox proportional hazards models. We combined ischemic stroke and TIA to increase power, which is reasonable since they have a similar pathophysiology.[23] Follow-up started at the date of MRI scan. We censored participants at date of stroke, date of TIA, date of death, end of follow-up, or January 1st 2014, whichever came first. Adjusted hazard ratio's (HR's) with 95% confidence intervals (CI's) were calculated adding total brain perfusion and retinal vessel diameters per standard deviation (SD) increase or decrease into the models. All models were adjusted for age and sex. In all models with vessel diameter as exposure, we adjusted for the other vessel diameter (venular diameter was adjusted for arteriolar diameter and arteriolar diameter for venular diameter). In the multivariable model, we additionally adjusted for study cohort, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication, smoking, diabetes mellitus, body mass index, and carotid stenosis. Effect modification between total brain perfusion and retinal vessel diameter was tested using an interaction term.

In order to further explore possible effect modification, we additionally performed a stratified analysis. Within tertiles of retinal diameter (venular and arteriolar), we examined the association between total brain perfusion and stroke or TIA, and within tertiles of total brain perfusion we examined the association between retinal vessel diameter and stroke. Finally, we categorized the participants based on both tertiles of retinal diameter and tertiles of cerebral perfusion and related these categories to the risk of stroke or TIA.

All analyses were done using IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY).

Results

The baseline characteristics of the study population are presented in Table 1. Participants had a mean age (±SD) of 58.8 (±7.1) years and 56.4% was women. After an average follow-up of 6.3 (±1.2) years, 29 participants had a stroke, and 48 a TIA.

Table 2 describes the association between total brain perfusion, retinal venular diameter, and retinal arteriolar diameter with stroke or TIA. We only observed an association of retinal venular diameter with stroke or TIA (multivariable adjusted HR per SD increase in venular diameter 1.39, 95% CI 1.07; 1.81). Total brain perfusion was not associated with stroke or TIA in the total population (HR 1.17, 95% CI 0.91; 1.49). However, stratified for age at median, we did find an association in people younger than 58.7 years (HR 1.61, 95% CI 1.01; 2.56). In the total population, we observed interactions between total brain perfusion and both the venular (p-value = 0.006) and arteriolar diameter (p-value = 0.020).

In Table 3, the results of the stratified analyses are shown. Total brain perfusion was only associated with stroke or TIA in people with the largest tertile of venular diameter (HR 1.70, 95% CI 1.12; 2.59) or the largest tertile of arteriolar diameter (HR 1.71, 95% CI 1.10; 2.68). Venular diameter was only associated with stroke or TIA in people with the lowest tertile of total brain

perfusion (HR 1.75, 95% CI 1.17; 2.60). Arteriolar diameter was not associated with stroke in any tertile of brain perfusion.

Combining all this information in one graph (Figure 2), it appeared that risk of stroke or TIA was mainly large in people with both the lowest tertile of cerebral perfusion and the highest tertile of venular diameter. The HR of being in tertile 1 of cerebral perfusion and tertile 3 of venular diameter, compared to the reference category (tertile 3 of cerebral perfusion and tertile 1 of venular diameter) was 2.11 (95% CI 0.77; 5.79). Since the group with tertile 1 of cerebral perfusion and tertile 3 of venular diameter stood out of the rest, we also compared this category with all other categories, which gave a HR of 1.93 (95% CI 1.07; 3.47). The pattern with arteriolar diameter was less clear.

Table 1. Baseline characteristics

	At risk for stroke or TIA N=3000
Age, years	58.8 (7.1)
Women	1691 (56.4%)
Systolic blood pressure, mmHg	134 (19)
Diastolic blood pressure, mmHg	82 (11)
Blood pressure lowering medication	674 (22.6%)
Total cholesterol, mmol/L	5.6 (1.0)
High-density lipoprotein cholesterol, mmol/L	1.4 (0.4)
Lipid-lowering medication	614 (20.6%)
Diabetes mellitus	243 (8.2%)
Smoking	
Never	878 (29.4%)
Former	1400 (46.9%)
Current	708 (23.7%)
Body mass index, kg/m^2	27.5 (4.3)
Carotid stenosis >50% on ultrasound	55 (1.8%)
Total brain perfusion, mL/min per 100 mL	57.3 (9.4)
Venular diameter, µm	238.3 (22.8)
Arteriolar diameter, µm	156.8 (15.9)

Abbreviations: N = number of persons included in study.
Data are presented as mean (standard deviations) or counts (percentages).
Percentages are calculated without missing data.

Table 2. Total brain perfusion and retinal vessel diameter and the risk of ischemic stroke or TIA

	Ischemic stroke or TIA n/N 77/3000		
	Model I HR (95% CI)	Model II HR (95% CI)	Model III HR (95% CI)
Total brain perfusion, per SD decrease	1.16 (0.91; 1.49)	1.16 (0.91; 1.49)	1.17 (0.91; 1.49)
Retinal venular diameter, per SD increase	1.40 (1.08; 1.81)	1.39 (1.07; 1.81)	1.39 (1.07; 1.81)
Retinal arteriolar diameter, per SD decrease	1.07 (0.82; 1.41)	1.06 (0.80; 1.42)	1.06 (0.80; 1.41)

Values are hazard ratios per standard deviation increase in the determinant with 95% confidence intervals.
Model I: Adjusted for age, sex, study cohort, and other retinal vessel if applicable.
Model II: As model I, additionally adjusted for systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication, smoking, diabetes mellitus, BMI, and carotid stenosis.
Model III: As model II, additionally adjusted for brain perfusion in analyses with vessel diameters, and for vessel diameters in analyses with brain perfusion.

Table 3. Total brain perfusion and retinal vessel diameter and the risk of ischemic stroke or TIA, within tertiles of retinal vessel diameter or brain perfusion
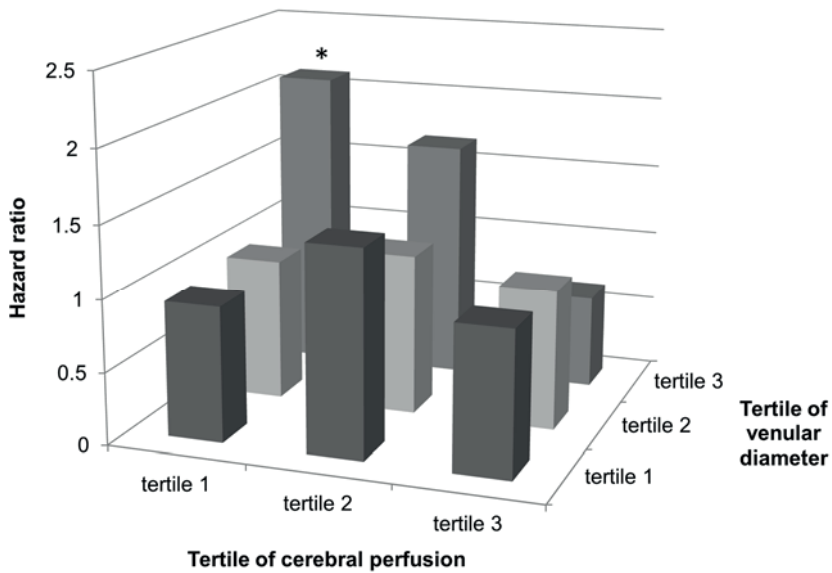
		Ischemic stroke or TIA		
		n/N	Model I HR (95% CI)	Model II HR (95% CI)
Venular diameter	Total brain perfusion			
Tertile 1	per SD decrease	26/1000	0.89 (0.59; 1.36)	0.88 (0.57; 1.35)
Tertile 2	per SD decrease	21/1000	0.97 (0.61; 1.53)	0.96 (0.60; 1.52)
Tertile 3	per SD decrease	30/1000	1.69 (1.11; 2.58)	1.70 (1.12; 2.59)
Total brain perfusion	Venular diameter			
Tertile 3	per SD increase	16/1000	0.89 (0.49; 1.62)	0.95 (0.51; 1.76)
Tertile 2	per SD increase	28/1000	1.36 (0.90; 2.05)	1.29 (0.84; 1.99)
Tertile 1	per SD increase	33/1000	1.70 (1.16; 2.51)	1.75 (1.17; 2.60)
Total brain perfusion	Arteriolar diameter			
Tertile 3	per SD decrease	16/1000	0.97 (0.52; 1.80)	0.97 (0.50; 1.87)
Tertile 2	per SD decrease	28/1000	1.12 (0.71; 1.76)	1.14 (0.72; 1.81)
Tertile 1	per SD decrease	33/1000	1.01 (0.66; 1.54)	1.05 (0.67; 1.63)
Arteriolar diameter	Total brain perfusion			
Tertile 3	per SD decrease	27/1000	1.74 (1.12; 2.69)	1.71 (1.10; 2.68)
Tertile 2	per SD decrease	20/1000	1.14 (0.70; 1.87)	1.14 (0.70; 1.86)
Tertile 1	per SD decrease	30/1000	0.89 (0.63; 1.28)	0.93 (0.64; 1.33)

Values are hazard ratios per standard deviation increase in the determinant with 95% confidence intervals.
Model I: Adjusted for age, sex, study cohort, and other retinal vessel.
Model II: Adjusted for age, sex, study cohort, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication, smoking, diabetes mellitus, BMI, carotid stenosis, and other retinal vessel.

Figure 2. Interaction brain perfusion and venular diameter and risk of ischemic stroke or TIA
Values are hazard ratio's compared to the reference category: tertile 3 of cerebral perfusion and tertile 1 of venular diameter. Adjusted for age, sex, study cohort, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication, smoking, diabetes mellitus, BMI, carotid stenosis, and other retinal vessel.
*Hazard ratio (HR) 2.11 (95% CI 0.77; 5.79) compared to reference category. HR 1.93 (95% CI 1.07; 3.47) compared to all other categories combined.

Discussion

In this population-based study, we observed that wider retinal venules were associated with an increased risk of stroke or TIA. Total cerebral perfusion was not associated with the risk of stroke in the total population. Interestingly, we observed an interaction between cerebral perfusion and both retinal venules and retinal arterioles for their risk of stroke or TIA. Total cerebral perfusion was associated with stroke or TIA in the highest tertile of venular diameter and in the highest tertile of arteriolar diameter. Correspondingly, venular diameter was only associated with stroke or TIA in the lowest tertile of cerebral perfusion.

A low cerebral blood flow[7] and a small venular diameter[8,24,25] were separately related to stroke in previous studies. The reason why we did not find an association of low cerebral perfusion and stroke in our total population may be explained by our different study population. We included a general population and the previous study included patients with severe stenosis in the intracranial vessels.[7] People in the previous study therefore had a larger impairment of the blood flow than people in our general population. The brain has compensatory mechanisms to keep the local blood perfusion intact for a long time, i.e. cerebral autoregulation, which may be sufficient if people do not have a severe stenosis.[26]

In people with cerebral small vessel disease, cerebral autoregulation can be impaired.[27] Although a diminished autoregulation by itself seemed not sufficient to increase the risk of stroke in a previous study,[28] it may increase the risk of stroke or TIA in combination with a low perfusion.[29] A high perfusion may compensate for a diminished autoregulation and a good autoregulation for a diminished perfusion. If both fail, however, this may lead to an increased risk of stroke and TIA. This is a possible explanation for our finding that a lower cerebral perfusion was associated with an increased risk of stroke or TIA in people with large retinal venules, reflecting small vessel disease, and that large retinal vessels were only associated with an increased risk of stroke in people with a low perfusion. It implies that in people with a stroke or TIA based on small vessel disease, also a source of diminished perfusion should be sought. For instance, large artery atherosclerosis.[7] In people with a low perfusion, the amount of small vessel disease should be examined. Another possible explanation is the risk factor load that may be higher in people with both a low perfusion and small vessel disease. However, associations remained after adjustment for many possible confounders. Furthermore, a diminished perfusion may relate to stroke mediated by small vessel disease,[6,17] although it is uncertain whether a diminished perfusion leads to small vessel disease or whether this association is inverse.[30] A final explanation therefore is that small vessel disease gives rise to white matter lesions[31] and that these can reduce the blood flow due to a diminished metabolic demand.[30] It may be that small vessel disease only relates to stroke if it is severe enough to demand a lower perfusion.

The finding that a low brain perfusion also associated with stroke in people with wider arterioles is actually the opposite of what we expected, since arteriolar narrowing is associated with atherosclerosis.[9,32] An explanation may be that arteriolar and venular diameter are highly correlated and people with wide venules therefore have wide arterioles.[32] However, we adjusted the analyses with arteriolar diameter for venular diameter and associations remained. Another explanation may be that arterioles keep the ability to dilate in response to a poor blood flow.[8,33] This may reflect an exhausted autoregulation or vasomotor reactivity.[33] If arterioles are fully widened in response to the usual blood flow in a person, they may not be able to widen any further in response to extra stimuli, which could lead to a stroke or TIA.[29,33]

Strengths of our study are the population-based setting and the thorough follow-up for stroke and TIA. A limitation is that pathophysiological subtypes were unavailable for many ischemic strokes. Therefore, we could not define whether the increased risk was the consequence of strokes based on large or small vessel disease. We even had a limited amount of stroke cases, so we had to pool the results of ischemic stroke and TIA. This seems reliable since stroke and TIA have the same etiology.[23] However, these findings should be replicated in a study with more power.

In conclusion, total brain perfusion and retinal vessel diameter interact in their risk of stroke or TIA. This suggests that a combination of a low brain perfusion and small vessel disease is necessary to increase the risk of stroke or TIA. If people have a stroke or TIA based on one of both, it may be useful to search for the other too. Future studies should examine the pathophysiological pathway of this effect and whether prediction of stroke can be improved taking both markers into account.

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Chapter 3.5

Mid- to late-life trajectories of blood pressure and the risk of stroke

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Abstract

Background and purpose
Hypertension is a major modifiable risk factor for stroke. Associations of blood pressure with incident stroke are mostly based on single or average blood pressure levels. However, this approach does not take into account long-term trajectories of blood pressure, which can vary considerably in the elderly.

Methods
Within the population-based Rotterdam Study, we examined trajectories of systolic blood pressure in 6745 participants (60.0% women) over an age-range from 55-106 years and jointly modeled their risk of stroke and competing causes of death using joint latent class mixed modeling.

Results
Four trajectories were identified. *Class 1* was characterized by blood pressure increasing gradually from on average 120 to 160 mmHg over five decades (N=4938). Compared to this class, *class 2,* characterized by a similar mid-life blood pressure, but a steep increase (N=822, increasing from 120 to 200 mmHg), and *class 4,* characterized by a high mid-life blood pressure (N=115, average 160 mmHg), had a higher risk of stroke and death. *Class 3,* characterized by a moderate mid-life blood pressure (N=870, average 140 mmHg), had a similar risk of death as *class 1*, but the highest risk of stroke.

Conclusions
Assessing trajectories of blood pressure provides a more nuanced understanding of the associations between blood pressure, stroke, and mortality. In particular, high blood pressure and rapidly increasing blood pressure patterns are associated with a high risk of stroke and death, while moderately high blood pressure is only related to an increased risk of stroke. Future studies should explore the potential etiologic significance of these patterns.

Introduction

Hypertension is a major treatable risk factor for stroke, with an estimated attributable risk of 35% to 50%.[1,2] Most studies that examined the association between hypertension and incident stroke used a single measurement or the average of blood pressure levels assessed over time. Results from such approaches have suggested that the risk of stroke increases with increasing blood pressure levels and that even prehypertension is associated with stroke.[3-6] Indeed, the current guideline to treat people above a certain target level of blood pressure (e.g. 150/90 mmHg[7] or 140/90 mmHg[8-10]) is largely based on such knowledge. However, long-term patterns (i.e. trajectories) of blood pressure may further influence stroke risk. Studies in young and middle aged adults showed that increases in blood pressure over long periods (10 to 30 years) are related to an increased risk of stroke and cardiovascular disease,[11-13] and that trajectories of higher blood pressure relate to a higher risk of subclinical atherosclerosis.[14] Trajectories in older people may vary even more, because it is particularly in later ages that arterial stiffness increases, which is associated with increases in blood pressure and blood pressure variability.[15-17] Furthermore, studies suggest that lower blood pressures might also be harmful in this population, leading to an increased risk of myocardial infarction or death.[18-20] This has particularly been observed for low diastolic blood pressures,[18,19] although low systolic blood pressures also seemed to be harmful in patients with vascular disease and diabetes.[20] To date, no study of long-term blood pressure trajectories in mid- to late-life has been conducted. Furthermore, it is unknown whether such trajectories relate to stroke. If we hope to empirically inform and refine prevention guidelines, a much-needed first step is to describe the prototypic and commonly observed patterns of blood pressure trajectories.

Therefore, the aim of our study was to identify long-term trajectories of blood pressure in a population-based study and to examine the risk of stroke within those trajectories. We focused on systolic blood pressure (SBP) because it is the best predictor of cardiovascular events.[21]

Methods

Setting and study population
This study was conducted within the prospective, population-based Rotterdam Study. Details regarding the objectives and design of the study have been described elsewhere.[22] Baseline examinations started in 1990 among 7983 people of 55 years and older residing in Ommoord, a suburb of Rotterdam, the Netherlands. Follow-up examinations take place every 3-4 years.

For the current study, data from five follow-up visits from 1990 to 2011 were used. Participants with no informed consent for follow-up data collection (n=226), prevalent stroke at baseline (n=243), no center visit prior to a stroke (n=668), and completely missing information on blood pressure and blood pressure-lowering medication (n=101) were excluded, resulting in 6745 participants eligible for the current analysis. Only measurements before occurrence of stroke were used. We had 6679, 5018, 3570, 2891, and 1499 measurements at each of the five center visits, respectively, totaling 19,657 measurements.

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study). All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

Assessment of blood pressure
During each visit, blood pressure was measured twice in the right arm, in sitting position, after a resting period of five minutes. The average of the two measurements was used in the analyses. Up until November 7th 2006, a Hawksley random-zero sphygmomanometer was used,[23] and for measurements after this date Omron M6 Comfort and Omron M7 devices were used.[24,25]

Assessment of stroke
Stroke was defined according to WHO criteria as a syndrome of rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death and no apparent cause other than of vascular origin.[26] This corresponds to ICD10 codes I61, I63, and I64. At baseline, history of stroke was assessed by interview and verified using medical records.[27] Subsequently, participants were continuously followed for occurrence of incident stroke, by digital linkage of the general practitioners' medical records with the study database. Nursing home physicians' medical records and general practitioners' medical records of participants who moved out of the Ommoord district were checked on a regular basis as well. Of all potential strokes, medical records from general practitioners and hospital discharge letters were collected and reviewed by research physicians. An experienced vascular neurologist verified the diagnoses.

Follow-up through January 1st 2013 was complete for 98.9% of potential person-years.[28]

Covariates
Covariates were assessed at each center visit. Details on the assessment of anthropometrics, cardiovascular risk factors (total cholesterol, high-density lipoprotein cholesterol, diabetes mellitus type 2, and smoking) and use of medication have been described previously.[29] Current alcohol use was assessed during each home interview and categorized into yes versus no. Use of blood pressure-lowering medication included the use of diuretics, beta blocking agents, calcium blockers, angiotensin receptor blockers, and ACE-inhibitors, if prescribed for the indication hypertension. The second center visit had limited examinations, and in particular had no assessment of cholesterol, high-density lipoprotein cholesterol and diabetes mellitus status. Therefore, measurements from the preceding center visit were carried forward.

Statistical analysis
We investigated the association of longitudinal trajectories in systolic blood pressure over age with the risk of stroke using a joint latent class mixed model. The main goal of a joint latent class mixed model is to describe the link between a continuous progression of diseases

through longitudinal markers such as blood pressure, and the incidence of clinical events.[30,31] The model uses all available information from the blood pressure and outcome measurements to model trajectories and co-occurring events like stroke and death.[30,31] Since hypertension has been associated with both ischemic and hemorrhagic strokes,[16] we examined the risk with all stroke types. Models were fit using the 'Jointlcmm' function of the 'lcmm' package in R.[32] The joint latent class mixed model assumes that people's blood pressure trajectories cluster in a set of mutually-exclusive patterns or 'latent classes'. That is, there are prototypical patterns in blood pressure trajectories that individuals follow. In the current analysis, the model differentiates the population into groups (latent classes) with different profiles of blood pressure, and links these classes to potential risk of stroke.[30,31,33] Trajectories of blood pressure over age from 55 years onwards were modeled using a class-specific linear mixed model with age as time.[34] Since blood pressure may have a non-linear pattern, we also added a class-specific quadratic age term. We included random intercepts and random slopes in all analyses. We added class-specific adjustments for sex and baseline blood-pressure lowering medication, since we expected these variables may influence the evolution of blood pressure. People entered at study baseline and were censored at date of stroke, date of death, last date of follow-up or January 1, 2013, whichever came first. The optimal number of classes was defined by the model with the lowest Bayesian information criterion (BIC).[31] Analyses were repeated using different random starting values to ensure convergence to the global maximum of the model.[31] We were primarily interested in stroke as outcome, but added stroke-free mortality since it is an important competing risk. The joint survival model was therefore defined by a two-parameter Weibull distribution with a class-specific baseline risk function for both stroke and the competing risk of mortality due to any other cause than stroke.[30,35] This includes cardiovascular deaths. The survival function was adjusted for sex and blood pressure lowering medication and in the multivariable model we additionally adjusted for visit-specific cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication, body mass index, smoking, alcohol use, diabetes mellitus type 2, and antithrombotic medication. Adjustments in the survival part of the analysis were only made for baseline characteristics. However, we do provide characteristics of later visits, in order to compare the evolution of these characteristics together with blood pressure. Analysis of covariance was used to study differences in baseline characteristics between subjects within the different estimated trajectory classes, adjusted for age and sex. Covariates were missing for up to a maximum of 4.6% across assessments. Missing values were imputed by the mean of 5 imputations, using multiple imputation based on the other covariates of the same visit. Class-specific trajectories of blood pressure or cumulative incidences of stroke or death were estimated for the mean value for all covariates. A Monte Carlo method was used to calculate the confidence intervals of the cumulative incidences.[32]

Sensitivity analyses
Sensitivity analyses were conducted to explore the robustness of our results to modeling decisions, missing data, and censorship. First, we inspected models based on one fewer and one greater trajectory than the model chosen based on lowest BIC. Second, we added a cubic age term. Third, to understand the robustness of our observed patterns to missing values and

censoring for stroke or death in between the measurements we repeated our analyses based on data from only the first 3 visits (from 1989 to 1999) in the people without missing values in these visits and examined the survival curves thereafter (from 1999 to 2013). Fourth, since few participants were alive and stroke-free after the age of 80, we wanted to understand whether our identified trajectories were similar in only younger ages. Therefore, we also repeated analyses in which we only included systolic blood pressure measurements assessed between the ages of 55 to 80 years. Finally, while latent class modeling is a useful data-reduction tool for understanding general patterns, individual membership in a class or trajectory is probabilistic. As a posterior check to see how observed trajectories of blood pressure of individual participants aligned with the identified trajectories in our final model, as well as to visually assess missing data patterns, we plotted the individual blood pressure values by most likely trajectory class, visit, outcome, and age.[36]

Results

Characteristics of the trajectory classes
Participants had a mean (± standard deviation) follow-up of 13.5 ±6.8 years, during which 1053 strokes occurred.

When investigating the trajectories, we found that the joint latent class model with 4 classes had the best fit, with the lowest BIC (Supplementary Table I). Mean posterior class membership probabilities (i.e. the probability that a person belongs to his or her most likely class) were at least 63% for each class in this model. Figure 1A shows the four identified trajectories of systolic blood pressure, based on the joint latent class mixed model. The largest class was characterized by a gradually increasing blood pressure, starting at on average 120 mmHg at the age of 55 years and increasing up to on average 160 mmHg at the age of 95 years (*class 1*, N=4938). A smaller class was characterized by a similar blood pressure at the age of 55 years, but a much steeper increase up to on average 200 mmHg (*class 2*, N=822). Two classes were characterized by a relatively higher baseline blood pressure: one of on average 140 mmHg, with modest variation over time (*class 3*, N=870), and the other of on average 160 mmHg, which decreased after age 65 (*class 4*, N=115). Supplementary Figure I shows the model-based trajectories compared to the mean observed values in the classes. The predicted values only diverge slightly from the observed values in people at old age. For instance, the observed values of the high baseline SBP class plateau at old age, whereas the predicted trajectory continues to increase.

People in *class 4* were more frequently men (Table 1). Mean and maximum baseline blood pressure assessments were highest in *class 4* followed by the *class 3* and *class 2*. Use of blood pressure-lowering medication was similar between classes at baseline, but at the end of follow-up the *class 3* and *class 4* had higher proportions of blood pressure-lowering medication users. Values on other covariates at each study visit are presented in Supplementary Table II. We found a large difference in frequency of current smokers among classes, with particularly higher frequencies in *class 2* and *class 4*.
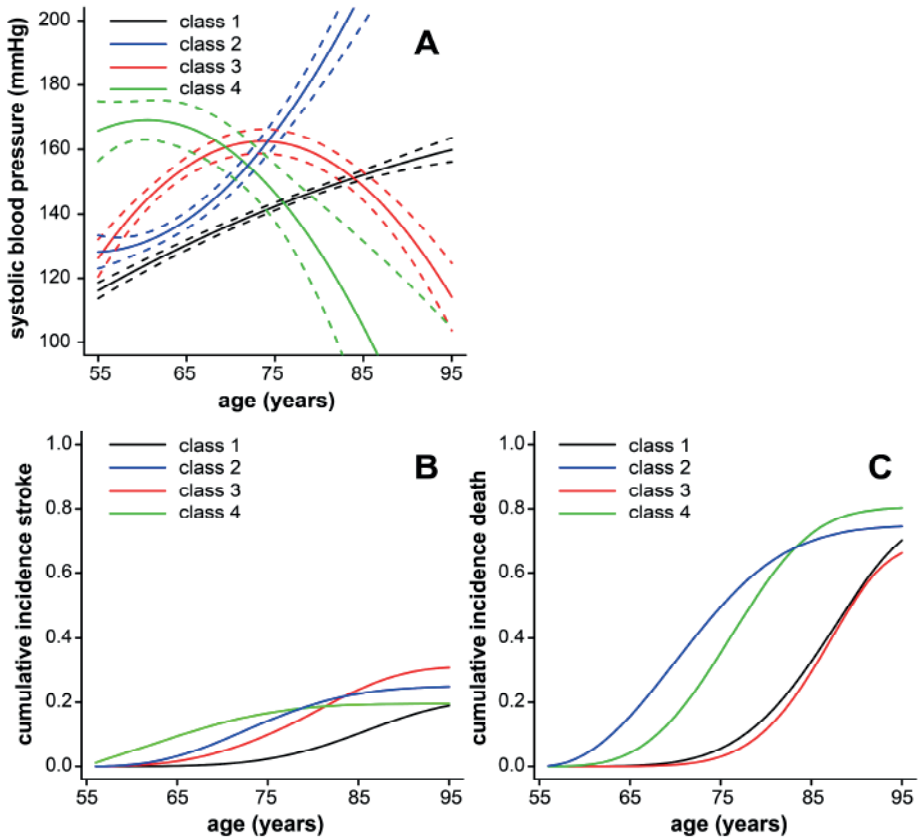
Figure 1. Trajectories of blood pressure and risk of stroke and competing causes of death
A: trajectories of systolic blood pressure over time, as predicted by the joint latent class mixed model. The solid line represents the average blood pressure in a class for the mean of covariates. Dotted lines represent confidence intervals. B: average class-specific cumulative incidence of stroke. C: average class-specific cumulative incidence of competing death due to any other cause than stroke. Cumulative incidences are plotted for the mean of covariates. Covariates are sex and blood-pressure lowering medication.

Risk of stroke and death for the separate trajectories
Relative to *class 1*, the increased risk of stroke was apparent in *class 4* beginning at age 55 years onwards, while increased stroke risk began later (approximately age 65) for *class 2* and *3* (Figure 1B). The three classes had a significantly and substantially higher risk of stroke than *class 1* (e.g., 4.7-13.6% compared to 0.7%). The estimated risks up to age 75 appeared higher in class 4 (13.6%) than in classes 2 (8.1%) and 3 (4.7%), although the wide confidence intervals limited interpretability (Supplementary Table III). *Class 2 and 4* also had the highest risk of dying through other causes (Figure 1C). The cumulative incidence curves in those classes plateaued around ages 75-85 years, at that time roughly all people in the classes were diagnosed as having a stroke or died. The risk of stroke in *class 3* continued to increase until older age. However, the risk of dying was lower than in *class 2* and *4*, similar to *class 1*.

At the end of follow-up, 2546 people (51.5%) in *class 1*, 575 (70.0%) people in *class 2*, 288 (33.1%) people in *class 3*, and 87 (75.7%) people in *class 4* died due to a non-stroke-related cause. Between 25 and 38% of non-stroke deaths in each class were due to cardiovascular events (Supplementary Figure II).

Multivariable-adjusted models were relatively similar, although some people's most probable class membership switched. Risks of stroke in *class 2* and *4* attenuated, whereas the risk became stronger in *class 3* (Figure 2).
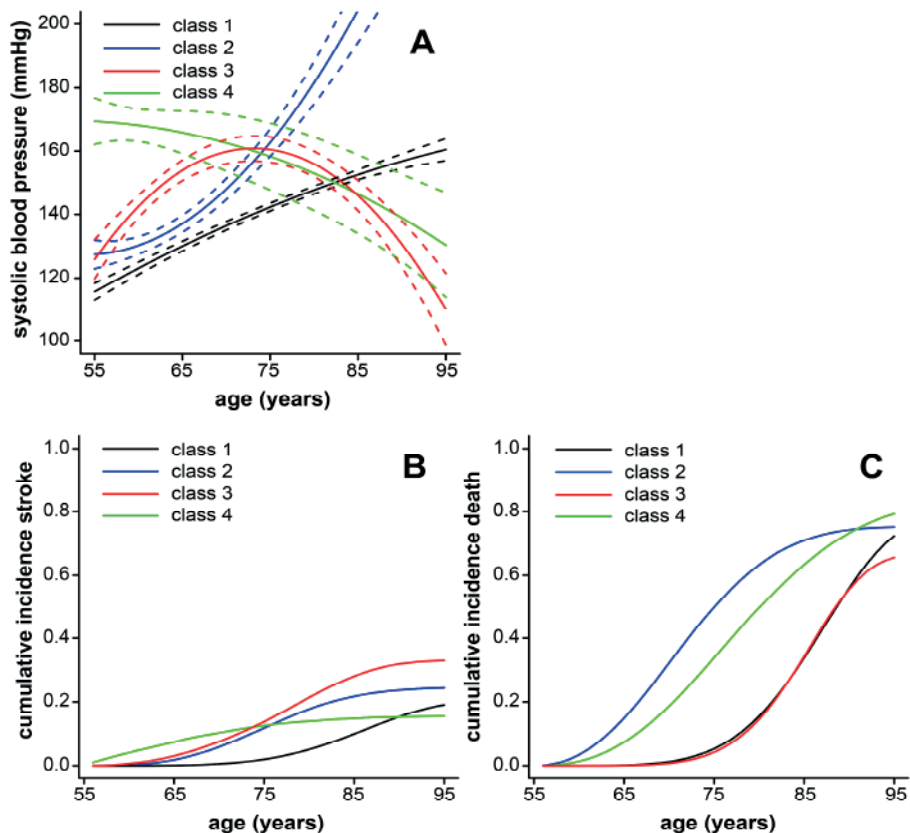
Figure 2. Trajectories of blood pressure and risk of stroke and competing causes of death, multivariable adjusted

A: trajectories of systolic blood pressure over time, as predicted by the joint latent class mixed model. The solid line represents the average blood pressure in a class for the mean of covariates. Dotted lines represent confidence intervals. B: average class-specific cumulative incidence of stroke. C: average class-specific cumulative incidence of competing death due to any other cause than stroke. Cumulative incidences are plotted for the mean of covariates. Covariates are sex, blood-pressure lowering medication, cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication, body mass index, smoking, alcohol use, diabetes mellitus type 2, and antithrombotic medication.

Table 1. Characteristics of the study population

Characteristic	Class 1 (N=4938)	Class 2 (N=822)	Class 3 (N=870)	Class 4 (N=115)
Age at entry, years	71.0 (9.4)[bcd]	64.5 (6.0)[acd]	65.7 (7.1)[abd]	62.0 (5.0)[abc]
Female	3017 (61.1%)[d]	470 (57.2%)[d]	511 (58.7%)[d]	48 (41.7%)[abc]
Mean systolic blood pressure, mmHg	140 (17)[bcd]	148 (22)[acd]	162 (14)[abd]	167 (20)[abc]
Maximum systolic blood pressure, mmHg	152 (20)[bcd]	161 (29)[acd]	177 (17)[abd]	184 (20)[abc]
Use of blood pressure-lowering medication				
Entry	1003 (20.5%)[bcd]	223 (27.4%)[a]	220 (25.5%)[a]	27 (24.1%)[a]
End	1686 (34.1%)[bcd]	271 (33.0%)[acd]	508 (58.4%)[ab]	71 (61.7%)[ab]
Characteristics at visit 1				
Mean systolic blood pressure, mmHg	135 (20)[bcd]	142 (23)[acd]	158 (20)[abd]	174 (20)[abc]
Smoking				
Past	1997 (41.9%)[bd]	310 (38.9%)[ac]	385 (45.1%)[bd]	51 (45.9%)[ac]
Current	974 (20.4%)[bcd]	286 (35.9%)[ac]	170 (19.9%)[abd]	47 (42.3%)[ac]
Current use of alcohol	2940 (78.7%)	565 (81.5%)	588 (79.7%)	84 (84.0%)
Total cholesterol, mmol/L	6.6 (1.2)[c]	6.7 (1.2)[c]	6.8 (1.2)[ab]	6.7 (1.1)
HDL cholesterol, mmol/L	1.4 (0.4)	1.3 (0.4)	1.4 (0.4)	1.3 (0.4)
Use of lipid-lowering medication	102 (2.1%)	21 (2.6%)	30 (3.5%)	3 (2.7%)
Diabetes mellitus	336 (7.4%)[bcd]	57 (7.4%)[ad]	63 (7.6%)[ad]	14 (13.0%)[abc]
Body mass index, kg/m²	26.2 (3.7)[cd]	26.1 (3.8)[cd]	26.8 (3.6)[ab]	27.1 (3.7)[ab]
Use of antithrombotic medication	224 (4.6%)[b]	46 (5.7%)[acd]	30 (3.5%)[b]	2 (1.8%)[b]
Age at the end of study period				
Age at stroke, years	84.8 (6.4)[bcd]	73.0 (6.7)[acd]	77.4 (5.6)[abd]	66.2 (6.7)[abc]
Age at death in people without stroke, years	86.0 (6.6)[bcd]	72.1 (6.0)[acd]	84.8 (4.9)[abd]	73.9 (5.3)[abc]
Age at end of follow-up for people alive and stroke-free, years	83.2 (5.8)[cd]	83.7 (4.6)[cd]	81.1 (4.1)[abd]	77.8 (2.7)[abc]

Abbreviations: N = number of participants, SBP = systolic blood pressure, HDL = high-density lipoprotein.

Values are presented as mean (standard deviation) or counts (percentages).

[a] Compared to Class 1, p-value <0.05 after age and sex adjustment – if applicable.
[b] Compared to Class 2, p-value <0.05 after age and sex adjustment – if applicable.
[c] Compared to Class 3, p-value <0.05 after age and sex adjustment – if applicable.
[d] Compared to Class 4, p-value <0.05 after age and sex adjustment – if applicable.

Sensitivity analyses

Visual assessment of individual blood pressure patterns by participants' most likely class membership, visit, age, and outcome suggested that the model-based patterns were unlikely to be influenced by missing data and that the described trajectories reflected observable patterns within and across individuals (Supplementary Figure II). On the other hand, differences in blood pressure over time seen between *class 1* and *2* were mainly apparent in older people. Further, people assigned to *class 4* often had a stroke or death at young age, with few people within this class having decreases in blood pressure, suggesting that the model-estimated decrease in this trajectory may be an artefact of a small number of survivors.

The three- and five-class model did not provide any additional insights. In the three-class model, no class resembling *class 4* was identified. In the five-class model, the class resembling *class 4* with respect to the high mid-life blood pressure was further subdivided into a class that was increasing and a class that was decreasing. For classes that heuristically overlapped with those identified in the four-class models, patterns of stroke and mortality risk were similar. The class with a high mid-life blood pressure that was increasing, was related to both stroke and death, while the class with a high mid-life blood pressure that was decreasing, was associated only with death; for both classes, numbers were low.

In the sensitivity analysis in which we only included people that completed the three first visits of blood pressure measurement, trajectories of blood pressure remained similar. Furthermore, we found a similar risk of death in the different classes. The risk of stroke was highest in *class 3* class, followed by *class 2*. *Class 4* did not have an increased risk of stroke (Supplementary Figure III).

In another sensitivity analysis in which trajectories were formed based only on measurements at the ages of 55 to 80 only, trajectories and associated risks of stroke were similar to our main findings (Supplementary Figure IV).

We report the results of trajectories that only allowed for a quadratic effect of age over time. As sensitivity analysis we did add a cubic age term, but this did not provide any additional insight (Supplementary Figure V).

Discussion

In this population-based study of people aged 55 years and older, we identified four trajectories of blood pressure: a class characterized by a gradually increasing blood pressure from approximately 120 to 160 mmHg (on average) over five decades (*class 1*); a class characterized by a more steep increase from an average of approximately 120 to 200 mmHg (*class 2*); a class characterized by a moderate blood pressure (140 mmHg) at mid-life and throughout (*class 3*); and a class characterized by a high blood pressure (160 mmHg) at mid-life that decreased (*class 4*). The class with a high blood pressure at midlife and the class with a steep increase had the highest risks of stroke and death through age 80 years. The class with a moderate blood pressure only had an increased risk of stroke, but not death, compared to the class with a low blood pressure at midlife and a gradual increase.

The trajectories that we identified extend results from prior studies of blood pressure trajectories in young to middle aged people. Those studies identified four to five parallel trajectories in which trajectories with long-term higher blood pressure related to more cardiovascular pathology.[12-14] In our older population, we also observed that the class with a high mid-life blood pressure had the highest risk of stroke and death compared to the class with the lowest blood pressure. However, a novel finding of our study is that the slope of increase was associated with an increasing risk of stroke and competing causes of death. Namely, we identified two classes characterized by equally low baseline blood pressure and increasing trajectories, but only the class characterized by steep increases had a high risk of stroke and death. Of note, the risks in that class were even similar to the class with a high mid-life blood pressure.

The trajectories and risk patterns identified in this paper could inform future research on etiology and potentially treatment guidelines, but the current study does not itself address such etiologic or treatment questions. Latent class mixture modeling is a useful data-reduction tool and is particularly helpful to describe prototypical and common patterns. It does not, however, account for time-dependent confounding or selection biases that may explain the trajectories and risks estimated. Therefore, the associations with stroke risk seen across our four trajectories could be due in part or whole to a number of non-causal explanations, including differences in health behaviors, healthcare utilization, and competing risks throughout the study period.[37,38] Moreover, findings from the classes are not necessarily applicable to individual participants' blood pressure effects, particularly because class membership is probabilistic. On the other hand, identifying the patterns described in our study are an important step, since they evoke new causal and treatment questions that can motivate future studies to explore the etiologic significance and predictive value of the associations. Questions raised by the patterns in our study are: would we reduce stroke risk if we recommended blood pressure medication to non-hypertensive middle-aged to older patients with fast increases in blood pressure? Or, would we reduce stroke risk if the current treatment level of 140/90 mmHg or 150/90 mmHg is lowered to also include people with moderately elevated blood pressure? In addition, trajectories may inform physicians about people that need further attention for their high risk of stroke or death.

Combining our study results with prior evidence, we can speculate to the biologic plausibility that treating middle-aged to older patients with (i) moderate blood pressure or (ii) fast increasing blood pressure could reduce strokes. The first is supported by previous studies that found a relation between prehypertension and stroke, and less of prehypertension with other vascular disease.[39-41] This suggests that the brain may be particular vulnerable to vascular damage. Whether it is likely that treatment of fast increasing blood pressure reduces stroke and death is less clear. It is known that vascular stiffness increases with ageing.[15,42] This leads to an increase in blood pressure and may explain the gradually increasing blood pressure trajectory in the largest part of the population.[15,42] However, this does not explain why another class was characterized by a much steeper increase. It may be that this trajectory reflects a higher vascular age.[43] This is supported by our findings that associations with stroke attenuated after adjusting for other cardiovascular risk factors. They may have stiffer vessels, which could lead to a steeper

increase or a blood pressure resistant to treatment.[44] The higher vascular age may then contribute to the increased risk of stroke and death. Furthermore, the steep increase in blood pressure may trigger rupture of an arteriole leading to a hemorrhagic stroke.[45] Knowing whether a fast increasing blood pressure causally relates to stroke and death is important, because people with fast increasing blood pressure may be missed using current guidelines, leading to undertreatment. Correspondingly, only 33% of the people in the fast increasing class used blood pressure-lowering medication at their end of follow-up. On the other hand, this could also mean that this class reflects people with more unhealthy behavior that are less adherent to medication themselves. Further research is necessary to unravel the etiological background of the trajectories. This should include the question whether some trajectories reflect advancing stages of vascular ageing. For example, with advancing vascular age people may move from an increasing trajectory to the trajectory of consistently high blood pressure.

Strengths of our study are the large study population, the use of repeated measures of blood pressure over a long follow-up, and the thorough collection of stroke assessments. However, our study also has some limitations. We only examined associations with all stroke, numbers were too small to examine stroke subtypes. Furthermore, we obtained a maximum of only five measurements of blood pressure per person. More measurements would probably lead to more precise results and possibly more or differently defined trajectories. It might also reduce the effect of regression to the mean and within-individual variability. Moreover, there may have been misclassification among classes due to individual variability or reliability in blood pressure assessments. Nevertheless, the trajectories may aid detection of high-risk persons even if the trajectories are in part explained by a white coat effect or variability. Additionally, the trajectories may be less reliable at very old ages when we had fewer measurements, although our trajectories were consistent up through age 80 whether or not later assessments were included in the model. Finally, we did not have information about blood pressure at earlier ages. Future studies should examine the lifetime trajectory of blood pressure and the risk of stroke.

In conclusion, treatment of blood pressure to reduce the risk of stroke is currently focused on blood pressure levels. In this population-based study on blood pressure trajectories, we had the novel finding that the trajectory with fast increasing blood pressure was related to a high risk of stroke and death. It shows that single values of blood pressure do not tell the whole story. This may inspire future studies to examine the predictive value of blood pressure trajectories for stroke and mortality. Additionally, future studies are needed to determine the etiologic significance of blood pressure changes. If the blood pressure slope is causally related to stroke and not just a reflection of an individual's health behavior, it may be a novel target for prevention.

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3

Supplementary information

Supplementary Table I. Summary of joint model fits

Number groups	Log-likelihood	N parameters	BIC	AIC	Score test (p-value)		Latent class proportion (%)
					Stroke	Death without stroke	
1 class	-104623	15	209380	209277	65 (0)	57 (0)	100
2 classes	-104427	25	209075	208904	14 (0)	23 (0)	11.18; 88.82
3 classes	-104331	35	208971	208732	9 (0.01)	10 (0.01)	11.96; 81.04; 7
4 classes	**-104266**	**45**	**208930**	**208623**	**7 (0.04)**	**4 (0.12)**	**73.21; 12.9; 1.7; 12.19**
5 classes	-104232	55	208949	208574	5 (0.10)	2 (0.36)	66.98; 3.17; 1.02; 14.2; 14.62

Abbreviations: N = number, BIC = Bayesian information criterion, AIC = Akaike's information criterion.
The BIC is the preferred criterion to define the optimal number of classes in mixture models.
The score test is a test for conditional independence. Conditional independence is rejected for a p-value <0.05.

Supplementary Table II. Characteristics of the study population

Characteristic	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
Class 1 (N=4938)	**N=4890**	**N=3581**	**N=2605**	**N=2179**	**N=1165**
Age at visit, years	71.0 (9.4)	70.9 (8.4)	73.0 (7.2)	76.1 (6.4)	79.7 (4.9)
Mean systolic blood pressure, mmHg	135 (20)	137 (20)	138 (18)	148 (19)	151 (20)
Use of blood pressure- lowering medication	1003 (20.5%)	817 (22.8%)	541 (20.8%)	660 (30.3%)	622 (53.4%)
Smoking					
Past	1997 (41.9%)	1525 (48.2%)	1366 (53.0%)	1291 (60.3%)	734 (63.3%)
Current	974 (20.4%)	669 (21.2%)	423 (16.4%)	273 (12.8%)	137 (11.8%)
Current use of alcohol	2940 (78.7%)	2336 (72.4%)	2136 (83.0%)	1798 (84.0%)	951 (81.9%)
Total cholesterol, mmol/L	6.6 (1.2)	6.6 (1.2)	5.8 (1.0)	5.6 (1.0)	5.3 (1.1)
High-density lipoprotein cholesterol, mmol/L	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)	1.5 (0.4)	1.5 (0.4)
Use of lipid-lowering medication	102 (2.1%)	79 (2.2%)	315 (12.1%)	433 (19.9%)	341 (29.3%)
Diabetes mellitus	336 (7.4%)	208 (6.2%)	267 (10.7%)	318 (14.6%)	141 (12.4%)
Body mass index, kg/m^2	26.2 (3.7)	26.3 (3.7)	26.7 (3.9)	27.2 (4.1)	27.2 (4.3)
Use of antithrombotic medication	224 (4.6%)	138 (3.9%)	621 (23.9%)	660 (30.3%)	453 (38.9%)

Class 2 (N=822)	N=813	N=596	N=324	N=187	N=48
Age at visit, years	64.6 (6.0)	65.7 (5.6)	68.7 (4.8)	73.5 (5.2)	78.7 (3.3)
Mean systolic blood pressure, mmHg	142 (23)	145 (24)	152 (23)	176 (26)	199 (15)
Use of blood pressure-lowering medication	223 (27.4%)	170 (28.5%)	93 (28.7%)	69 (36.9%)	24 (50.0%)
Smoking					
Past	310 (38.9%)	231 (42.5%)	141 (44.1%)	85 (46.7%)	28 (58.3%)
Current	286 (35.9%)	186 (34.3%)	102 (31.9%)	52 (28.6%)	6 (12.5%)
Current use of alcohol	565 (81.5%)	409 (74.6%)	259 (80.9%)	144 (79.1%)	40 (83.3%)
Total cholesterol	6.7 (1.2)	6.7 (1.2)	5.8 (1.0)	5.8 (1.0)	5.9 (1.2)
High-density lipoprotein cholesterol, mmol/L	1.3 (0.4)	1.3 (0.4)	1.3 (0.4)	1.4 (0.4)	1.5 (0.4)
Use of lipid-lowering medication, mmol/L	21 (2.6%)	15 (2.5%)	43 (13.3%)	35 (18.7%)	16 (33.3%)
Diabetes mellitus	57 (7.4%)	37 (6.5%)	47 (15.6%)	39 (20.9%)	11 (23.9%)
Body mass index, kg/m²	26.1 (3.8)	26.3 (3.8)	26.6 (4.3)	27.6 (4.7)	28.1 (4.4)
Use of antithrombotic medication	46 (5.7%)	25 (4.2%)	63 (19.4%)	47 (25.1%)	16 (33.3%)

Class 3 (N=870)	N=864	N=747	N=578	N=483	N=270
Age at visit, years	65.7 (7.1)	67.0 (6.6)	70.9 (6.3)	73.8 (5.2)	78.4 (3.9)
Mean systolic blood pressure, mmHg	158 (20)	159 (18)	162 (20)	166 (20)	161 (22)
Use of blood pressure-lowering medication	220 (25.5%)	243 (32.5%)	223 (38.6%)	295 (61.1%)	235 (87.0%)
Smoking					
Past	385 (45.1%)	350 (51.5%)	311 (54.7%)	285 (60.6%)	163 (60.6%)
Current	170 (19.9%)	129 (19.0%)	83 (14.6%)	53 (11.3%)	28 (10.4%)
Current use of alcohol	588 (79.7%)	527 (76.2%)	478 (84.0%)	390 (83.0%)	212 (78.5%)
Total cholesterol, mmol/L	6.8 (1.2)	6.8 (1.2)	5.8 (0.9)	5.5 (0.9)	5.1 (1.1)
High-density lipoprotein cholesterol, mmol/L	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)
Use of lipid-lowering medication	30 (3.5%)	31 (4.1%)	101 (17.5%)	132 (27.3%)	110 (40.7%)
Diabetes mellitus	63 (7.6%)	52 (7.3%)	90 (16.1%)	105 (21.7%)	57 (21.8%)
Body mass index, kg/m²	26.8 (3.6)	26.7 (3.5)	27.2 (3.8)	28.1 (4.0)	27.9 (4.1)
Use of antithrombotic medication	30 (3.5%)	27 (3.6%)	136 (23.5%)	172 (35.6%)	143 (53.0%)

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| Class 4 (N=115)	N=112	N=94	N=63	N=42	N=16
Age at visit, years	62.0 (5.0)	63.8 (4.6)	67.6 (4.5)	71.5 (4.5)	75.5 (2.5)
Mean systolic blood pressure, mmHg	174 (20)	170 (23)	156 (28)	148 (28)	142 (29)
Use of blood pressure- lowering medication	27 (24.1%)	37 (39.4%)	44 (69.8%)	36 (85.7%)	16 (100%)
Smoking					
Past	51 (45.9%)	44 (51.2%)	38 (62.3%)	26 (66.7%)	11 (68.8%)
Current	47 (42.3%)	31 (36.0%)	17 (27.9%)	9 (23.1%)	4 (25.0%)
Current use of alcohol	84 (84.0%)	69 (77.5%)	52 (85.2%)	33 (84.6%)	15 (93.8%)
Total cholesterol	6.7 (1.1)	6.6 (1.1)	5.7 (0.9)	5.2 (0.9)	4.9 (0.7)
High-density lipoprotein cholesterol, mmol/L	1.3 (0.4)	1.3 (0.4)	1.3 (0.3)	1.3 (0.3)	1.4 (0.4)
Use of lipid-lowering medication, mmol/L	3 (2.7%)	3 (3.2%)	13 (20.6%)	15 (35.7%)	6 (37.5%)
Diabetes mellitus	14 (13.0%)	11 (12.0%)	13 (21.3%)	11 (26.2%)	1 (6.7%)
Body mass index, kg/m^2	27.1 (3.7)	27.2 (3.7)	28.4 (5.4)	29.0 (5.4)	28.1 (3.6)
Use of antithrombotic medication	2 (1.8%)	2 (2.1%)	15 (23.8%)	24 (57.1%)	9 (56.3%)

Abbreviations: N = number of participants, SBP = systolic blood pressure.
Values are presented as mean (standard deviation) or counts (percentages).

Supplementary Table III. Cumulative incidences of stroke and competing causes of death for trajectories of blood pressure

Event	Class 1 (N=4938)	Class 2 (N=822)	Class 3 (N=870)	Class 4 (N=115)
Stroke				
Age 60	0.007 (0.002; 0.022)	0.52 (0.13; 1.78)	0.25 (0.02; 2.18)	4.83 (0.81; 23.7)
Age 70	0.71 (0.42; 1.20)	8.14 (4.06; 14.1)	4.74 (1.49; 12.3)	13.6 (3.92; 30.7)
Age 80	5.48 (4.17; 7.15)	19.3 (12.5; 26.7)	16.7 (9.71; 25.9)	18.4 (5.94; 37.7)
Age 90	15.3 (13.1; 17.5)	24.2 (17.1; 32.3)	28.8 (21.1; 36.7)	19.4 (6.37; 39.9)
Death				
Age 60	0.006 (0.003; 0.012)	3.73 (2.20; 5.86)	0.0004 (0.00003; 0.005)	0.39 (0.07; 1.67)
Age 70	1.37 (1.00; 1.89)	32.7 (26.5; 39.3)	0.49 (0.14; 1.53)	15.7 (7.54; 26.5)
Age 80	15.5 (13.3; 18.0)	62.5 (54.8; 69.9)	11.4 (6.51; 17.7)	56.9 (41.5; 69.4)
Age 90	53.7 (51.0; 56.5)	73.4 (65.1; 80.4)	52.5 (44.0; 60.8)	78.7 (58.9; 91.6)

Values are cumulative incidences (%) for the mean of covariates. Covariates are sex and use of blood pressure-lowering medication.
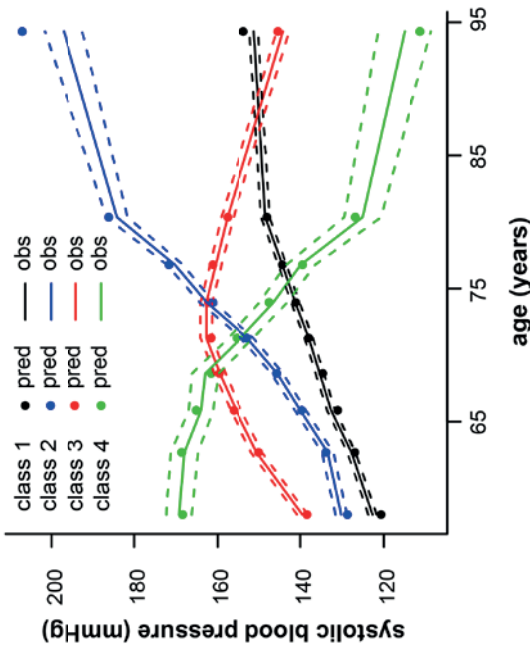
Supplementary Figure I. Comparison of model-based trajectories to class-specific mean values
This plot compares the model-based trajectories (dots) to the observed class-specific mean values (solid line) with 95% confidence intervals (dotted lines). It shows whether the predicted trajectories follow the observed values within the classes well.
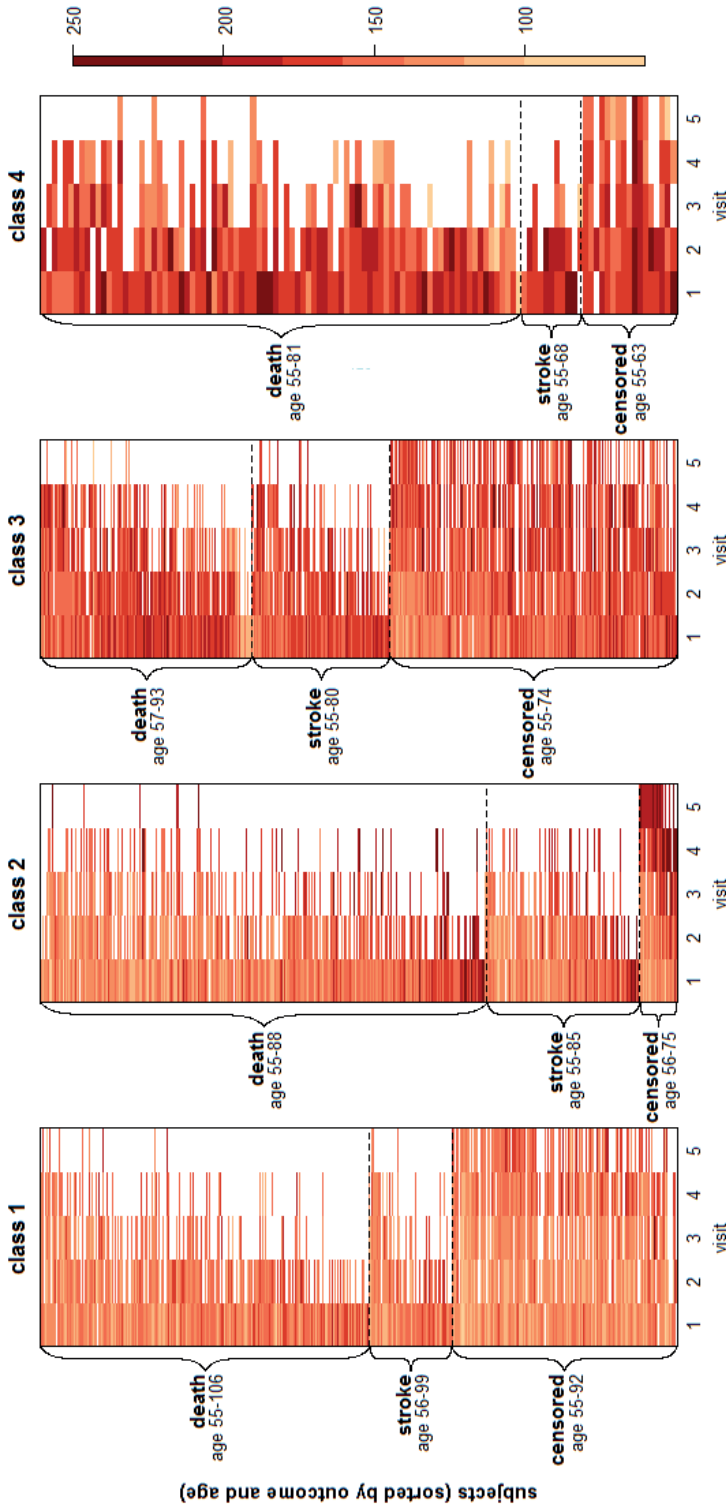
Supplementary Figure II. Lasagna plot of participants in trajectory groups, sorted by outcome

Rows represent individual subjects of the study. Colors are blood pressure values (in mmHg) darkening with increasing blood pressure, as described in the legend. The upper panel of each lasagna plot represents values in people that died, the middle panel of people that had a stroke, the lower of people that were censored. Within each panel participants were sorted by ascending age from top to bottom.

In class 1, 633 of 2546 non-stroke-related deaths (24.9%) were due to a cardiovascular cause; in class 2, 144 of 575 non-stroke-related deaths (25.0%) were cardiovascular; in class 3, 88 of 288 (30.6%); in class 4, 33 of 87 (37.9%).
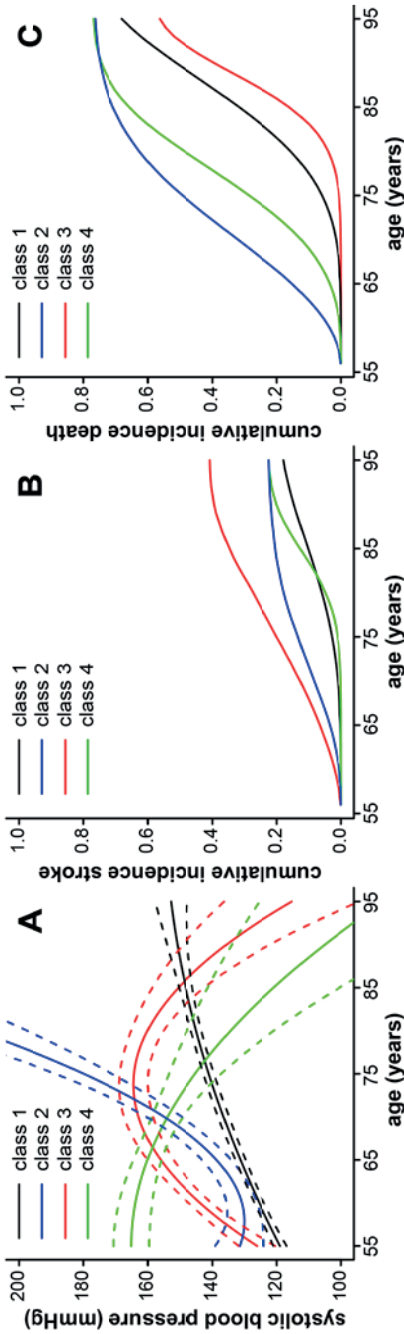
Supplementary Figure III. Trajectories of blood pressure and risk of stroke and competing causes of death, only including people with first three visits completed and starting follow-up time thereafter

A: trajectories of blood pressure, as predicted by the joint latent class mixed model. The solid line represents the average blood pressure in a class for the mean of covariates. Dotted lines represent confidence intervals. B: average class-specific cumulative incidence of stroke. C: average class-specific cumulative incidence of competing death due to any other cause than stroke. Cumulative incidences are plotted for the mean of covariates. Covariates are sex and blood-pressure lowering medication.
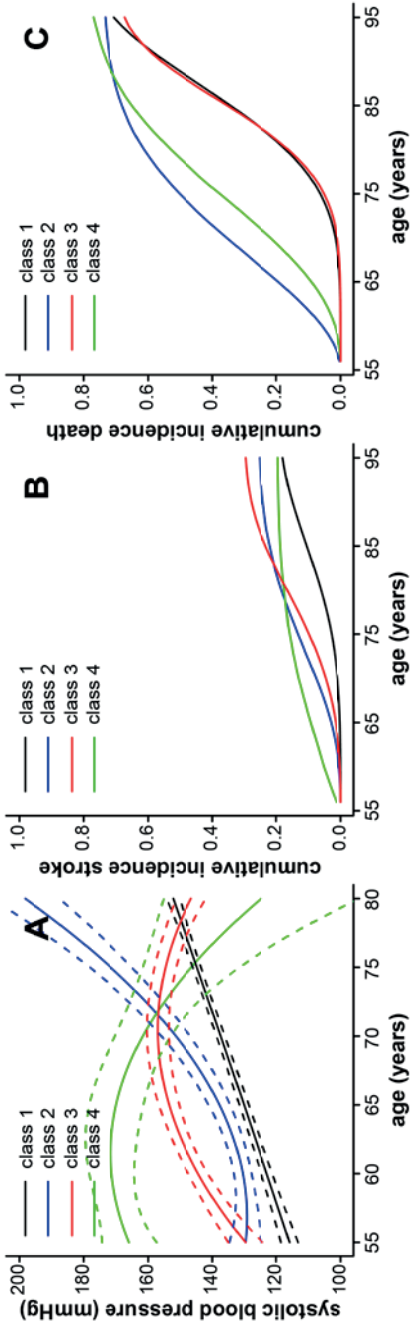
Supplementary Figure IV. Trajectories of blood pressure and risk of stroke and competing causes of death, only including measurements from age 55 to 80

A: trajectories of systolic blood pressure over time, as predicted by the joint latent class mixed model. The solid line represents the average blood pressure in a class for the mean of covariates. Dotted lines represent confidence intervals. B: average class-specific cumulative incidence of stroke. C: average class-specific cumulative incidence of competing death due to any other cause than stroke. Cumulative incidences are plotted for the mean of covariates. Covariates are sex and blood-pressure lowering medication.
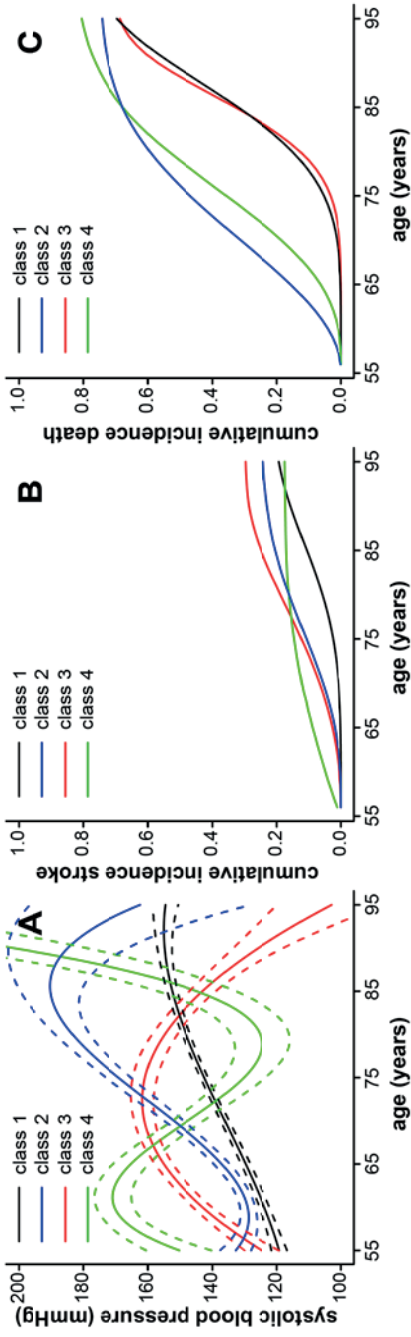
Supplementary Figure V. Trajectories of blood pressure and risk of stroke and competing causes of death, including a cubic age term
A: trajectories of systolic blood pressure over time, as predicted by the joint latent class mixed model. The solid line represents the average blood pressure in a class for the mean of covariates. Dotted lines represent confidence intervals. B: average class-specific cumulative incidence of stroke. C: average class-specific cumulative incidence of competing death due to any other cause than stroke. Cumulative incidences are plotted for the mean of covariates. Covariates are sex and blood-pressure lowering medication.

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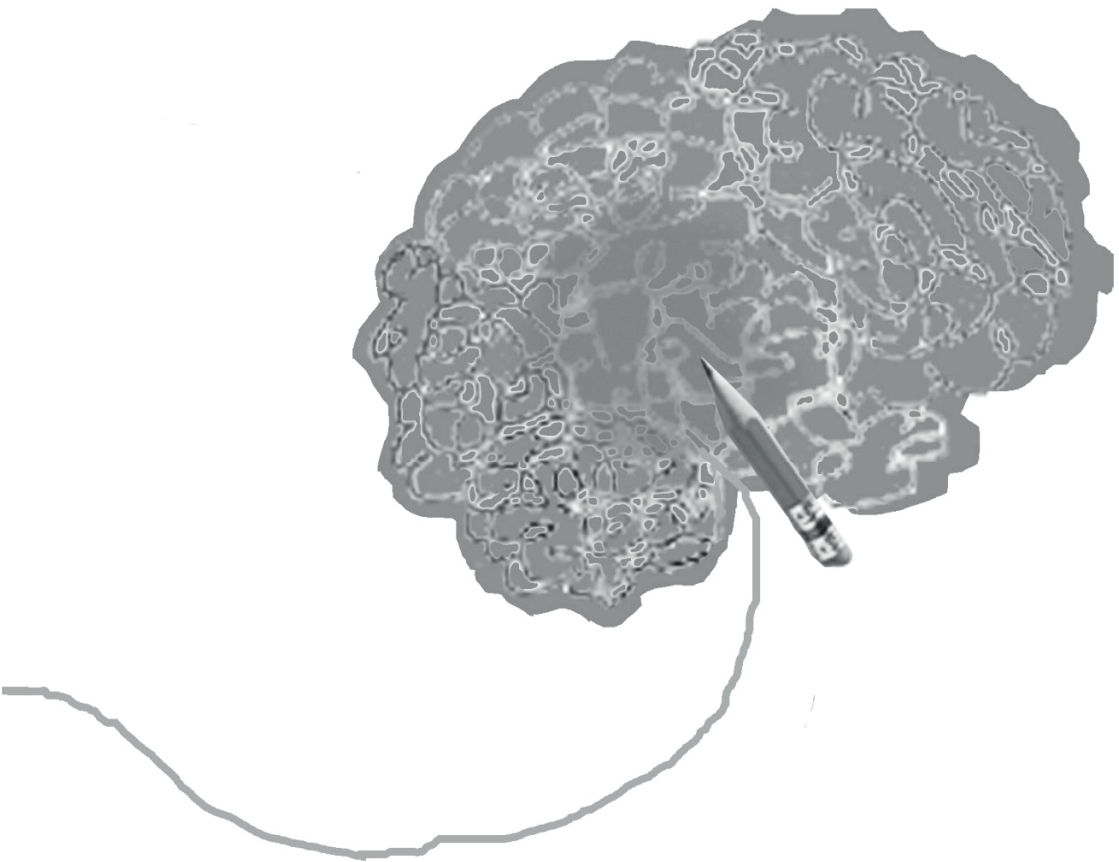
Chapter 4

Non-cardiovascular disease and stroke

Chapter 4.1

Chronic obstructive pulmonary disease and the risk of stroke

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Abstract

Background and purpose
Worldwide, chronic obstructive pulmonary disease (COPD) and stroke are leading causes of death. Increasing evidence suggests an association between both diseases, either caused by an increased atherosclerosis risk in patients with COPD, or as a consequence of shared risk factors between stroke and COPD. Our aim was to examine the associations between COPD and subtypes of stroke in the general population and to explore the role of cardiovascular risk factors and exacerbations on these associations.

Methods
Within the prospective population-based Rotterdam Study, we followed 13115 participants without history of stroke for occurrence of stroke. Follow-up started in 1990-2008 and ended in 2012. COPD was related to stroke using a time-dependent Cox proportional hazard model.

Results
COPD was diagnosed in 1566 participants. During 126347 person-years, 1250 participants suffered a stroke, of which 701 were ischemic and 107 hemorrhagic. Adjusted for age, age^2, and sex, COPD was significantly associated with all stroke (HR 1.20, 95% CI 1.00; 1.43), ischemic stroke (HR 1.27, 95% CI 1.02; 1.59), and hemorrhagic stroke (HR 1.70, 95% CI 1.01; 2.84). Adjusting for cardiovascular risk factors gave similar effect sizes. In contrast, additional adjusting for smoking attenuated the effect sizes: HR 1.09 (95% CI 0.91; 1.31) for all stroke, HR 1.13 (95% CI 0.91; 1.42) for ischemic stroke, and HR 1.53 (95% CI 0.91; 2.59) for hemorrhagic stroke. Following an acute severe exacerbation subjects with COPD had a 6.66-fold (95% CI 2.42; 18.20) increased risk of stroke.

Conclusions
Our cohort study demonstrated a higher risk of both ischemic and hemorrhagic stroke in subjects with COPD, and revealed the importance of smoking as a shared risk factor.

Introduction

Chronic obstructive pulmonary disease (COPD) and stroke are both leading causes of morbidity and mortality worldwide.[1,2] COPD is primarily characterized by persistent airflow limitation that is associated with an abnormal inflammatory response of the lungs to noxious particles or gases, but is also frequently associated with systemic effects and/or comorbidities. The most prevalent comorbidities in patients with COPD encompass hypertension, ischemic heart disease, chronic heart failure, osteoporosis, muscle weakness, depression, and lung cancer.[3-7] Since comorbidities have a major impact on the severity and prognosis of COPD, the recent ATS/ERS Research Statement on COPD launched an urgent call for studies to confirm or exclude associations between specific comorbidities and COPD.[8] In addition, studies to elucidate the pathobiological mechanisms linking COPD to its comorbidities are eagerly awaited.

A few previous population-based studies found associations between COPD and stroke.[9-13] However, important limitations were their cross-sectional design,[10-13] lack of information about possible confounders,[10,13] and absence of analysis of stroke subtypes.[9-13] Therefore, several important issues remain unanswered. First, it is unknown whether the observed associations between COPD and stroke are causal, for instance through inflammation[5,14] or hypoxia,[15,16] aggravated by COPD exacerbations,[17] or whether they are merely due to confounding by shared risk factors. Considering that smoking is the main risk factor for COPD and a risk factor for stroke, this might be an important confounder.[7,18] Other confounders could be cardiovascular risk factors and concomitant diseases such as diabetes and hypertension.[4,7,18] Second, it is unknown whether associations are only present for ischemic stroke, or also for hemorrhagic stroke, and whether they are present for different subtypes of ischemic stroke.[19] Given that the pathophysiology and preventive options for ischemic and hemorrhagic stroke are different, it is necessary to study associations with both subtypes separately.

Therefore, the aim of our prospective population-based study was to examine the associations between COPD and stroke, taking into account the different subtypes of stroke. Furthermore, to gain more insight into the pathophysiology of these associations we investigated the role of smoking, other cardiovascular risk factors, serum high-sensitivity C-reactive protein (hs-CRP), carotid intima-media thickness (cIMT), and exacerbations.

Methods

Setting and study population
This study was conducted within the Rotterdam Study, a large prospective population-based cohort study, which aims to assess incidence of, and risk factors for chronic diseases in the elderly. Initially, from 1990 onwards 7983 persons aged 55 years and older and living in the Ommoord district in the city of Rotterdam were included (Rotterdam Study I). The cohort was extended in 2000 with 3011 participants who moved into the study district or had become 55 years or older since the start of the study (Rotterdam Study II). In 2006, the study was further extended with 3932 persons that were aged 45 years and older (Rotterdam Study III). Follow-

up examinations take place every three to four years. Details regarding the objective and design of the Rotterdam Study have been described elsewhere.[20]

The study population consisted of participants who gave informed consent for follow-up and had no history of stroke at baseline. Patients with physician diagnosed asthma or potential asthma-COPD overlap syndrome (ACOS) were excluded. ACOS was defined as a physician diagnosis of COPD in subjects with previously diagnosed asthma. The Rotterdam Study has been approved by the medical ethics committee of the Erasmus MC, Rotterdam (02/2015), and the review board of The Netherlands Ministry of Health, Welfare and Sports (3098760). A written informed consent was obtained from all participants.

Assessment of COPD

The diagnosis of COPD was based on an obstructive spirometry (proportion of the forced vital capacity exhaled in the first second (FEV_1/FVC) < 0.7, assessed pre-bronchodilator) during the research center visits or in absence of spirometry, on information collected continuously from medical records of general practitioners and lung physicians.[21,22] All participants with physician-diagnosed COPD were validated through thorough examination of medical files and specialists' letters. Date of incident COPD was defined as the date of obstructive lung function examination, the date of COPD diagnosis in the medical records, or the date of a first COPD medication prescription in someone with established COPD, whichever came first. Medication use was obtained through automated linkage with pharmacy filled prescription data. The follow-up for COPD was complete until January 1st, 2012 for 99.1% of potential person-years. Patients were classified as having frequent exacerbations if they had on average and rounded two or more moderate or severe exacerbations a year. Moderate exacerbations were defined as needing a course of steroids and/or antibiotics and severe exacerbations as needing hospitalization.[23] The high-risk period following an exacerbation was defined as 1 day to 7 weeks after start of the exacerbation, conform a previous study.[17] Follow-up for exacerbations was complete until December 31st, 2010.

The severity of COPD was assessed in people with an interpretable lung function test at the research center in 2002-2008 (n=4141). We used the GOLD 2013 criteria,[24] including the percentage predicted of the forced expiratory volume in 1s (FEV1), the exacerbation rate, and the grade of dyspnea. Dyspnea score was based on 5 dyspnea-questions and scored from 0 (never dyspneic) to 5 (even dyspneic at rest).

In a subgroup of 1158 participants with lung function measurements between 2009-2012, we measured the diffusion capacity of the lungs using carbon monoxide (DL_{CO}) and corrected for the haemoglobin concentration ($DL_{CO,c}$).

Assessment of stroke

Stroke was defined according to the World Health Organization criteria as syndrome of rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or leading to death, with no apparent cause other than of vascular origin.[25] Prevalent stroke at baseline was assessed using the home interview and verified by reviewing medical records. From baseline onwards, all participants were continuously followed-up for

occurrence of stroke through automatic linkage of general practitioners' medical records with the study database. Additionally, nursing home physicians' medical records and medical records from general practitioners of participants who moved out of the Ommoord district were inspected on a regular basis. Research physicians reviewed potential strokes using hospital discharge letters and information from general practitioners and an experienced neurologist verified the stroke diagnoses.[26] Strokes were subclassified in ischemic or hemorrhagic stroke based on neuroimaging reports. If no neuroimaging was performed, strokes were classified as unspecified. Ischemic strokes were further subclassified in two ways. They were subclassified in cortical or lacunar ischemic stroke, based on neuroimaging reports or clinical symptoms.[27] Furthermore, they were subclassified according to TOAST criteria based on the diagnostic workup mentioned in medical records.[28] The follow-up for stroke was complete until January 1st, 2012 for 96.3% of potential person-years.

Covariates

Covariates were measured at baseline of each cohort. Smoking status and medication use were assessed during a home interview. Smoking was categorized into current, past, and never smoking. Cigarette pack-years were calculated as the duration of smoking in years, multiplied by the number of smoked cigarettes, divided by 20.[22] Additionally, cardiovascular risk factors were measured during a visit at the study center.[29] Blood pressure was measured twice in sitting position on the right arm with a random-zero sphygmomanometer. The average of the two consecutive measurements was used in the analyses. Hypertension was defined as a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg and/or the use of blood pressure-lowering medication with the indication hypertension. Pulse oxygen saturation (SaO2) was measured twice on the right index finger. The average of the two measurements was used in the analysis. A low pulse oxygen saturation was defined as ≤95%. Total cholesterol, high-density lipoprotein (HDL) cholesterol, and hs-CRP were measured in serum. Diabetes mellitus was defined as having an overnight fasting glucose level 7.0 mmol/L or higher, a non-fasting glucose level of 11.0 mmol/L or higher, or use of antidiabetic medication. Carotid intima-media thickness (cIMT) was measured by ultrasonography of the left and right carotid arteries, performed with a 7.5 MHz linear array transducer (ATL UltraMark IV; Advanced Technology Laboratories, Bethel, Washington). The maximal cIMT, which is the mean of maximal measurements from the near and far walls of both the left and right sides, was used for analysis.[29] The presence of atrial fibrillation at baseline was based on medical records from general practitioners and on the ECG performed during the baseline visit at the study center.[30]

Statistical analyses

Analysis of covariance was used to study differences in baseline characteristics between subjects with incident versus prevalent COPD and between subjects with COPD (incident or prevalent) versus subjects without COPD during follow-up, adjusted for age and sex. Associations between COPD and stroke were evaluated using Cox proportional hazards regression. COPD was added as time-varying covariate in the analysis, which captured persons

with prevalent COPD at baseline and persons that developed COPD during follow-up. Participants were censored at date of stroke, date of death, last date of follow-up, or January 1st, 2012, whichever came first. We examined associations of COPD with both ischemic and hemorrhagic stroke and additionally with cortical or lacunar ischemic stroke and TOAST subclassifications of ischemic stroke. All models were adjusted for age, age squared (age^2), and sex. To determine effects of inflammation, cardiovascular risk factors, and in particular smoking on the associations, we constructed the following consecutive models: model I adjusted for age, age^2 and sex; model II adjusted for age, age^2, sex, and CRP; model III adjusted for age, age^2, sex, and cIMT; model IV adjusted for age, age^2, sex, and hypertension; model V adjusted for age, age^2, sex, and anticoagulant or antiplatelet medication; model VI adjusted for age, age^2, sex, and the cardiovascular risk factors: systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication, total cholesterol, HDL cholesterol, lipid-lowering medication, diabetes mellitus, BMI, and atrial fibrillation; model VII adjusted for age, age^2, sex, and smoking; model VIII adjusted for age, age^2, sex, and pack-years, and model IX adjusted for all covariates. In a subgroup of participants we also adjusted for pulse oxygen saturation. CRP was natural log transformed because of skewed distribution.

As additional analysis we related COPD with stroke categorizing COPD as diagnosed based on lung function or medical records and analyzed the risk of COPD in the participants with ACOS that were excluded for the other analyses. Furthermore, we categorized COPD as with few or many exacerbations and related that to the risk of stroke. In another analysis we examined the risk of stroke during the high-risk period of an exacerbation itself. This high risk period was defined conform an earlier study as starting one day after the start of an exacerbation, until 7 weeks after the exacerbation.[17] To examine the risk of stroke in this period, we added exacerbations as time-dependent covariate to the Cox proportional hazards models, in which people were considered at high risk starting one day after the start of an exacerbation, until 7 weeks after the exacerbation. If subjects had multiple exacerbations they were considered at risk for 7 weeks after each of those. The reference period was formed by the remaining follow-up time in which subjects did not suffer from exacerbations. For this analysis we only included participants with COPD and censored them at date of stroke, date of death, last date of follow-up, or February 19th (7 weeks after the end of exacerbation follow-up), whichever came first.

In a subgroup of people with lung function in 2002-2008, we examined the risk of stroke according to the severity of COPD as measured by the GOLD criteria and according to the severity of airflow limitation as measured by FEV1.

Missing data on covariates (for cIMT 21.5%, for all covariates 14.1% or less) were imputed based on the other covariates using multiple imputation with 5 imputation sets.

We constructed cumulative incidence curves according to the Kaplan-Meier method, starting at date of COPD for the people with COPD and at baseline for the reference population.

To examine the impact of cardiovascular risk factors for the risk of stroke in subjects with COPD, we calculated the population attributable risks (PARs) using the Interactive Risk Attributable Program version 2.2 (US National Cancer Institute),[31] conform a previous study.[32] This program calculates hazard ratio's (HR's) with 95% CI based on a Poisson model and enables

the calculation of PARs with 95% CI. We calculated the PARs in people with prevalent COPD at baseline, because at this time the risk factors were assessed. We assessed the PARs of the major risk factors for stroke, namely hypertension, smoking, diabetes mellitus, and atrial fibrillation, and of the combination of these risk factors, adjusted for age, age^2, sex, and the other risk factors if applicable. For the missing data, the mean of the 5 imputation sets as described above was used.

All analyses were done using IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY), R Statistical Software version 3.1.2 (The R Foundation for Statistical Computing, Vienna, Austria), and IRAP version 2.2 (US National Cancer Institute).

Results

General characteristics
Participants who did not give informed consent for collection of follow-up data (n=238), or had a history of stroke at baseline (n=453), or a history of asthma or Asthma and COPD Overlap Syndrome (ACOS) at baseline (n=1120) were excluded. Consequently, 13115 participants were eligible for analysis (*Figure 1*).
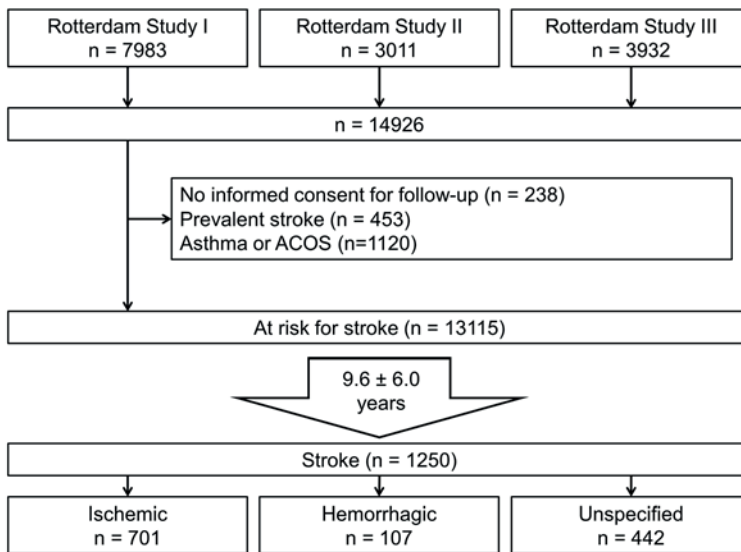
Figure 1. Study population

Baseline characteristics of the study population are presented in *Table 1*. Follow-up duration was on average 9.6 (±6.0) years. 538 participants had COPD at baseline and 1028 participants developed COPD during follow-up. 1298 (82.9%) diagnoses were based on spirometry performed either at the research center (868 cases since January 2002) or by a pulmonologist

(430 subjects). In 268 (17.1%) subjects the diagnosis of COPD was made by their general practitioner. The mean age at baseline (±SD) was 67.0 years (±9.3) for persons with prevalent COPD, 65.2 (±7.4) for persons who developed COPD during follow-up, and 65.8 (±10.6) for persons without COPD. Persons with COPD were more often male and (current) smokers.

In the subgroup of participants with lung function measurements in 2002-2008, we found that the mean FEV1 was 110% (±18%) in participants without COPD, 76% (±20%) in participants with prevalent COPD and 100% (±22%) in participants who developed COPD during follow-up.

1250 subjects suffered a stroke during follow-up. 701 strokes were classified as ischemic stroke, and 107 as hemorrhagic stroke. In the 442 participants in whom no neuroimaging was performed, their stroke was classified as unspecified. Of all ischemic strokes, 40.2% was classified as cortical, and 7.3% was classified according to the TOAST criteria as based on large artery atherosclerosis.

Table 1. Baseline characteristics

	No COPD N=11549	Prevalent COPD N=538	Incident COPD N=1028
Age, years	65.8 (10.6)	67.0 (9.3)[ab]	65.2 (7.4)[a]
Female	6971 (60.4%)	250 (46.5%)[a]	449 (43.7%)[a]
Smoking			
Never	4052 (35.9%)	80 (15.0%)[a]	151 (14.8%)[a]
Past	4797 (42.5%)	240 (44.9%)[b]	410 (40.2%)[a]
Current	2449 (21.7%)	214 (40.1%)[a]	459 (45.0%)[a]
Pack-years for cigarette smokers	22.9 (22.2)	37.0 (26.8)[a]	34.5 (24.3)[a]
Systolic blood pressure, mmHg	138 (22)	139 (21)	137 (21)
Diastolic blood pressure, mmHg	77 (12)	77 (12)[b]	76 (12)[a]
Use of blood pressure-lowering medication	2573 (22.4%)	120 (22.3%)	207 (20.2%)
Hypertension	5964 (56.0%)	278 (57.1%)	519 (52.4%)
Total cholesterol, mmol/L	6.2 (1.2)	6.1 (1.2)[b]	6.3 (1.2)[a]
HDL-cholesterol, mmol/L	1.37 (0.39)	1.43 (0.44)[ab]	1.34 (0.36)
Use of lipid-lowering medication	1047 (9.1%)	62 (11.6%)[ab]	74 (7.2%)[a]
Use of anticoagulant drugs	345 (3.0%)	23 (4.3%)	32 (3.1%)
Use of antiplatelet drugs	587 (5.1%)	44 (8.2%)[a]	61 (5.9%)
Diabetes mellitus	922 (9.1%)	55 (11.9%)[b]	67 (7.1%)[a]
Body mass index	26.9 (4.0)	26.4 (4.1)[a]	26.0 (3.8)[a]
Atrial fibrillation	405 (4.1%)	33 (7.3%)[ab]	31 (3.3%)
Carotid intima-media thickness	0.98 (0.20)	1.03 (0.22)[a]	1.01 (0.19)[a]
hs–CRP, mg/L[c]	1.5 (0.6 – 3.1)	2.2 (0.9 – 4.3)[ab]	1.8 (0.8 – 3.7)[a]
Pulse oxygen saturation <95%	250 (13.2%)	26 (25.0%)[a]	27 (16.5%)

Abbreviations: N = number of persons included in study; HDL = high-density lipoprotein; hs-CRP = high-sensitivity C-reactive protein.

Data are presented as mean (standard deviations) or counts (percentages).

[a] Significantly different between persons with and without COPD, after sex and age adjustment – if applicable.

[b] Significantly different between persons with prevalent and incident COPD, after sex and age adjustment – if applicable.

[c] Median and inter-quartile range because of skewed distribution.

Association between COPD and the risk of stroke

In Figure 2 we show associations between COPD and the risk of stroke. Adjusting for age, age squared (age^2), and sex, we found a significant association of COPD with all stroke (HR 1.20, 95% CI 1.00; 1.43), ischemic stroke (HR 1.27, 95% CI 1.02; 1.59), and hemorrhagic stroke (HR 1.70, 95% CI 1.01; 2.84) (Figure 2, Figure 3). Associations were slightly, but not significantly weaker in subjects with COPD diagnosed based on spirometry (HR 1.16, 95% CI 0.95; 1.42) compared to COPD subjects diagnosed based by their general practitioner (HR 1.31, 95% CI 0.95; 1.82).

All stroke

	Hazard Ratio
Basic: age, age^2, and sex	1.20 [1.00 , 1.43]
Basic + hs-CRP	1.17 [0.98 , 1.39]
Basic + cIMT	1.16 [0.97 , 1.39]
Basic + hypertension	1.21 [1.01 , 1.44]
Basic + antithrombotics	1.19 [1.00 , 1.42]
Basic + CV risk factors	1.21 [1.01 , 1.44]
Basic + smoking	1.09 [0.91 , 1.31]
Basic + packyears	1.09 [0.91 , 1.30]
Full model	1.07 [0.89 , 1.27]

Ischemic stroke

	Hazard Ratio
Basic: age, age^2, and sex	1.27 [1.02 , 1.59]
Basic + hs-CRP	1.24 [0.99 , 1.55]
Basic + cIMT	1.23 [0.98 , 1.54]
Basic + hypertension	1.29 [1.03 , 1.61]
Basic + antithrombotics	1.27 [1.01 , 1.58]
Basic + CV risk factors	1.29 [1.04 , 1.62]
Basic + smoking	1.13 [0.91 , 1.42]
Basic + packyears	1.14 [0.91 , 1.43]
Full model	1.10 [0.88 , 1.38]

Hemorrhagic stroke

	Hazard Ratio
Basic: age, age^2, and sex	1.70 [1.01 , 2.84]
Basic + hs-CRP	1.69 [1.00 , 2.83]
Basic + cIMT	1.69 [1.01 , 2.83]
Basic + hypertension	1.71 [1.02 , 2.85]
Basic + antithrombotics	1.67 [1.00 , 2.80]
Basic + CV risk factors	1.64 [0.98 , 2.74]
Basic + smoking	1.53 [0.91 , 2.59]
Basic + packyears	1.57 [0.93 , 2.66]
Full model	1.45 [0.86 , 2.45]

Hazard Ratio (95%CI): 0.75 1.00 2.00 4.00

Figure 2. COPD and the risk of stroke (N=13115)
Values are hazard ratios with 95% confidence intervals. Hs-CRP = high-sensitivity C-reactive protein, cIMT = carotid intima-media thickness, CV = cardiovascular. All models were adjusted for age, age^2, and sex. Antithrombotics include both antiplatelet drugs and anticoagulants. Cardiovascular risk factors are systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication, diabetes mellitus, body mass index, and atrial fibrillation.
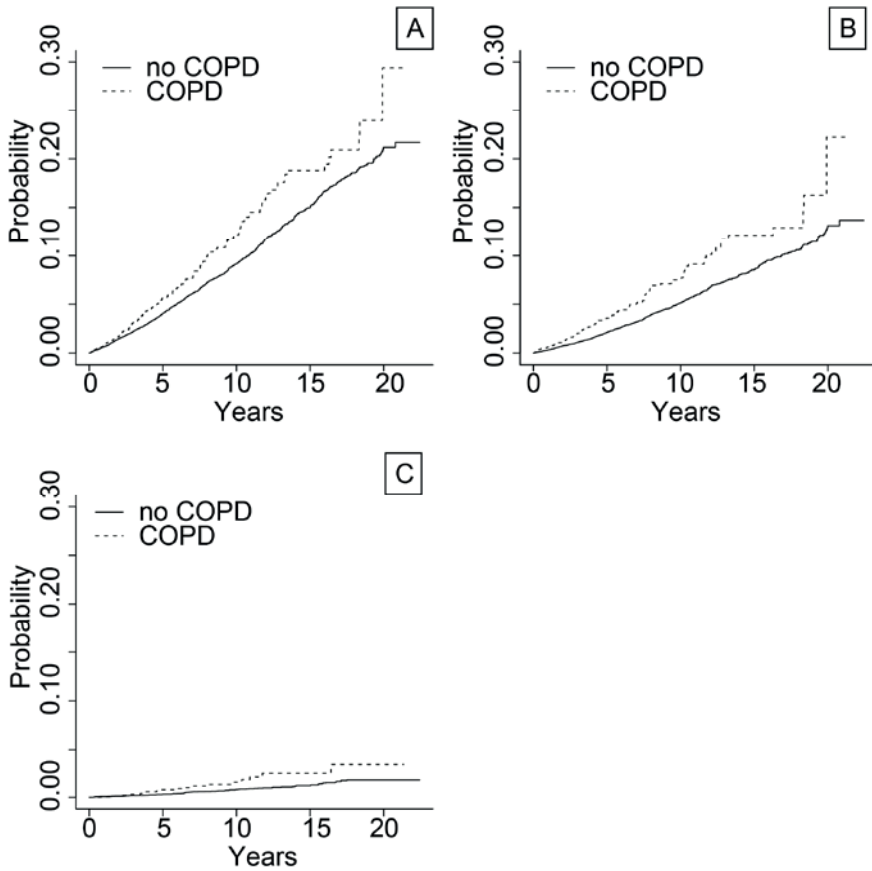
Figure 3. Cumulative incidence curve of stroke according to COPD status
A: all stroke, B: ischemic stroke, C: hemorrhagic stroke.

Influence of covariates

Adjusting for serum hs-CRP as marker for systemic inflammation or cIMT as marker for carotid atherosclerosis both led to a modest change of the associations (HR for all stroke additionally adjusted for hs-CRP 1.17, 95% CI 0.98; 1.39, HR for all stroke additionally adjusted for cIMT 1.16, 95% CI 0.97; 1.39) (Figure 2). Adjustment for hypertension or all cardiovascular risk factors other than smoking did not change the associations. Adjusting for pulse oxygen saturation in a subgroup had a modest effect (data not shown). However, additional adjusting for smoking clearly attenuated the associations: HR all stroke 1.09 (95% CI 0.91; 1.31), ischemic stroke 1.13 (95% CI 0.91; 1.42), hemorrhagic stroke 1.53 (95% CI 0.91; 2.59). Adjustment for cumulative smoking exposure (i.e. pack-years) had a similar effect as adjustment for smoking status. Although not statistically significant, effect sizes of COPD and ischemic stroke were higher for persons with a cortical stroke (HR adjusted for age, age^2, sex, and smoking 1.36, 95% CI 0.97;

1.90) and persons with an ischemic stroke based on large artery atherosclerosis (HR adjusted for age, age^2, sex, and smoking 1.78, 95% CI 0.89; 3.57).

In subjects with COPD, similar to what was observed in the general population,[32] we found that hypertension was the most important modifiable risk factor, with a PAR of 0.30 (95% CI 0.09; 0.65), adjusted for age, age^2, sex, smoking, diabetes, and atrial fibrillation (Supplementary Table I).

Influence of exacerbations

The stratification for frequent exacerbators did not show a significantly higher risk in COPD subjects with a mean of two or more exacerbations a year (HR adjusted for age, age^2, sex and smoking 1.13, 95% CI 0.80; 1.59), compared to COPD subjects without exacerbations or less than two a year (HR 1.06, 95% CI 0.86; 1.29) (Supplementary Table II).

Interestingly, we observed that 22 of the 145 strokes that occurred in patients with COPD, occurred within 7 weeks after the start of an acute exacerbation of COPD. 11 strokes were classified as ischemic and 11 as unspecified. Among 1530 subjects with COPD, 329 COPD subjects did not suffer from any exacerbation during follow-up. The remaining 1201 COPD subjects had a total of 11904 exacerbations. Stratified for severity of exacerbations we found that 11373 moderate exacerbations occurred in 1197 subjects and 531 severe exacerbations in 268 subjects. The risk of any stroke following an acute exacerbation period (within 7 weeks after start of the exacerbation) was not significantly increased compared to a stable period (HR 1.28, 95% CI 0.81; 2.02). Importantly, the risk of stroke after a severe exacerbation of COPD was significantly higher than during stable disease (HR 6.66, 95% CI 2.42; 18.20), adjusted for age, age^2, sex and smoking (Table 2).

Table 2. The risk of stroke within 7 weeks after the onset of an acute exacerbation in patients with COPD compared to stable disease

	Stroke n/N^a 142/1530	
	Model I	Model II
No exacerbation / stable period	1 (reference)	1 (reference)
Exacerbation period (n = 11904)^b	1.30 (0.82; 2.05)	1.28 (0.81; 2.02)
Moderate exacerbation period (n = 11373)^b	1.10 (0.67; 1.81)	1.09 (0.66; 1.79)
Severe exacerbation period (n = 531)^b	7.02 (2.57; 19.18)	6.66 (2.42; 18.20)

^{a} n = number of strokes; N = number of persons included in study.
^{b} n = number of exacerbations.
Values are hazard ratios with 95% confidence intervals.
Model I: Adjusted for age, age^2, and sex.
Model II: Adjusted for age, age^2, sex, and smoking.

Influence of severity

With increasing severity of airflow limitation as measured by FEV1, we found an increased risk of stroke (Supplementary table III). When we combined the severity of airflow limitation with exacerbation rate and grade of dyspnea, we found that the risk of stroke was increased in subjects with GOLD classification A and was even higher in subjects with GOLD classification B

(Table 3). For the classes C and D we did not find significant results, although for class C the effect size was similar to class B. The risk was not further increased for COPD subjects belonging to group D.

Table 3. COPD severity based on the GOLD 2013 classification and the risk of stroke.

	Stroke		
	n/N	Model I	Model II
No COPD	91/3589	1 (reference)	1 (reference)
GOLD A	19/308	1.74 (1.06; 2.87)	1.64 (0.99; 2.71)
GOLD B	14/146	2.32 (1.31; 4.11)	2.13 (1.20; 3.79)
GOLD C	3/34	2.38 (0.75; 7.60)	2.07 (0.65; 6.63)
GOLD D	4/64	1.62 (0.59; 4.44)	1.43 (0.86; 2.40)

Abbreviations: n = number of cases; N = number of persons included in study.
Values are hazard ratios with 95% confidence intervals.
Model I: Adjusted for age, age^2, and sex.
Model II: Adjusted for age, age^2, sex, and smoking.
GOLD A: mild/moderate airflow limitation, exacerbation rate <2/year, dyspnea < grade 2.
GOLD B: mild/moderate airflow limitation, exacerbation rate <2/year, dyspnea >= grade 2.
GOLD C: severe/very severe airflow limitation or exacerbation rate >=2/year, or severe exacerbation rate >=1/year, and dyspnea < grade 2.
GOLD D: severe/very severe airflow limitation or exacerbation rate >=2/year, or severe exacerbation rate >=1/year, and dyspnea >= grade 2.

Discussion

In this large prospective population-based cohort study, we found that COPD was associated with a higher risk of ischemic stroke as well as hemorrhagic stroke. Associations between COPD and stroke were no longer significant after adjusting for smoking, although effect sizes remained high for cortical ischemic stroke, hemorrhagic stroke and ischemic stroke based on large artery atherosclerosis. Furthermore, we found that subjects with COPD were significantly at higher risk of stroke following severe exacerbations.

Our results showed that people with COPD have an approximately 20% increased risk of stroke, which was similar to findings from previous studies.[10-13] Only one study found a much stronger risk, but this was driven by the younger age groups until 54 years.[9]

However, the association between COPD and stroke is weaker than expected in the light of our previous findings. For instance, carotid artery plaques were twice as frequent in subjects with COPD, whereas we only found an overall 20% increased risk of stroke.[22] Competing risks might partially explain this discrepancy. Previous studies showed that in people with COPD the risk of cardiovascular disease is higher than the risk of stroke, implicating that either COPD subjects die due to a cardiac cause before a stroke might occur, or the cardiovascular treatment started for cardiac disease might prevent subsequent stroke.[9-13,23] Yet, although not statistically significant after adjustment for smoking, the hazard ratio of COPD on ischemic stroke based on large artery atherosclerosis was 1.8 and on cortical ischemic stroke 1.4, suggesting that there might be link between COPD and ischemic stroke through large vessel disease.

An important novelty of our study is that we examined for the first time associations with hemorrhagic stroke. Most important causes of hemorrhagic strokes are hypertension, small vessel disease, and use of anticoagulants.[33] Adjustment for hypertension or anticoagulants at baseline did not change our association, suggesting independent effects. This indicates small vessel disease as a likely explanation, which is further supported by our previous finding that COPD was related to microbleeds, with a similar OR of 1.7.[34] Nevertheless, residual confounding too remains a possibility, either due to single measurements versus lifelong exposures or therapy initiation after baseline.

In summary, our results support findings of previous studies that people with COPD have higher risk of both large vessel[22,35] and small vessel[34,36,37] damage.

Still, the question remains: What is the underlying mechanism of the association between COPD and stroke? Our results indicate that smoking is the strongest explanatory factor since effect estimates remained more or less constant after adjustment for other cardiovascular risk factors, but were highly attenuated after adjustment for smoking. The HR for stroke in people with COPD decreased from 1.19 to 1.09 and became non-significant. Although one previous study still found an OR of 1.6 (95% CI 1.1; 2.3),[11] our results were in line with two other studies that did not find a significant effect of COPD on stroke in older subjects after adjusting for smoking.[9,12] Since the effect size of COPD independent of smoking is quite small and the prevalence of COPD is lower than prevalence of hypertension or smoking, presence of COPD adds little to the global population attributable risk of stroke.[32]

Nonetheless, we still found effect estimates varying from 1.4 to 1.8 after adjustment for smoking, namely for associations of COPD with hemorrhagic stroke, and with ischemic strokes that were cortical or from the atherosclerotic subtype. This could mean that there still is residual confounding by smoking or that smoking is not the only explanatory factor and that the potential causal role of other explanatory factors like inflammation should be further investigated. Especially during COPD exacerbations, pulmonary inflammation can lead to systemic inflammation,[5] eliciting instable atherosclerotic plaques and a pro-thrombotic state, and eventually a stroke.[14,38,39] This is supported by recent studies showing that in patients with COPD carotid arterial plaque burden is increased and more prone to rupture, independent from smoking status.[22,40,41] In our current study we could not find strong evidence for an effect of chronic systemic inflammation, as we only found a modest attenuation of the association between COPD and stroke after adjusting for serum hs-CRP at baseline. This was in line with a previous study on lung function and the risk of stroke in which adjustment for hs-CRP did not change the effect.[42] However, hs-CRP at baseline might not be representative for the systemic inflammation that occurs during follow-up and during exacerbations. Importantly, following an acute severe exacerbation the risk of stroke was sixfold higher than during stable disease, suggesting that acute inflammation might lead to a higher risk of stroke. Otherwise, this might be a result of hypoxia during exacerbations. Hypoxia could lead to endothelial dysfunction which might predispose to stroke.[15,16,43] We could not confirm or refute this possibility, because we only had information about hypoxia at baseline in a limited group of participants and not during exacerbations. However, we did find that participants with GOLD class B had a higher risk of stroke compared to class A, suggesting that dyspnea might lead to a higher risk. We did

not find the same for class D, but this might again be explained by the low numbers (i.e. lack of power) or competing risks. People with very severe COPD might indeed die before a stroke can occur.

More research is necessary to define whether associations are completely due to the shared risk factor smoking or whether other mechanisms such as systemic inflammation or hypoxia still play a role. Furthermore, future studies should define if, and to what extent, ischemic and hemorrhagic stroke share the same underlying mechanisms. In addition to smoking cessation, further research into preventive options in patients with COPD, such as aggressive control of cardiovascular risk factors such as hypertension, is necessary to reduce the risk of stroke. Hypertension remains, similar as in subjects without COPD, the most important modifiable risk factor for stroke.

The strengths of our study are the longitudinal design with long-term follow-up, the population-based setting, and the thorough prospective collection of COPD and stroke information during follow-up. This study also has limitations. We adjusted for baseline covariates, whereas the status of those variables might change during follow-up. This might have led to residual confounding. Moreover, we did not adjust for seasonality. Although our stroke data did not show a clear seasonal pattern, winter and cold weather are associated with an increased risk of exacerbations[44] and might increase risk of stroke because of hemoconcentration due to peripheral vasoconstriction and a raised blood pressure.[45] Another limitation is that not all strokes could be subclassified in ischemic or hemorrhagic, and not all ischemic strokes into lacunar or cortical or according to etiologic cause based on the TOAST criteria, because we were limited by the diagnostic workup performed in stroke cases. Furthermore, not all COPD cases were identified similarly. Some COPD cases were diagnosed based on spirometry either pre-bronchodilator at the research center or by the pulmonologist, others were diagnosed by their general practitioner. This might have led to some misclassification. However, associations with stroke in both groups were not significantly different.

In conclusion, we found that COPD was associated with both ischemic and hemorrhagic stroke. Our study reveals the importance of smoking as shared risk factor and implicates that clinicians should be aware of the higher risk of both stroke subtypes in subjects with COPD, especially following severe exacerbations.

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Supplementary information

Supplementary Table I. Population attributable risks of presumed etiologic factors for stroke in patients with COPD at baseline (n=538)

Risk factors	Classification	Prevalence (%)	HR (95% CI)	PAR (95% CI)
Hypertension		57.1%	1.77 (0.94; 3.33)	0.30 (0.09; 0.65)
Smoking	Past	45.4%	0.91 (0.39; 2.17)	0.03 (0.00; 1.00)
	Current	39.8%	1.23 (0.51; 2.97)	
Diabetes mellitus		10.8%	1.46 (0.63; 3.37)	0.05 (0.00; 0.41)
Atrial fibrillation		6.9%	1.03 (0.36; 2.96)	0.003 (0.00; 1.00)
Total				0.35 (0.06; 0.81)

Abbreviations: PAR = population attributable risk; HR = hazard ratio.
Values are hazard ratios (95% confidence intervals) or population attributable risks (95% confidence intervals). All analyses were adjusted for age, age^2, sex, hypertension, smoking, diabetes mellitus, and atrial fibrillation, where appropriate.

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Supplementary Table II. COPD exacerbations and the risk of stroke

	Stroke n/N 1248/12983		Ischemic stroke n/N 699/12983		Hemorrhagic stroke n/N 107/12983	
	Model I	Model II	Model I	Model II	Model I	Model II
No COPD	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
COPD <2 exacerbations	1.15 (0.94; 1.40)	1.06 (0.86; 1.29)	1.24 (0.96; 1.59)	1.11 (0.86; 1.43)	1.94 (1.13; 3.34)	1.76 (1.02; 3.05)
COPD >= 2 exacerbations	1.28 (0.91; 1.79)	1.13 (0.80; 1.59)	1.28 (0.83; 1.98)	1.10 (0.71; 1.70)	0.83 (0.20; 3.38)	0.72 (0.18; 2.95)

Abbreviations: n = number of cases; N = number of persons included in study.
Values are hazard ratios with 95% confidence intervals.
Model I: Adjusted for age, age^2, and sex.
Model II: Adjusted for age, age^2, sex, and smoking.

Supplementary Table III. Severity of airflow limitation measured by FEV1 and the risk of stroke

		Stroke	
	n/N	Model I	Model II
FEV1			
≥100%	44/2473	1 (reference)	1 (reference)
90-100%	26/660	1.57 (0.96; 2.57)	1.50 (0.92; 2.43)
80-90%	19/414	1.59 (0.91; 2.76)	1.49 (0.83; 2.65)
70-80%	13/187	2.26 (1.19; 4.28)	2.07 (1.09; 3.93)
60-70%	3/121	0.71 (0.22; 2.30)	0.63 (0.19; 2.04)
≤60%	12/126	2.82 (1.45; 5.47)	2.43 (1.24; 4.77)
Per 10% decrease	117/3981	1.06 (0.97; 1.15)	1.03 (0.95; 1.12)

Abbreviations: n = number of cases; N = number of persons included in study; FEV1 = forced expiratory volume in 1 second.
Values are hazard ratios with 95% confidence intervals.
Model I: Adjusted for age, age^2, and sex.
Model II: Adjusted for age, age^2, sex, and smoking.

Chapter 4.2

Anxiety and the risk of stroke
Marileen L.P. Portegies, Michiel J. Bos, Peter J. Koudstaal, Albert Hofman, Henning W. Tiemeier,
M. Arfan Ikram

Abstract

Background and purpose
It is unclear whether anxiety is a risk factor for stroke. We assessed the association between anxiety and the risk of incident stroke.

Methods
This population-based cohort study was based on two rounds of the Rotterdam Study. Each round was taken separately as baseline. In 1993-1995, anxiety symptoms were measured using the HADS-A. In 2002-2004, anxiety disorders were assessed using the M-CIDI. Participants were followed for incident stroke until January 2012.

Results
In the sample undergoing HADS-A (N=2,625; mean age at baseline 68.4 years), 332 strokes occurred during 32,720 years of follow-up. HADS-A score was not associated with the risk of stroke during complete follow-up (adjusted HR 1.02, 95% confidence interval (CI) 0.74; 1.43, for HADS-A ≥8 compared to HADS-A <8), although we did find an increased risk after a shorter follow-up of 3 years (adjusted HR 2.68, 95% CI 1.33; 5.41). In the sample undergoing M-CIDI (N=8,662; mean age at baseline 66.1 years), 340 strokes occurred during 48,703 years of follow-up. Participants with any anxiety disorder had no higher risk of stroke than participants without anxiety disorder (adjusted HR 0.95, 95% CI 0.64; 1.43). We also did not observe an increased risk of stroke for the different subtypes of anxiety.

Conclusions
Anxiety disorders were not associated with stroke in our general population study. Anxiety symptoms were only related to stroke in the short-term, which needs further exploration.

Introduction

An increasing body of evidence suggests that anxiety is associated with the risk of coronary heart disease.[1,2] Literature on the association between anxiety and stroke is scarce and inconsistent. Whereas one study found no association between generalized anxiety disorder (GAD) and stroke,[3] another study showed that the risk of stroke increased with increasing anxiety symptom score.[4] Therefore, these findings require further corroboration. We assessed the association between anxiety and the risk of stroke in the general population.

Methods

Setting and study population
The Rotterdam Study is a prospective population-based cohort study, with repeat examination rounds every 3 to 4 years.[5] The original cohort (RS-I) started in 1990 and was expanded twice in 2000 (RS-II) and 2006 (RS-III). Currently, the study consists of 14,926 participants aged ≥45 years.[5]

Anxiety symptoms were assessed once, in the 2nd round of the RS-I subcohort (1993-1995). This round was attended by 6,315 participants. A random half of these participants (N=3,060) was invited to undergo the Hospital Anxiety and Depression Scale – Anxiety (HADS-A), of whom 2,975 were sufficiently screened on anxiety symptoms. We excluded participants without informed consent for retrieval of follow-up data (n=22), with prevalent stroke (n=84) or with prevalent (n=38) or missing data on dementia (n=206). Therefore, 2,625 participants were eligible for the analysis.

Anxiety disorders were assessed in the 4th round of the RS-I subcohort, the 2nd round of the RS-II subcohort, and the 1st round of the RS-III subcohort (2002-2004), using the Munich version of the Composite International Diagnostic Interview (M-CIDI). Of the 9,996 participants that attended these rounds, 9,974 underwent the interview. Of these, 9,426 participants had a valid anxiety assessment. After excluding participants without informed consent (n=62), with prevalent stroke (n=364), with prevalent (n=52) or missing data on dementia (n=280), or incomplete follow-up (n=6), 8,662 participants were eligible for this analysis. In total, 1,460 participants were overlapping across the sample that received the HADS-A and the sample that received the M-CIDI.

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study). All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

Assessment of anxiety
We used the HADS-A to measure anxiety symptoms. Participants filled in the questionnaire by themselves. The HADS-A is a subscale of the HADS,[6] a brief questionnaire that is used for the self-assessment of anxiety (HADS-A) and depressive symptoms (HADS-D). It measures anxiety

including 7 items. The total score ranges from 0-21, and the HADS-A has an optimal balance between sensitivity and specificity for identifying anxiety disorder cases at a cutoff score of ≥8.[6] We also used a cutoff score of >11, which includes fewer false positives.[7]

The 1-year prevalence of anxiety disorders was assessed using a slightly adapted version of the M-CIDI.[8] The M-CIDI was administered by trained interviewers. They were trained by a medical doctor (HT), who followed a formal training in M-CIDI by the Wittchen group in Dresden.[9] This score does not allow for a personal interpretation of the participant's answers. Rather a computerized diagnostic algorithm was used to calculate the following anxiety disorders according to the DSM-IV-TR criteria: generalized anxiety disorder, panic disorder, agoraphobia, social phobia, and specific phobia.[8]

Assessment of stroke

Stroke was defined according to World Health Organization criteria.[10] After enrollment, participants were continuously monitored for incident stroke through automated linkage of the study database with medical records from general practitioners. Additional information was obtained from hospital records as described previously.[10] Strokes were classified as ischemic or hemorrhagic based on neuroimaging reports. Strokes were classified as unspecified if no neuroimaging was performed.[10] The follow-up was complete until January 1, 2012, for 93.1% of potential person-years.

Assessment of covariates

We considered age, sex, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication, smoking, diabetes mellitus type 2, body mass index, and myocardial infarction as potential confounders and their assessment has been described previously.[5,11] Information about cholesterol, lipid-lowering medication, and diabetes mellitus was unavailable for the visit of the HADS cohort and was therefore obtained from one visit earlier. Depressive symptoms were obtained from the HADS Depression subscale (HADS-D).[6]

Statistical analysis

We calculated hazard ratios (HRs) and 95% confidence intervals (CIs) with Cox proportional hazards models using IBM SPSS Statistics version 21. We analyzed participants based on HADS-A score (continuously per standard deviation (SD) and categorically using score ≥8 versus score <8 and score ≥11 versus score <11) and based on M-CIDI (at least one disorder [generalized anxiety disorder, panic disorder, agoraphobia, social phobia, or specific phobia] versus no disorder, and the separate subtypes of anxiety disorder versus no disorder). Participants were censored at date of stroke, date of death, last date of follow-up or January 1, 2012. Additionally, we constructed Kaplan-Meier curves to detect possible short-term effects. Covariates were missing up to a maximum of 9% and imputed based on the other covariates using multiple imputation with 5 imputation sets.

Results

Baseline characteristics of the study samples are presented in Table 1. Average (±SD) follow-up in the sample undergoing HADS-A was 12.5 (±5.3) years during which 332 strokes occurred: 204 ischemic and 30 hemorrhagic. Average follow-up in the sample undergoing M-CIDI was 5.6 (±2.1) years during which 340 strokes occurred: 237 ischemic and 40 hemorrhagic.

Table 1. Baseline characteristics

	Anxiety symptoms	Anxiety disorders
	HADS-A sample (N=2,625)	M-CIDI sample (N=8,662)
Age, years	68.4 (8.4)	66.1 (10.5)
Female sex	1453 (55.4%)	4999 (57.7%)
Percentage with HADS-A ≥8	343 (13.1%)	NA
Percentage with HADS-A ≥11	147 (5.6%)	NA
HADS-A, score	3.7 (3.6)	NA
Any anxiety disorder[a]	NA	728 (8.4%)
Generalized anxiety disorder	NA	224 (2.6%)
Panic disorder	NA	79 (0.9%)
Agoraphobia	NA	301 (3.5%)
Social phobia	NA	104 (1.2%)
Specific phobia	NA	143 (1.7%)
Systolic blood pressure, mmHg	139 (22)	142 (22)
Diastolic blood pressure, mmHg	77 (11)	81 (11)
Use of blood pressure-lowering medication	585 (23.9%)	2564 (29.7%)
Total cholesterol, mmol/L	6.6 (1.2)	5.6 (1.0)
High-density lipoprotein cholesterol, mmol/L	1.4 (0.4)	1.4 (0.4)
Use of lipid-lowering medication	72 (2.7%)	1869 (21.7%)
Body mass index	26.3 (3.6)	27.7 (4.3)
Smoking		
Current	567 (22.3%)	1728 (20.0%)
Past	1243 (48.9%)	4476 (51.7%)
Never	732 (28.8%)	2456 (28.4%)
Diabetes mellitus	153 (6.0%)	1062 (12.4%)
Myocardial infarction	118 (4.5%)	409 (4.7%)

Abbreviations: NA = not available, HADS-A = Hospital Anxiety and Depression Scale anxiety subscale, M-CIDI = Munich version of the Composite International Diagnostic Interview.
Data are presented as mean (standard deviations) or counts (percentages).
[a] Generalized anxiety disorder, panic disorder, agoraphobia, social phobia, or specific phobia.

Neither anxiety symptoms nor anxiety disorders were associated with the risk of stroke after complete follow-up (Table 2, Supplementary Table I): the multivariate adjusted HR was 1.02 (95% CI 0.74; 1.43) for anxiety symptoms (HADS-A≥8 compared to HADS-A<8) and 0.95 (95% CI 0.64; 1.43) for any versus no anxiety disorder. The stricter dichotomization of HADS-A (≥11 versus <11) resulted in stronger effects, though still not significant. We also did not observe any

association for the separate subtypes of anxiety disorder or when we examined anxiety symptoms on a continuous scale (Supplementary Table II, Table 2).

Inspection of the survival curve (Supplementary Figure I) pointed towards a possible short-term effect for anxiety symptoms. Indeed, an increased HADS was associated with an increased risk of stroke in the first 3 years of follow-up (HR 2.55, 95% CI 1.45; 4.46 for HADS-A ≥8 compared to HADS-A <8) (Supplementary Table III). After additional adjustment for depressive symptoms, the association remained similar.

Table 2. Anxiety and the risk of stroke

	Any stroke			Ischemic stroke		
	n/N	Model 1 HR (95% CI)	Model 2 HR (95% CI)	n/N	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Anxiety symptoms						
HADS-A score						
HADS-A<8	291/2282	reference	reference	184/2282	reference	reference
HADS-A≥8	41/343	1.06 (0.76; 1.48)	1.02 (0.74; 1.43)	20/343	0.85 (0.54; 1.36)	0.84 (0.52; 1.33)
HADS-A<11	313/2478	reference	reference	193/2478	reference	reference
HADS-A≥11	19/147	1.14 (0.71; 1.81)	1.16 (0.73; 1.86)	11/147	1.15 (0.63; 2.13)	1.16 (0.63; 2.14)
per SD	332/2625	1.05 (0.95; 1.18)	1.05 (0.94; 1.17)	204/2625	1.00 (0.87; 1.16)	1.00 (0.86; 1.16)
Anxiety disorders						
No disorder	314/7934	reference	reference	219/7934	reference	reference
Any disorder	26/728	0.97 (0.65; 1.45)	0.95 (0.64; 1.43)	18/728	0.95 (0.59; 1.55)	0.94 (0.58; 1.52)

Abbreviations: n = number of strokes, N = number of participants at risk, SD = standard deviation, HADS-A = Hospital Anxiety and Depression Scale anxiety subscale, HR = hazard ratio.
Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication, diabetes mellitus, myocardial infarction, smoking, and body mass index.

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Discussion

In this prospective population-based cohort study, we found no long-term association between anxiety and the risk of stroke.

Two previous cohort studies assessed the association between anxiety and stroke: in one study, the risk of stroke increased with increasing anxiety symptom score,[4] whereas in the other study, psychological distress was associated with stroke, but not generalized anxiety disorder.[3] A reason for the differences may be the different scores to assess anxiety. Anxiety symptoms coincide with depressive symptoms and stress symptoms. Vice versa, depressive symptoms and stress symptoms have been related to stroke before.[12,13] In our study, we only detected a short-term increased risk of stroke in people with anxiety symptoms, independent of depressive symptoms. This may be a true effect with possible mechanisms being activation of the hypothalamic-pituitary-adrenal axis and sympathetic nervous system or adverse health behaviors.[1] This is further supported by the finding that a stricter dichotomization of the anxiety symptom score resulted in stronger effects. Moreover, there may have been some non-differential misclassification in our assessment of anxiety, which could have resulted in an underestimation of the true effect. In contrast, the lack of a long-term effect argues against a true effect. Moreover, we found no associations with anxiety disorders, for which any mechanism should have a similar effect. An alternative explanation may thus be reversed causality, as suggested previously for mortality.[14] Given that patients with stroke have a high cardiovascular risk, they may experience a worsening health in the years preceding stroke and get anxious in response.

Strengths of our study are the population-based setting, the long follow-up period, the stringent stroke monitoring and the limited loss of follow-up. A limitation is that lacking neuroimaging after stroke, 20% of all strokes remained of unspecified subtype. Additionally, we did not perform a formal evaluation of the reliability of our anxiety assessments. Furthermore, we may have missed weak associations: assuming $\alpha=0.05$, our study had a power of 0.77 to detect the HR of 1.43 that was found previously for high versus low anxiety (in our study HADS-A score ≥8 versus <8).[4,15]

In conclusion, anxiety disorders were not associated with stroke in this general population study. Anxiety symptoms were only associated to stroke in the short term, which needs further exploration.

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Supplementary information
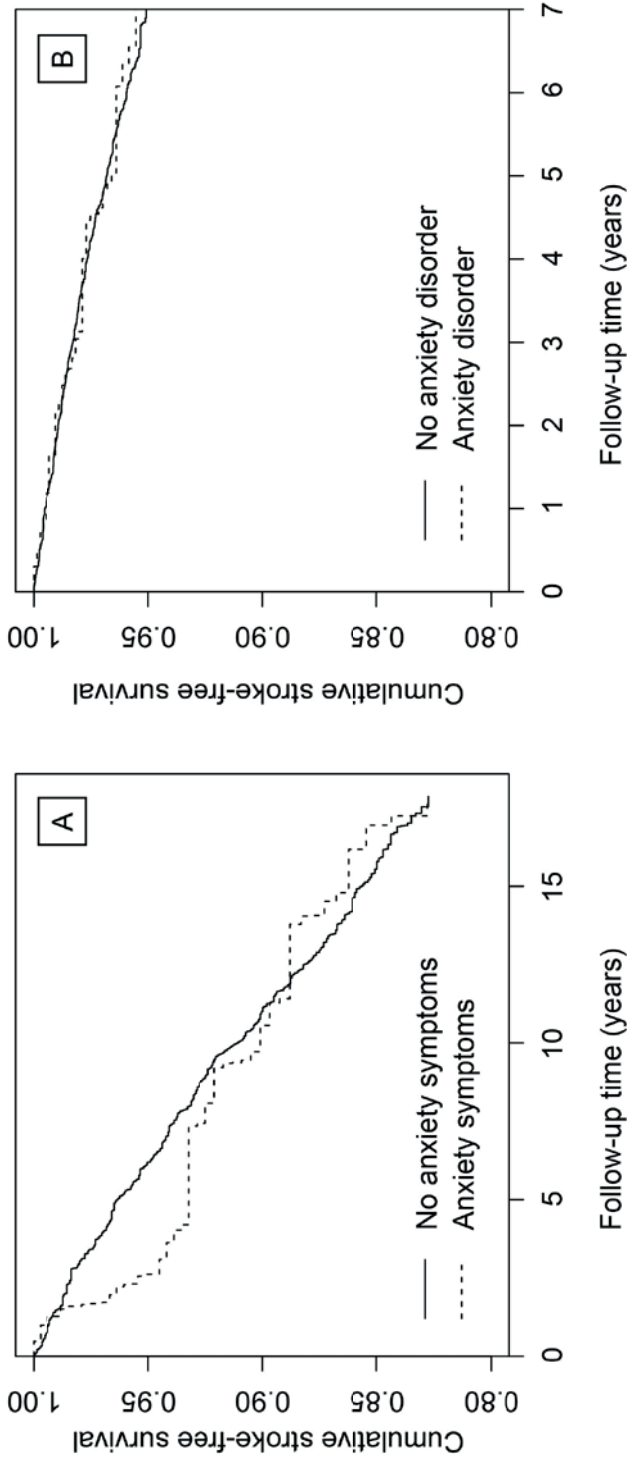
Supplementary Figure I. Survival curve
A: Cumulative stroke-free survival of people with anxiety symptoms (HADS-A ≥ 8) versus without anxiety symptoms (HADS-A < 8). B: Cumulative stroke-free survival of people with anxiety disorder versus without anxiety disorder, based on M-CIDI.

Supplementary Table I. Anxiety symptoms and risk of hemorrhagic stroke

		Hemorrhagic stroke	
	n/N	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Anxiety symptoms			
HADS-A score			
per SD	30/2625	0.98 (0.67; 1.44)	0.96 (0.65; 1.42)
HADS-A<8	26/2282	reference	reference
HADS-A≥8	4/343	1.22 (0.42; 3.53)	1.09 (0.37; 3.19)
HADS-A<11	30/2478	reference	reference
HADS-A≥11	0/147	NA	NA
Anxiety disorders			
No	37/7934	reference	reference
Any	3/728	0.90 (0.28; 2.95)	0.85 (0.26; 2.77)

Abbreviations: n = number of strokes; N = number of participants in study; HADS-A = Hospital Anxiety and Depression Scale anxiety subscale; HR = hazard ratio.
Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication, diabetes mellitus, myocardial infarction, smoking, and body mass index.

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Supplementary Table II. Subtypes of anxiety disorder and the risk of stroke

Subtype of anxiety disorder	Any stroke		Ischemic stroke	
	n/N	HR (95% CI)	n/N	HR (95% CI)
No anxiety disorder	314/7934	reference	219/7934	reference
Generalized anxiety disorder	5/224	0.64 (0.26; 1.55)	4/224	0.73 (0.27; 1.98)
Panic disorder	3/79	1.83 (0.58; 5.74)	2/79	1.64 (0.40; 6.63)
Agoraphobia	15/301	1.11 (0.66; 1.86)	9/301	0.96 (0.49; 1.88)
Social phobia	1/104	0.31 (0.04; 2.24)	1/104	0.44 (0.06; 3.12)
Specific phobia	6/143	1.09 (0.49; 2.46)	5/143	1.31 (0.54; 3.20)

Abbreviations: n = number of strokes; N = number of participants at risk; NA = not applicable, HR = hazard ratio.
Adjusted for age, sex, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication, diabetes mellitus, myocardial infarction, smoking, and body mass index.

Supplementary Table III. Anxiety symptoms and risk of stroke, during 3 years of follow-up

Type of stroke	Any stroke				Ischemic stroke			
	n/N	Model 1 HR (95% CI)	Model 2 HR (95% CI)	Model 3 HR (95% CI)	n/N	Model 1 HR (95% CI)	Model 2 HR (95% CI)	Model 3 HR (95% CI)
HADS-A score per SD	63/2625	1.32 (1.07; 1.62)	1.29 (1.04; 1.60)	1.29 (0.96; 1.73)	43/2625	1.32 (1.02; 1.70)	1.31 (1.01; 1.70)	1.38 (0.96; 1.98)
HADS-A score <8	45/2282	reference	reference	reference	31/2282	reference	reference	reference
HADS-A score ≥8	18/343	2.79 (1.60; 4.85)	2.55 (1.45; 4.46)	2.68 (1.33; 5.41)	12/343	2.79 (1.42; 5.47)	2.67 (1.35; 5.28)	3.10 (1.31; 7.31)

Abbreviations: n = number of strokes; N = number of participants in study; HADS-A = Hospital Anxiety and Depression Scale anxiety subscale; HADS-D = Hospital Anxiety and Depression Scale depression subscale; HR = hazard ratio.
Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication, diabetes mellitus, myocardial infarction, smoking, and body mass index. Model 3: additionally adjusted for HADS-D.
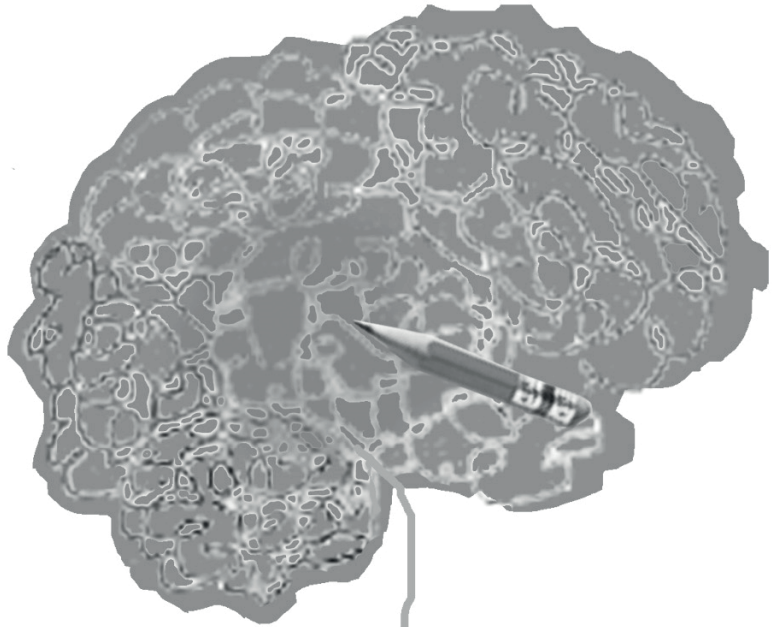
Chapter 5

Challenges after stroke: stroke recognition and the long-term consequences of stroke

Chapter 5.1

Left-sided strokes are more often recognized than right-sided strokes

Marileen L.P. Portegies, Mariana Selwaness, Albert Hofman, Peter J. Koudstaal, Meike W. Vernooij, M. Arfan Ikram

Abstract

Background and Purpose
Left-sided strokes are reported to be more common than right-sided strokes, but it is unknown whether they occur more often or are simply recognized more easily by clinicians. In a large unselected community-dwelling population we examined the frequency of clinical left- and right-sided strokes and transient ischemic attacks (TIAs) and compared it with the frequency of left- and right-sided infarcts on MRI.

Methods
This study was conducted within the population-based Rotterdam Study. Between 1990-2012, 13,894 participants were followed-up for first-ever stroke and TIA. MRI scans were performed within a random subgroup of 5081 persons and were rated for presence of supratentorial cortical and lacunar infarcts. We compared frequencies of left- and right-sided strokes, TIAs, or MRI-infarcts using binomial and Fisher exact tests.

Results
After a mean follow-up of 9.6 (±6.0) years, 1252 persons suffered a stroke, of which 704 were ischemic, and 799 participants had a TIA. Within the subgroup with MRI, we identified 673 infarcts. Ischemic strokes were more frequently left-sided (57.7%, 95% CI 53.7; 61.6) than right-sided, similar to TIAs (57.8% left-sided, 95% CI 53.4; 62.3). In contrast, we found no left-right difference in distribution of infarcts on MRI (51.9% left-sided, 95% CI 48.1; 55.6).

Conclusions
Clinical ischemic strokes and TIAs are more frequently left-sided than right-sided, whereas this difference is not present for infarcts on MRI. This suggests that left-sided strokes and TIAs are more easily recognized. Consequently, there should be more attention for symptoms of right-sided strokes and TIAs.

Introduction

Several hospital-based studies have reported that left-sided strokes are more frequent than right-sided strokes.[1-3] A predilection for the left side may be explained by characteristics of the atherosclerotic plaque in the left carotid artery or by anatomy.[3] The finding that isolated aphasia is a typical presentation of cardioembolic stroke or transient ischemic attack (TIA) also suggests that cardiac thrombi may preferably affect the left hemisphere.[4] Another hypothesis is that the strokes in hospitals are a selection of strokes with symptoms that are better recognized or perceived as more severe. Left-sided strokes might be referred more frequently, because they lead to clear symptoms like aphasia, whereas right-sided strokes may lead to less explicit symptoms like hemineglect or spatial disorientation.[1,5]

Previous MRI studies also suggested that right-sided strokes are more often unnoticed, since they found more right-sided silent infarcts in persons with carotid stenosis and atrial fibrillation.[6,7] An important advantage of MRI-studies is that these not only detect clinical strokes, but also clinically silent infarcts, thereby providing a better estimate of the true distribution of left- and right-sided infarcts. To our knowledge, no study has compared the distribution of clinical strokes to that of MRI-defined cerebral infarcts within an unselected community-dwelling population. This can distinguish between an actual higher frequency of left-sided infarcts versus a higher frequency of clinically recognized strokes.

In a population-based study we investigated the frequency of left- and right-sided ischemic strokes and TIAs and compared this to the frequency of left- and right-sided cerebral infarcts on MRI.

Methods

Population
This study was embedded within the prospective population-based Rotterdam Study. Details regarding the objective and design of the Rotterdam Study have been described elsewhere.[8] The study started in 1990 among 7983 subjects aged 55 years and older residing in Ommoord, a suburb of Rotterdam, the Netherlands. In 2000 the cohort was extended with 3011 participants who had become 55 years of age or moved into the study district. In 2006 another 3932 subjects living in Ommoord were included, aged 45-54 years. After excluding 256 participants that did not give informed consent for collection of follow-up information and excluding 776 persons with prevalent stroke or TIA, 13894 participants (mean age at baseline 65.5±10.3 years, 59.3% female) were followed-up for first ever stroke and TIA. MRI scans were performed in 5081 participants (mean age 64.2±11.1, 55.0% female) of the Rotterdam Scan Study, which is a substudy of the Rotterdam Study that started in 2005.[9] The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. A written informed consent was obtained from all participants.

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Assessment of clinical stroke and TIA

History of stroke and transient ischemic attack (TIA) was assessed using home interviews and confirmed reviewing medical records. From baseline onwards persons were continuously followed-up for occurrence of stroke and TIA through automatic linkage of general practitioners' files with the study database. Furthermore, nursing home physicians' files and files from general practitioners of participants that moved out of the study area, were checked on a regular basis. Of all potential strokes and TIAs, hospital discharge letters and information from general practitioner was collected. Research physicians reviewed the information and an experienced vascular neurologist verified the strokes according to World Health Organization criteria.[10] Strokes were further classified into ischemic or hemorrhagic on basis of neuroimaging reports.[11] If no neuroimaging was performed, strokes were classified as unspecified. We defined TIAs as temporary attacks with presence of focal symptoms, which are attributable to dysfunction of one arterial territory of the brain.[12] Assessment of the hemispheric side of strokes and TIAs was based on clinical symptoms and CT/MRI-images described in the medical records. Follow-up for both stroke and TIA was complete until January 1st, 2012 for 96.3% of potential person-years.

Cerebral infarcts on MRI

All scans were performed on a 1.5T MRI scanner (General Electric Healthcare, Milwaukee, USA).[9] Infarcts were rated on FLAIR, PD-weighted, and T1-weighted sequences by experienced raters under supervision of a neuroradiologist. Lacunar infarcts were defined as focal lesions ≥ 3 mm and <15 mm in size with the same signal characteristics as CSF on all sequences, and with a hyperintense rim on the FLAIR sequence.[9]

To assess symptomatic and silent infarcts, we coupled our MRI-dataset with the clinical stroke and TIA dataset. We recorded an MRI-infarct as symptomatic if there was a previous clinical ischemic stroke or TIA (either a first-ever event or a recurrent event) matching with the hemispheric side of the MRI-infarct. Similarly, to adjudicate an MRI-infarct as silent, there should be no previous clinical ischemic stroke or TIA matching with the hemispheric side of that MRI-infarct.

Statistical analysis

We restricted our analyses to the cerebral hemispheres. We calculated frequencies of first-ever left- and right-sided strokes, TIAs, and MRI-infarcts with 95% confidence intervals (CIs). These frequencies were compared with the expected frequency of 50 percent using a binomial test. Furthermore, the Fisher's exact test was used to compare distribution of clinical stroke and TIA side with distribution of infarcts on MRI. For strokes and TIAs and the combination ischemic stroke and TIA we included only the first event. We included all MRI-infarcts, because we could not separate recent from chronic infarcts, also if a participant had infarcts on both sides. As sensitivity analysis we performed linear mixed models to account for multiple MRI-infarcts in one person. All analyses were done using IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY).

Results

During 9.6 ±6.0 years of follow-up, 1252 participants suffered a clinical stroke and 799 a TIA (mean age 78.7 ±9.2, 61.6% female). 588 ischemic strokes and 465 TIAs occurred supratentorially (Figure 1).
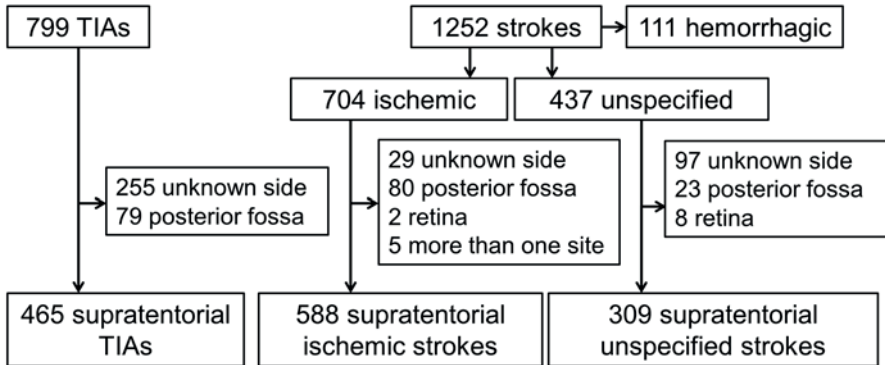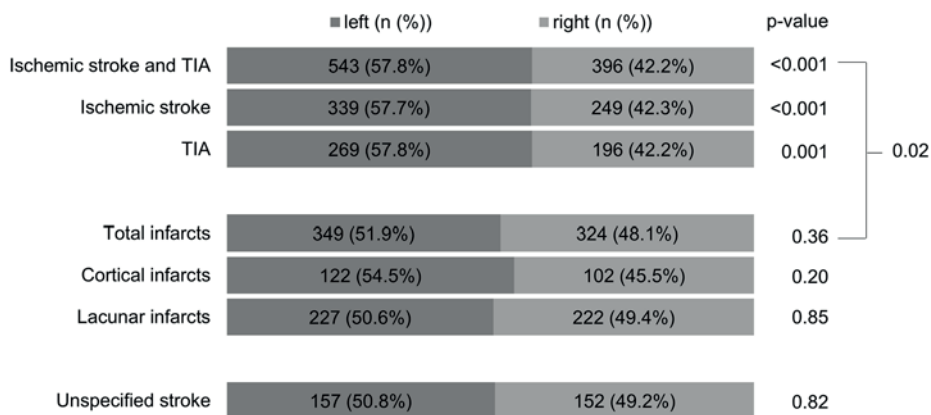

Figure 1. Flowchart of the study population

We identified cerebral infarcts on MRI in 510 participants. Participants with only infratentorial infarcts were excluded (n=110). Of the remaining 400 participants, 166 had infarcts only on the left side, 149 only on the right side, and 85 on both sides. The location was not registered for 7 cortical and 5 lacunar infarcts. In total we identified 673 supratentorial infarcts, of which 224 were cortical and 449 lacunar.

Ischemic strokes occurred more often left- than right-sided (57.7% left-sided, 95% CI 53.7; 61.6), similar to TIAs (57.8% left-sided, 95% CI 53.4; 62.3). We did not find a significant left-right difference for cerebral infarcts on MRI (51.9% left-sided, 95% CI 48.1; 55.6, Figure 2). Importantly, direct comparison revealed that clinical ischemic stroke and TIA were significantly more frequently left-sided than infarcts on MRI (p=0.02). Figure 2 and Supplementary Figure I also show the left-right distribution for various subtypes of clinical strokes and MRI-infarcts (e.g. lacunar versus cortical; symptomatic versus silent MRI-infarct).

Calculating the percentage of MRI-infarcts using linear mixed models did not change our results in any way (data not shown).

	left (n (%))	right (n (%))	p-value
Ischemic stroke and TIA	543 (57.8%)	396 (42.2%)	<0.001
Ischemic stroke	339 (57.7%)	249 (42.3%)	<0.001
TIA	269 (57.8%)	196 (42.2%)	0.001
Total infarcts	349 (51.9%)	324 (48.1%)	0.36
Cortical infarcts	122 (54.5%)	102 (45.5%)	0.20
Lacunar infarcts	227 (50.6%)	222 (49.4%)	0.85
Unspecified stroke	157 (50.8%)	152 (49.2%)	0.82

0.02

Figure 2. Frequency of left- and right-sided clinical strokes, TIAs, and cerebral infarcts on MRI
Data are presented as counts (percentages). p-values are presented for the left-right difference and for the difference in distribution between clinical stroke and TIA and MRI-infarcts.

Discussion

In this population-based study, we found that clinical ischemic strokes and TIAs occur more often left-sided than right-sided, whereas we did not find such a difference for cerebral infarcts on MRI.

Previous studies found a similar higher incidence of clinical left-sided strokes and TIAs, but did not compare their results to infarcts on MRI, which includes clinically silent infarcts. These studies therefore did not help to distinguish between a true higher incidence of left-sided stroke due to a predilection of infarcts for the left side versus a difference in recognition of left- and right-sided strokes.[1-3] In our study, clinical ischemic strokes and TIAs were more frequently left-sided than right-sided. This was different from the distribution of infarcts on MRI. This suggests that left-sided strokes are recognized better or perceived as more severe whilst right-sided strokes are missed. This might be the consequence of complex right-sided symptoms such as hemineglect and spatial disorientation. Furthermore, patients might not present themselves to the hospital because of anosognosia. However, this is speculative, as we do not routinely examine our participants to detect symptoms that might have been missed.

To determine the impact of missing right-sided strokes, future studies should examine differences in long-term disability between left- and right-sided strokes.

Strengths of this study are the thorough collection of strokes and the availability of MRI in a subgroup of the study population. A limitation is that we could not compare frequencies of clinical strokes and TIAs with frequencies of MRI-infarcts in exactly the same population, because of small overlap. Also, participants with a severe stroke probably did not visit the study center for MRI scanning because of their physical limitations. This might have led to some selection bias in the MRI population. Furthermore, due to lack of clinical neuroimaging after stroke, not all strokes could be classified into ischemic or hemorrhagic and remained unspecified. Many of those unspecified strokes were not referred to hospital, partly because

they were perceived as less severe. Left-sided strokes were not more frequent in this group, which supports our other results that left-sided strokes are perceived as more severe and referred to a hospital more often. A final consideration is that the distribution of left-sided MRI-infarcts was not exactly 50-50, especially for cortical MRI-infarcts. It is therefore possible, that there also is a small, but true predilection for left-sided strokes, which remained obscured in our study due to insufficient power.

In conclusion, our findings suggest that the higher frequency in clinical left-sided strokes and TIAs compared with right-sided events is in a large part due to a better recognition of those. Consequently, there should be more attention for symptoms of right-sided strokes and TIAs.

5

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Supplementary information

	left (n (%))	right (n (%))	p-value
Total infarcts	349 (51.9%)	324 (48.1%)	0.36
Symptomatic infarcts	54 (55.1%)	44 (44.9%)	0.36
Silent infarcts	295 (51.3%)	280 (48.7%)	0.56

Supplementary Figure I. Frequencies of symptomatic and silent infarcts on MRI
Data are presented as counts (percentages). p-values are presented for the left-right difference.

5

Chapter 5.2

Role of prestroke vascular pathology in long-term prognosis after stroke

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Abstract

Background and purpose
Mortality after stroke remains high for years, mostly due to cardiovascular causes. Given that cardiovascular pathology plays an important role in causing the initial stroke, such pre-stroke pathology might also influence the prognosis after stroke. Within the population-based Rotterdam Study, we examined the proportion of deaths after stroke that are attributable to pre-existent cardiovascular risk factors before stroke (the population attributable risk (PAR)).

Methods
We examined 1,237 first-ever stroke patients and 4,928 stroke-free persons (between 1990-2012), matched on age, sex, examination round and stroke date (index date). Cardiovascular risk factors measured on average 4 years before index date were used as determinants. Persons were continuously followed-up for mortality (average 6 years) after the index date. We calculated separate and combined PAR of hypertension, total cholesterol, HDL cholesterol, BMI, diabetes mellitus, smoking, TIA, and atrial fibrillation.

Results
919 stroke patients and 2,654 stroke-free persons died. The combined PAR in persons with stroke was 27% (95% CI 14-45%) and in stroke-free persons 19% (95% CI 12-29%). PARs of diabetes mellitus, smoking, and atrial fibrillation were higher in persons with stroke compared to the reference group due to a higher prevalence of risk factors. Additionally, people with atrial fibrillation and stroke had a higher hazard ratio for death compared to those with only atrial fibrillation.

Conclusions
A quarter of deaths after stroke could theoretically be prevented with rigorous cardiovascular prevention and treatment, but this should preferably start before stroke occurrence. Additionally, research into factors explaining the remaining deaths needs to be encouraged.

Introduction

Stroke is the second leading cause of death worldwide.[1] Many patients die in the acute phase after stroke,[2] but the risk of death remains high for years, particularly due to cardiovascular deaths.[3-6] Accumulation of cardiovascular pathology is a slow process spanning many years and it is likely that such pathology does not only cause stroke,[7] but also plays a role in mortality after stroke. This could mean that preventive and therapeutic measures for cardiovascular health, which are often implemented following a stroke,[8] may be starting too late.

Most studies investigating risk factors for mortality after stroke were performed in clinical settings among stroke patients.[9-16] In such settings, the influence of reverse causality cannot be disentangled from the true effect of these risk factors. In other words, a stroke itself induces changes in risk factors, such as blood pressure, cholesterol, and diabetes mellitus, which then are not a true reflection of their status prior to the stroke.[17-19]

Therefore, the aim of our study was to examine the role of pre-stroke cardiovascular risk factors in mortality after stroke. We first calculated mortality rates in persons with and without stroke. Subsequently, we estimated the impact of pre-stroke cardiovascular risk factors on the risk of mortality using the population attributable risk (PAR). This shows the maximum proportion of deaths that can theoretically be prevented with complete elimination of the cardiovascular risk factors, assuming that risk factors are causally linked and completely modifiable.[20] Furthermore, we compared this to the PAR in stroke-free participants.

Materials and methods

Setting

This study was conducted within the prospective, population-based Rotterdam Study. Details regarding the objectives and design of the study have been described elsewhere.[21] In brief, baseline examinations started in 1990 among 7,983 persons of 55 years and older residing in Ommoord, a suburb of Rotterdam, the Netherlands. Examinations took place every 3-4 years, which means participants were invited for a visit in 1990-1993, 1993-1995, 1997-1999, 2002-2004, and 2009-2011. In 2000, the cohort was extended with 3,011 persons that had become 55 since the start of the study or moved into the research area. They were invited for a visit in 2000-2001, 2004-2005, and 2011-2012. In 2006 another 3,932 subjects were included, aged 45 years and older. These participants visited the center in 2006-2008.

For the current study, participants with prevalent stroke (n=453) or who did not give informed consent for collection of follow-up information (n=238) were excluded. Consequently, 14,235 participants were at risk for stroke. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the "Wet Bevolkings-onderzoek: ERGO (Population Studies Act: Rotterdam Study)". All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

Assessment of stroke and mortality

History of stroke was assessed during the home interview at baseline and confirmed by reviewing medical records.[22] Strokes were defined according to World Health Organization criteria.[23] From baseline onwards, participants were continuously followed-up for stroke and death by automatic linkage of general practitioners' medical records with the study database. In addition, nursing home physicians' medical records, municipal records and medical records from general practitioners of participants who moved out of the Ommoord district were examined on a regular basis. Potential strokes were reviewed by research physicians and verified by an experienced vascular neurologist.

Follow-up for stroke was complete for 96.3% of potential person-years and for vital status for 99.2% of potential person-years.

Assessment of cardiovascular risk factors

History of smoking and medication use were assessed during the home interview. Smoking status was categorized as current, former, and never smoking. Physical examinations were performed during the visits at the study center.[24] Body mass index was calculated as weight divided by length squared. Underweight was defined as a body mass index <18.5. Hypertension was defined as a systolic blood pressure ≥140mmHg and/or a diastolic blood pressure ≥90mmHg and/or the use of blood pressure-lowering medication with the indication hypertension.[25] Hypercholesterolemia was defined as a total cholesterol >6.2 mmol/L.[26] A low high-density lipoprotein (HDL)-cholesterol was defined as a HDL-cholesterol ≤1.0 mmol/L.[26] Diabetes mellitus was defined as a fasting glucose level ≥7.0 mmol/L, non-fasting glucose level ≥11.0 mmol/L, or use of antidiabetic medication.[27]

Occurrence of transient ischemic attacks (TIAs) and atrial fibrillation was assessed through active follow-up and verified using standardized definitions similar to the follow-up for stroke.[28,29] However, follow-up for atrial fibrillation was only complete until 2010, which means that people with atrial fibrillation after 2010 were missed.

Statistical analysis

Cardiovascular risk factors obtained during the closest available examination preceding the stroke were used for the analyses. In every visit we had information about BMI and smoking status. Cholesterol and glucose (necessary for diabetes mellitus status) were measured in each visit, except from the second center visit (1993-1995) of the first cohort. Blood pressure was also measured in each visit, but the hypertension variable including blood pressure medication with the indication of hypertension is not available for the fifth visit of the first cohort (2009-2011) and the third visit of the second cohort (2011-2012). Therefore information on these missing variables was obtained from a visit earlier. Participants were continuously followed up for occurrence of TIA and atrial fibrillation. Hence, presence of TIA or atrial fibrillation at any moment before stroke was used in the analysis.

To compare risks and risk factors of death between participants with stroke and a stroke-free group, we first defined an exposed group: all persons who suffered an incident stroke. For this exposed group the at risk time for mortality started at the stroke date. Then, we defined a

non-exposed or stroke-free group. This non-exposed group consisted of 4 participants per stroke case, randomly matched to this case on age (within 1 year), sex, and visiting the same examination round, using an incidence density sampling approach.[30] This non-exposed group was free of stroke until the stroke date of their matched case (index date) and their at risk time for mortality started at the index date. Note that persons could be included as non-exposed several times and could become exposed later on. Further of note, this is different from a case-control design.

We used Poisson regression to calculate age and sex adjusted mortality rates and rate ratios for persons with and without stroke, starting at the index date. As sensitivity analysis we also calculated cardiovascular and non-cardiovascular mortality rates separately.

Associations between pre-stroke cardiovascular risk factors and mortality after stroke were calculated using the Interactive Risk Attributable Program (IRAP).[31] This program calculates hazard ratio's (HRs) with 95% confidence intervals (CI) based on a Poisson model and enables the calculation of population attributable risks (PARs) with 95% CI. We provide logit transformed 95% CI accompanying the PARs as these are more easily interpretable and more stringent with regard to our combined PAR estimate.[32] The PAR is estimated adjusting for confounding using the following formulas:

$$\text{PAR} = 1 - \sum_{i=1}^{I} \sum_{j=1}^{J} \rho_{ij} \, R_{i|j}^{-1}$$

where

$$R_{i|j} = \frac{\Pr(D = 1|X = x_i, C = c_j)}{\Pr(D = 1|X = x_1, C = c_j)}$$

and

$$\rho_{ij} = \Pr\left(X = x_i, C = c_j | D = 1\right)$$

with D=1 denoting presence of disease, X denoting exposure with i levels, and C denoting a confounder with j levels. We calculated the PAR for each risk factor separately and calculated the combined PAR. The combined PAR included the PARs that were associated with mortality in the expected direction, meaning that the HR was higher than 1. PARs cannot be calculated for a HR below one, because this will result in a PAR that cannot be interpreted.[33]

We checked the proportional hazards assumption by inspecting log minus log plots. Follow-up started at the index date. Information on the investigated cardiovascular risk factors was missing up to a maximum of 9.7% and was imputed based on the other covariates using multiple imputation with 5 imputation sets. The mean of these 5 imputation sets was used to implement in the Interactive Risk Attributable Program. In each model, we adjusted for age, sex, time between center date and index date, and for the categories of hypertension, cholesterol, HDL cholesterol, BMI, diabetes mellitus, smoking, and atrial fibrillation, if appropriate. As sensitivity analysis we also calculated the PARs for cardiovascular and non-cardiovascular mortality. We explored potential effect modification by presence of stroke by using an interaction term of the stroke status with the potential risk factor. Differences in prevalence of risk factors between participants with stroke and stroke-free participants and

between men and women were calculated using the Chi-Square test. Interactions with sex were tested adding an interaction term of the dependent variable with sex.

All analyses were performed using IRAP version 2.2 (US National Cancer Institute), IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY) and SAS version 9.3 (SAS Institute Inc., Cary, NC).

Results

Characteristics of the study population

Of the 14,235 participants at risk for stroke that were followed-up for a mean (±SD) of 9.6 (±6.0) years, 1,325 participants suffered a stroke. 1,243 of these participants had a center visit before the stroke. Due to old age, 6 of those participants could not be matched and another 8 stroke cases could only be matched to 1 or 2 stroke-free participants. Eventually we had 1,237 participants with stroke and 4,928 participants without stroke eligible for analysis (Figure 1). Because people could serve as control multiple times and could become case later in time, these control participants consisted out of 3,377 study participants. 2,079 were included only once, 1,298 were included multiple times, of which 378 became a case later in time.
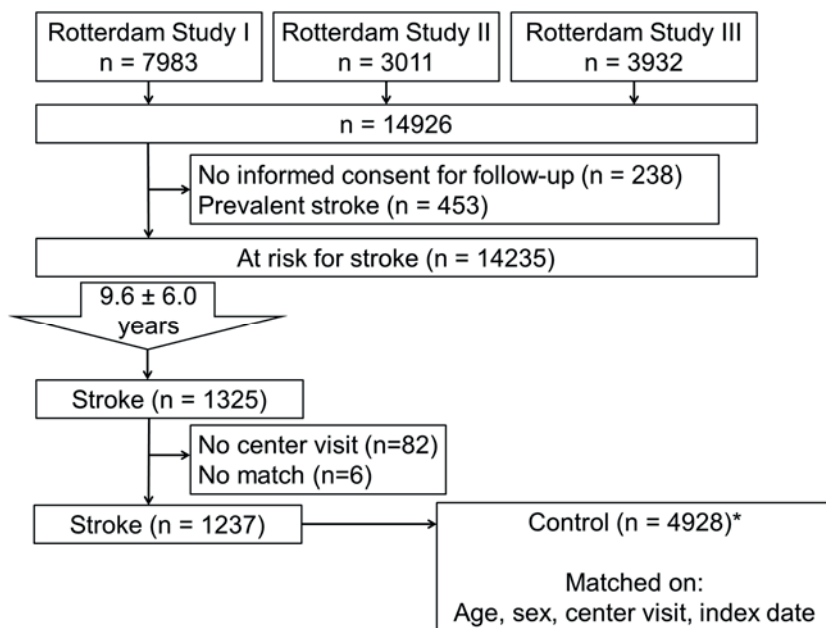
Figure 1. Study population
*Consists of 3377 participants: 2079 were included only once, 1298 were included multiple times of which 378 became a case later in time.

Mean age of persons with stroke was 79.9 (± 8.7) years, 60.4% was female, and cardiovascular risk factors were measured for a mean of 3.7 (± 3.2) years before stroke. Due to matching, these statistics were similar in persons without stroke (Table 1).

Table 1. Baseline characteristics

	Persons with stroke N=1237	Matched persons without stroke N=4928
Age at index date (years)	79.9 (8.7)	79.8 (8.7)
Time-interval between center visit and index date (years)	3.7 (3.2)	3.7 (3.4)
Female	747 (60.4%)	2978 (60.4%)
Hypertension	1007 (85.2%)	3653 (76.2%)
Blood pressure-lowering medication	575 (47.7%)	1989 (41.3%)
Cholesterol		
Hypercholesterolemia	443 (39.6%)	1913 (41.6%)
Lipid-lowering medication	107 (9.6%)	486 (10.6%)
Low HDL cholesterol	225 (19.9%)	771 (16.7%)
Body mass index		
<18.5	9 (0.8%)	35 (0.8%)
18.5-25	327 (30.1%)	1455 (32.5%)
>=25	751 (69.1%)	2992 (66.8%)
Diabetes mellitus	212 (18.7%)	525 (11.4%)
Smoking		
Never	328 (29.3%)	1651 (36.3%)
Past	515 (46.0%)	2197 (48.3%)
Current	276 (24.7%)	698 (15.4%)
TIA	194 (15.7%)	407 (8.3%)
Atrial fibrillation	203 (18.4%)	484 (10.6%)

Abbreviations: N = number of persons at risk; HDL = high-density lipoprotein; TIA = transient ischemic attack.
Percentages are calculated without missing values.
Data are presented as mean (standard deviations) or counts (percentages).

Mortality rates

During on average 3.8 (± 4.4) years (median [5th – 95th percentile range]: 2.3 [0 – 13.1] years), 919 persons with a stroke died, 515 (56.0%) due to a cardiovascular cause. During 6.5 (± 4.7) years (median [5th – 95th percentile range]: 5.5 [0.6 – 15.8] years), 2,654 persons without a stroke died, 806 (30.4%) due to a cardiovascular cause. Mortality rates after stroke were highest in the first 30 days (2.56 events per person-year, 95% CI 2.22; 2.95) and remained stable after one year around 0.14 (95% CI 0.13; 0.15) events per person-year. These mortality rates were 51.7 (95% CI 34.3; 78.0) times higher compared to the matched stroke-free group in the first 30 days and remained almost two times higher (Mortality rate ratio (MRR) 1.9, 95% CI 1.7; 2.1)) from 1 year onwards after the stroke (Figure 2). The increased risk of death in the first 30 days was mainly due to cardiovascular causes (MRR 183.7; 95% CI 81.7; 413.4) compared to the reference population). Similarly, the increased risk of death after 1 year was mainly due to cardiovascular causes (MRR 2.4, 95% CI 2.0; 2.9) compared to the reference population), but stroke patients also had a higher risk of non-cardiovascular deaths (MRR 1.6, 95% CI 1.4; 1.9) (Supplementary Figure I).
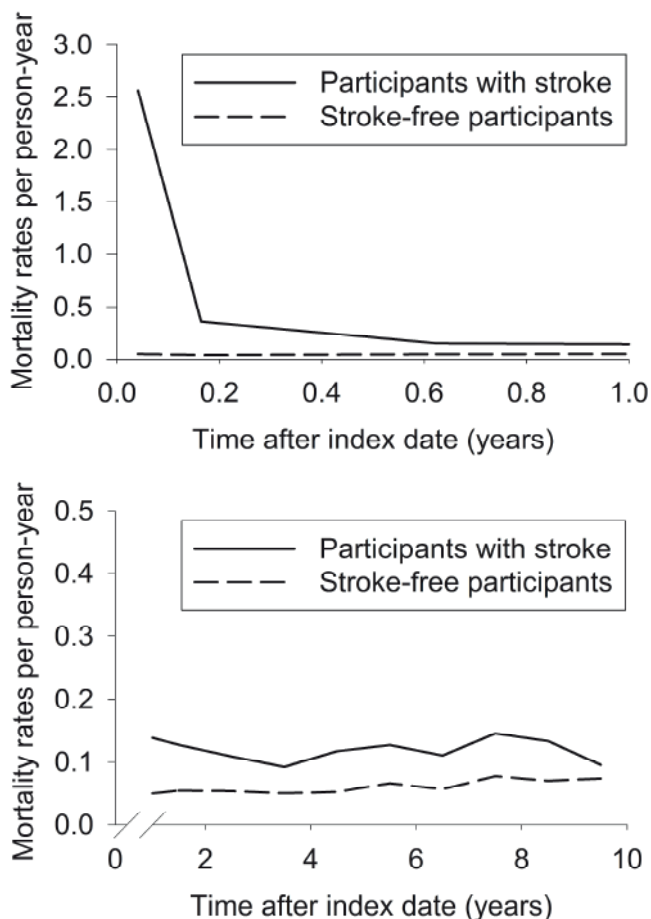
Figure 2. Mortality rates
Age- and sex adjusted mortality rates in the first year (left) or one year after baseline (right).

Population attributable risk

Persons with stroke had more often hypertension, low HDL cholesterol, diabetes mellitus, previous TIA or atrial fibrillation, and smoked more compared to stroke-free persons (Table 2).

In persons with stroke strongest harmful associations were found for underweight (HR 2.06, 95% CI 1.05; 4.03), diabetes mellitus (HR 1.48, 95% CI 1.24; 1.75), atrial fibrillation (HR 1.44, 95% CI 1.21; 1.70), current smoking (HR 1.40, 95% CI 1.15; 1.72), and hypertension (HR 1.07, 95% CI 0.88; 1.31). In combination with the prevalence, the most important modifiable risk factors were smoking (PAR 0.13, 95% CI 0.06; 0.25), diabetes mellitus (PAR 0.06, 95% CI 0.04; 0.10), atrial fibrillation (PAR 0.06, 95% CI 0.03; 0.09), and hypertension (PAR 0.06, 95% CI 0.00; 0.57). Smaller attributable risks were found for a low HDL cholesterol (PAR 0.02, 95% CI 0.00; 0.10) and being underweight (PAR 0.01, 95% CI 0.00; 0.02). The total proportion of deaths that was attributable to these modifiable risk factors combined was 0.27 (95% CI 0.14; 0.45) (Table 2).

Table 2. Cardiovascular risk factors and risk of mortality after stroke

Risk factors	Persons with stroke n/N = 919/1237			Persons without stroke n/N = 2654/4928		
	Prevalence (%)	HR (95% CI)	PAR (95% CI)	Prevalence (%)	HR (95% CI)	PAR (95% CI)
Hypertension	84.8%[a]	1.07 (0.88; 1.31)	0.06 (0.00; 0.57)	76.2%[a]	1.09 (0.99; 1.20)	0.06 (0.02; 0.19)
Cholesterol						
Hypercholesterolemia	39.9%	0.88 (0.77; 1.02)	NA	41.8%	1.03 (0.95; 1.12)	0.001 (0.00; 1.00)
Treated	8.6%	0.60 (0.44; 0.81)[a]		9.9%	0.83 (0.70; 0.98)[a]	
Low HDL cholesterol	18.5%[a]	1.12 (0.94; 1.33)	0.02 (0.00; 0.10)	15.9%[a]	1.17 (1.06; 1.29)	0.03 (0.01; 0.05)
BMI						
<18.5	0.7%	1.84 (0.93; 3.63)[a]	NA	0.7%	0.72 (0.45; 1.17)[a]	NA
>=25	70.7%	0.84 (0.73; 0.97)[a]		68.5%	0.95 (0.88; 1.04)	
Underweight	0.7%	2.06 (1.05; 4.03)[a]	0.01 (0.00; 0.02)	0.7%	0.74 (0.46; 1.20)[a]	NA
Diabetes mellitus	17.3%[a]	1.48 (1.24; 1.75)	0.06 (0.04; 0.10)	10.8%[a]	1.35 (1.20; 1.52)	0.03 (0.02; 0.05)
Smoking						
Past	47.7%	1.16 (0.98; 1.37)	0.13 (0.06; 0.25)	49.1%	1.02 (0.93; 1.12)	0.05 (0.02; 0.13)
Current	22.5%[a]	1.40 (1.15; 1.72)		14.4%[a]	1.41 (1.24; 1.60)	
TIA	15.7%[a]	0.93 (0.78; 1.12)	NA	8.3%[a]	1.00 (0.87; 1.13)	NA
Atrial fibrillation	16.8%[a]	1.44 (1.21; 1.70)	0.06 (0.03; 0.09)	10.1%[a]	1.27 (1.14; 1.43)	0.03 (0.02; 0.05)
Total			**0.27 (0.14; 0.45)**			**0.19 (0.12; 0.29)**

Abbreviations: n = number of deaths, N = number at risk; HR = hazard ratio; PAR = population attributable risk; HDL = high-density lipoprotein; BMI = body mass index; TIA = transient ischemic attack; NA = not applicable because the HR is smaller than 1.

Values are hazard ratios (95% confidence intervals) or population attributable risks (95% confidence intervals).

Adjusted for age, sex, time between center visit and index date, and for the categories of hypertension, cholesterol, HDL cholesterol, BMI, diabetes mellitus, smoking, and atrial fibrillation, if appropriate.

[a]Statistically different between persons with and without stroke (p<0.05).

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Table 3. Cardiovascular risk factors and risk of cardiovascular mortality after stroke

Risk factors	Persons with stroke n/N = 515/1237			Persons without stroke n/N = 806/4928		
	Prevalence (%)	HR (95% CI)	PAR (95% CI)	Prevalence (%)	HR (95% CI)	PAR (95% CI)
Hypertension	84.8%[a]	1.02 (0.79; 1.34)	0.02 (0.00; 1.00)	76.2%[a]	1.29 (1.07; 1.56)	0.19 (0.09; 0.35)
Cholesterol						
Hypercholesterolemia	39.9%	0.97 (0.80; 1.16)	NA	41.8%	1.12 (0.96; 1.29)	0.05 (0.01; 0.21)
Treated	8.6%	0.65 (0.44; 0.96)		9.9%	0.95 (0.71; 1.27)	
Low HDL cholesterol	18.5%[a]	1.12 (0.88; 1.42)	0.02 (0.00; 0.18)	15.9%[a]	1.25 (1.04; 1.49)	0.04 (0.02; 0.09)
BMI						
<18.5	0.7%	1.61 (0.65; 4.00)	NA	0.7%	0.88 (0.36; 2.14)	0.08 (0.02; 0.26)
>=25	70.7%	0.83 (0.68; 1.00)[a]		68.5%	1.13 (0.97; 1.32)[a]	
Underweight	0.7%	1.82 (0.74; 4.49)	0.004 (0.00; 0.03)	0.7%	0.81 (0.34; 1.97)	NA
Diabetes mellitus	17.3%[a]	1.77 (1.42; 2.20)	0.09 (0.06; 0.14)	10.8%[a]	1.55 (1.27; 1.90)	0.05 (0.03; 0.08)
Smoking						
Past	47.7%	1.16 (0.93; 1.45)[a]	0.14 (0.06; 0.31)	49.1%	1.30 (1.09; 1.53)[a]	0.17 (0.10; 0.27)
Current	22.5%[a]	1.55 (1.19; 2.02)		14.4%[a]	1.61 (1.27; 2.05)	
TIA	15.7%[a]	1.08 (0.86; 1.36)	0.01 (0.00; 0.25)	8.3%[a]	0.89 (0.70; 1.13)	NA
Atrial fibrillation	16.8%[a]	1.78 (1.45; 2.20)	0.10 (0.06; 0.15)	10.1%[a]	1.54 (1.27; 1.87)	0.06 (0.03; 0.09)
Total			**0.32 (0.15; 0.55)**			**0.49 (0.37; 0.61)**

Abbreviations: n = number of deaths, N = number at risk; HR = hazard ratio; PAR = population attributable risk; HDL = high-density lipoprotein; BMI = body mass index; TIA = transient ischemic attack; NA = not applicable because the HR is smaller than 1.
Values are hazard ratios (95% confidence intervals) or population attributable risks (95% confidence intervals).
Adjusted for age, sex, time between center visit and index date, and for the categories of hypertension, cholesterol, HDL cholesterol, BMI, diabetes mellitus, smoking, and atrial fibrillation, if appropriate.
[a]Statistically different between persons with and without stroke (p<0.05).

In the reference population the PARs of diabetes mellitus (0.03, 95% CI 0.02; 0.05), atrial fibrillation (0.03, 95% CI 0.02; 0.05), and smoking (0.05, 95% CI 0.02; 0.13) were lower compared to the PARs of participants with stroke. For diabetes mellitus and smoking hazard ratios were similar compared to persons with stroke, but the prevalence of both was lower. For atrial fibrillation both the prevalence and the hazard ratio were lower, although the HR only borderline significant (HR 1.27; 95% CI 1.14; 1.43, p for interaction 0.10). In persons without stroke no attributable risk for underweight could be calculated, due to an inversed hazard ratio (HR 0.75, 95% CI 0.46; 1.20, p for interaction 0.02). The PAR for hypertension was similar in the reference population (0.06; 95% CI 0.02; 0.19). In total, the proportion of deaths attributable to cardiovascular risk factors in the reference population was 0.19 (95% CI 0.12; 0.29) (Table 2).

The risk of cardiovascular deaths attributable to cardiovascular risk factors was lower in stroke patients (0.32; 95% CI 0.15; 0.55) compared to the reference population (0.49; 95% CI 0.37; 0.61). In the reference population we found that more cardiovascular deaths were attributable to hypertension, cholesterol, and BMI, whereas in the stroke population more deaths were attributable to diabetes and atrial fibrillation (Table 3). In contrast, in stroke patients more non-cardiovascular deaths were attributable to cardiovascular risk factors than in the reference population (PAR 0.21, 95% CI 0.05; 0.55) in stroke population, PAR 0.07 (95% CI 0.02; 0.22) in reference population) (Supplementary Table I).

In Supplementary Table II and III the stratified analyses for men and women are presented. Men smoked more compared to women and women with a low HDL cholesterol had a higher risk of death after stroke compared to men. In total, the proportion of death attributable to cardiovascular risk factors was 0.29 (95% CI 0.13; 0.53) in men with stroke, 0.42 (95% CI 0.29; 0.57) in men without stroke, 0.25 (95% CI 0.18; 0.34) in women with stroke, and 0.14 (95% CI 0.06; 0.29) in women without stroke. Largest differences between men and women were found for hypertension. In men with stroke more deaths were attributable to hypertension (PAR 0.22, 95% 0.07; 0.52), compared to in men without stroke (PAR 0.07, 95% CI 0.01; 0.28). In women with stroke no deaths were attributable to hypertension, whereas in women without stroke we found a PAR of 0.05 (95% CI 0.01; 0.29). In women we found stronger PARs for diabetes, smoking, and a low HDL cholesterol compared to men, but only a low HDL cholesterol had a significantly higher HR in women compared to men.

Discussion

We found that participants with stroke had a long-term mortality rate that was two-fold higher compared with stroke-free persons. This is partly due to cardiovascular risk factors already present before stroke, since 27% deaths after stroke were attributable to these factors. In total, this was only 8% higher than in people without stroke. Deaths after stroke were mainly cardiovascular, but only 32% of those deaths were attributable to cardiovascular risk factors before stroke, compared to 49% in stroke-free persons. Nevertheless, attributable risks of diabetes mellitus and atrial fibrillation were almost twice as high in persons with stroke compared with stroke-free persons for both all cause and cardiovascular mortality.

Despite the changes in stroke treatment over the last decades with improvement of incidence and mortality rates,[22,34-36] the two-fold higher mortality rate in persons with stroke compared with stroke-free persons is similar to what was found a decade earlier[3-5,12] and in young adults.[6] In other words, the improvement in healthcare of stroke patients has not exceeded the improvement in healthcare experienced in the general population. Hence, the room for improving long-term prognosis after stroke remains substantial. An important contribution of our study is that we investigated and quantified to what extent pre-existent cardiovascular risk factors can contribute to post-stroke survival.

Main risk factors for death after stroke in our study were smoking, diabetes, atrial fibrillation, and in men hypertension. Smoking and diabetes were previously also identified as risk factors, in a study examining factors before stroke,[37] as well as in studies that started at stroke onset and might be influenced by reversed causality.[10, 13, 14] Results with respect to atrial fibrillation were conflicting, some studies did find an increased risk of death,[11,13,16] other studies did not. [10,14] Differences might be due to adjustments for severity, which could actually be a mediator in the pathway, although one study found an effect independent from severity.[13] Hypertension was previously not found as an important risk factor, but the studies did not stratify for sex.[10,13,15,16]

A novelty of our study is that we measured PAR and compared this to a reference population. Although cardiovascular risk factor management is an important cornerstone of treatment after stroke,[8] we found that a quarter of deaths after stroke is attributable to cardiovascular risk factors. An explanation might be that pathologic changes leading to those deaths were already present before the stroke, which is supported by the fact that the risk factors were measured years before the stroke. Aggressive risk factor control after stroke might therefore be too late. Yet, the proportion of cardiovascular deaths attributable to cardiovascular risk factors was lower in the stroke population compared to the reference population. To properly interpret these results, a few considerations need to be taken into account. First, the stroke population suffered many more cardiovascular deaths than the reference population, which is obvious since stroke itself is a cardiovascular disease. Second, this larger number also means that the absolute number of cardiovascular deaths in stroke patients due to cardiovascular risk factors is higher than the reference population, even though the relative proportions (attributable risks) were inversed. Similar reasoning holds for the opposite difference in attributable risks for non-cardiovascular mortality. A third consideration to properly interpret our findings on cardiovascular mortality is that patients with stroke are more aggressively treated after the index date compared to the reference population. That might have reduced some of the harmful effect of cardiovascular risk factors. More importantly, our findings emphasize that other factors too should be explored to understand the causes of death following stroke. This is further emphasized by the 73% of deaths that remain unexplained. Such novel factors may include other pre-stroke risk factors, e.g. genetic factors; post-stroke factors, e.g. proper medical care, response to treatment, or frailty caused by the stroke; and characteristics of the stroke itself, e.g. hemorrhagic transformation or size of the stroke.[11,38,39]

At the same time, we have to consider that the PAR for diabetes mellitus, smoking, and atrial fibrillation was almost twice as high in people with stroke compared to stroke-free people. Differences in PAR can be caused by a difference in effect size or a difference in prevalence.[33] The PAR in our study was mostly driven by the higher prevalence of diabetes mellitus, smoking, and atrial fibrillation in people with stroke, probably because these factors are also important risk factors for the stroke itself.[40]

Interestingly, we found effect modification of stroke for the effect of atrial fibrillation and underweight with stronger effect sizes for death in people with stroke compared to people without stroke. This might be due to more severe strokes and more complications after stroke in people with atrial fibrillation.[41] For people with underweight, the increased risk of death after stroke might be due to frailty in combination with dysphagia.[37,42] Underweight participants might be more vulnerable to undernutrition. Furthermore they may reflect people with a high morbidity and increased risk of both stroke and death, e.g. patients with cancer. This is supported by the finding that especially non-cardiovascular deaths are attributable to underweight. However, due to low numbers these results have to be interpreted with caution.

Strengths of this study are the long follow-up, the availability of cardiovascular risk factors measured before stroke occurrence which avoids reversed causality, and that we could compare people with stroke to a reference population from the same study population. This study also has some limitations. For instance, the PAR might be an overestimation of the amount of deaths that could be prevented, as for some risk factors optimal treatment cannot completely reduce the harmful effect.[40,43] Irreversible damage might already have occurred before starting of the optimal treatment. Moreover, we only used a single measurement of the cardiovascular risk factor before stroke, while the long-term pattern (i.e. trajectory) of a risk factor before and after stroke might further influence the risk of dying. However, we were interested in the effect of pre-stroke risk factors on death after stroke and adding post-stroke information might obscure this effect. We also missed information about physical activity, which is an important risk factor for stroke[44] and possibly for stroke mortality.[37] So our estimates might be an underestimation of the effect of cardiovascular risk factors. Another limitation is that we did not measure severity of stroke, which is an important risk factor for mortality.[11] This could have led to some residual confounding. However, severity is likely to be an intermediate as well in the causal pathway and adjusting might in fact lead to over-adjustment. Furthermore, not all persons with stroke (6.2%) had visited our research center before the stroke occurred. This could have led to selection bias if persons that did come to the center were more healthy. A final consideration is that we had missing values up to 9.7% per variable. We imputed these missing values to prevent selection bias, but this might have led to some misclassification. Furthermore, atrial fibrillation follow-up was only complete until 2010. This could have led to misclassification in participants with an index date after 2010, since their atrial fibrillation status was outdated.

In conclusion, the long-term risk of mortality after stroke remains increased and is almost twice as high compared to persons without stroke. A quarter of stroke deaths might be prevented with optimal cardiovascular prevention and treatment of mainly diabetes, atrial fibrillation, and smoking, starting before stroke. These findings underline the importance of

primary prevention, as it does not only reduce the risk of stroke, but also its long-term prognosis. At the same time, equally important, our findings indicate the need of finding factors that explain the remaining three-quarter of deaths and the doubled risk of death in people with stroke compared to those without stroke, because it cannot only be explained by cardiovascular risk factors.

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Supplementary information
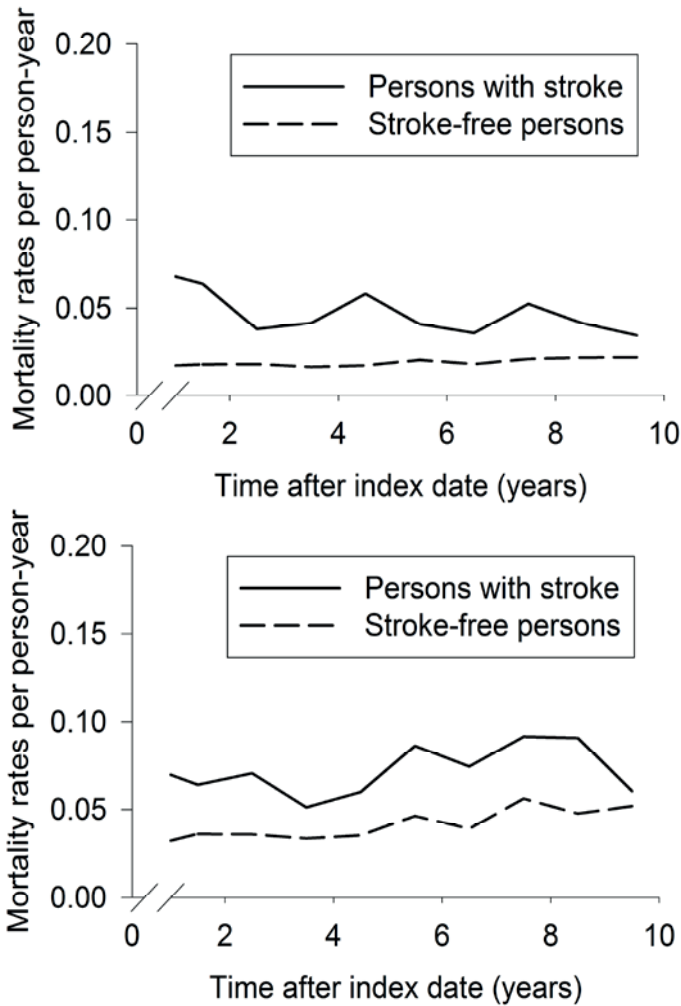
Supplementary Figure I. Cardiovascular and non-cardiovascular mortality rates
Age- and sex adjusted cardiovascular mortality rates (left) and non-cardiovascular mortality rates (right).

Supplementary Table I. Cardiovascular risk factors and non-cardiovascular mortality after stroke

Risk factors	Persons with stroke n/N = 404/1237			Persons without stroke n/N = 1848/4928		
	Prevalence (%)	HR (95% CI)	PAR (95% CI)	Prevalence (%)	HR (95% CI)	PAR (95% CI)
Hypertension	84.8%[a]	1.13 (0.84; 1.51)	0.10 (0.01; 0.63)	76.2%[a]	1.02 (0.91; 1.14)	0.01 (0.00; 0.85)
Cholesterol						
Hypercholesterolemia	39.9%	0.80 (0.64; 0.98)	NA	41.8%	1.00 (0.90; 1.09)	NA
Treated	8.6%	0.54 (0.34; 0.86)		9.9%	0.78 (0.63; 0.96)	
Low HDL cholesterol	18.5%[a]	1.12 (0.87; 1.45)	0.02 (0.00; 0.19)	15.9%[a]	1.14 (1.01; 1.28)	0.02 (0.01; 0.06)
BMI						
<18.5	0.7%	2.18 (0.79; 6.03)	NA	0.7%	0.67 (0.38; 1.19)	NA
>=25	70.7%	0.86 (0.69; 1.07)		68.5%	0.89 (0.81; 0.98)	
Underweight	0.7%	2.40 (0.88; 6.56)	0.01 (0.001; 0.03)	0.7%	0.72 (0.41; 1.27)	NA
Diabetes mellitus	17.3%[a]	1.14 (0.87; 1.51)	0.02 (0.002; 0.15)	10.8%[a]	1.26 (1.09; 1.46)	0.02 (0.01; 0.05)
Smoking						
Past	47.7%	1.14 (0.88; 1.48)	0.10 (0.02; 0.37)	49.1%	0.92 (0.82; 1.03)	NA
Current	22.5%[a]	1.23 (0.90; 1.68)		14.4%[a]	1.32 (1.14; 1.54)	
TIA	15.7%[a]	0.76 (0.57; 1.01)	NA	8.3%[a]	1.04 (0.89; 1.22)	0.004 (0.00; 0.18)
Atrial fibrillation	16.8%[a]	1.02 (0.76; 1.36)	0.003 (0.00; 1.00)	10.1%[a]	1.16 (1.00; 1.33)	0.02 (0.01; 0.05)
Total			0.21 (0.05; 0.55)			0.07 (0.02; 0.22)

Abbreviations: n = number of deaths, N = number at risk; HR = hazard ratio; PAR = population attributable risk; HDL = high-density lipoprotein; BMI = body mass index; TIA = transient ischemic attack; NA = not applicable because the HR is smaller than 1.

Values are hazard ratios (95% confidence intervals) or population attributable risks (95% confidence intervals).

Adjusted for age, sex, time between center visit and index date, and for the categories of hypertension, cholesterol, HDL cholesterol, BMI, diabetes mellitus, smoking, and atrial fibrillation, if appropriate.

[a]Statistically different between persons with and without stroke (p<0.05).

Supplementary Table II. Cardiovascular risk factors and risk of mortality after stroke, men

Risk factors	Men with stroke n/N = 351/490			Men without stroke n/N = 1003/1950		
	Prevalence (%)	HR (95% CI)	PAR (95% CI)	Prevalence (%)	HR (95% CI)	PAR (95% CI)
Hypertension	82.4%[a]	1.35 (0.99; 1.86)	0.22 (0.07; 0.52)	71.8%[ab]	1.10 (0.95; 1.28)	0.07 (0.01; 0.28)
Cholesterol						
Hypercholesterolemia	26.9%[b]	0.69 (0.54; 0.88)[ab]	NA	29.4%[b]	1.00 (0.87; 1.15)[a]	NA
Treated	9.8%	0.53 (0.33; 0.83)		12.6%[b]	0.85 (0.67; 1.08)	
Low HDL cholesterol	29.2%[ab]	0.90 (0.71; 1.14)[ab]	NA	24.7%[ab]	1.25 (1.09; 1.43)[a]	0.06 (0.03; 0.11)
BMI						
<18.5	0.4%	1.57 (0.38; 6.43)	NA	0.5%	0.50 (0.21; 1.22)	NA
>=25	70.0%	0.81 (0.64; 1.02)		67.0%	0.94 (0.82; 1.08)	
Underweight	0.4%	1.74 (0.43; 7.09)	0.002 (0.00; 0.06)	0.5%	0.51 (0.21; 1.25)	NA
Diabetes mellitus	17.3%[a]	1.31 (0.99; 1.75)	0.04 (0.01; 0.13)	10.5%[a]	1.41 (1.16; 1.72)	0.03 (0.02; 0.06)
Smoking						
Past	64.5%[ab]	0.92 (0.57; 1.50)	NA	69.6%[ab]	1.38 (1.09; 1.76)[b]	0.31 (0.18; 0.48)
Current	30.2%[ab]	1.17 (0.71; 1.93)		20.4%[ab]	1.99 (1.53; 2.60)[b]	
TIA	16.7%[a]	1.10 (0.83; 1.46)	0.02 (0.00; 0.27)	7.3%[a]	0.89 (0.70; 1.12)	NA
Atrial fibrillation	15.3%[a]	1.72 (1.30; 2.28)[a]	0.07 (0.04; 0.13)	11.0%[a]	1.16 (0.96; 1.39)[a]	0.02 (0.00; 0.07)
Total			**0.29 (0.13; 0.53)**			**0.42 (0.29; 0.57)**

Abbreviations: n = number of deaths; N = number at risk; HR = hazard ratio; PAR = population attributable risk; HDL = high-density lipoprotein; BMI = body mass index; TIA = transient ischemic attack; NA = not applicable because the HR is smaller than 1.

Values are hazard ratios (95% confidence intervals) or population attributable risks (95% confidence intervals).

Adjusted for age, sex, time between center visit and index date, and for the categories of hypertension, cholesterol, HDL cholesterol, BMI, diabetes mellitus, smoking, and atrial fibrillation, if appropriate.

[a]Statistically different between persons with and without stroke (p<0.05).

[b]Statistically different between men and women (p<0.05).

Supplementary Table III. Cardiovascular risk factors and risk of mortality after stroke, women

Risk factors	Women with stroke n/N = 568/747			Women without stroke n/N = 1651/2978		
	Prevalence (%)	HR (95% CI)	PAR (95% CI)	Prevalence (%)	HR (95% CI)	PAR (95% CI)
Hypertension	86.3%[a]	0.89 (0.69; 1.15)	NA	79.0%[ab]	1.07 (0.94; 1.21)	0.05 (0.01; 0.29)
Cholesterol						
Hypercholesterolemia	48.3%[b]	1.04 (0.87; 1.24)[b]	NA	50.0%[b]	1.04 (0.94; 1.15)	0.01 (0.00; 0.84)
Treated	7.9%	0.69 (0.46; 1.03)		8.1%[b]	0.80 (0.63; 1.02)	
Low HDL cholesterol	11.5%[b]	1.55 (1.20; 1.99)[ab]	0.05 (0.02; 0.09)	10.1%[b]	1.10 (0.95; 1.27)[a]	0.01 (0.00; 0.06)
BMI						
<18.5	0.9%	1.92 (0.86; 4.26)	NA	0.9%	0.86 (0.48; 1.52)	NA
>=25	71.2%	0.84 (0.70; 1.01)		69.5%	0.95 (0.86; 1.06)	
Underweight	0.9%	2.17 (0.99; 4.77)	0.01 (0.00; 0.03)	0.9%	0.89 (0.50; 1.57)	NA
Diabetes mellitus	17.3%[a]	1.69 (1.36; 2.11)	0.08 (0.05; 0.13)	11.0%[a]	1.31 (1.13; 1.53)	0.03 (0.02; 0.05)
Smoking						
Past	36.7%[b]	1.21 (1.00; 1.45)	0.11 (0.06; 0.22)	35.7%[b]	0.98 (0.88; 1.09)[b]	0.01 (0.00; 0.19)
Current	17.4%[ab]	1.48 (1.13; 1.92)		10.4%[ab]	1.29 (1.08; 1.53)[b]	
TIA	15.0%[a]	0.84 (0.66; 1.06)	NA	8.9%[a]	1.05 (0.89; 1.24)	0.00 (0.00; 0.13)
Atrial fibrillation	17.8%[a]	1.32 (1.07; 1.63)	0.05 (0.02; 0.10)	9.5%[a]	1.36 (1.18; 1.58)	0.03 (0.02; 0.06)
Total			**0.25 (0.18; 0.34)**			**0.14 (0.06; 0.29)**

Abbreviations: n = number of deaths; N = number at risk; HR = hazard ratio; PAR = population attributable risk; HDL = high-density lipoprotein; BMI = body mass index; TIA = transient ischemic attack; NA = not applicable because the HR is smaller than 1.
Values are hazard ratios (95% confidence intervals) or population attributable risks (95% confidence intervals).
Adjusted for age, sex, time between center visit and index date, and for the categories of hypertension, cholesterol, HDL cholesterol, BMI, diabetes mellitus, smoking, and atrial fibrillation, if appropriate.
[a]Statistically different between persons with and without stroke (p<0.05).
[b]Statistically different between men and women (p<0.05).

5

Chapter 5.3

Prestroke vascular pathology and the risk of recurrent stroke and post-stroke dementia
Marileen L.P. Portegies, Frank J. Wolters, Albert Hofman, M. Kamran Ikram, Peter J. Koudstaal, M. Arfan Ikram

Abstract

Background and purpose
Improved survival after stroke has increased the necessity to understand factors that determine the risk of long-term clinical hazards after stroke, such as recurrent stroke and dementia. This risk may be largely influenced by exposure to cardiovascular risk factors before the initial stroke. Within the population-based Rotterdam Study, we determined the proportion of recurrent strokes and post-stroke dementia cases that are attributable to pre-stroke cardiovascular risk factors (i.e. the population attributable risk (PAR)).

Methods
We followed 1,237 first-ever stroke patients and 4,928 stroke-free participants, matched on age, sex, examination round, and stroke date (index date), for the occurrence of a new stroke or dementia. We calculated incidence rates in both groups using Poisson regression, and estimated the individual and combined PAR of pre-stroke cardiovascular risk factors (measured on average 4 years before stroke) for both outcomes.

Results
Short-term incidence rates of recurrent stroke and dementia were particularly high, but from one to ten years after stroke, stroke patients retained a threefold increased risk of recurrent stroke and an almost twofold increased risk of dementia compared to people without stroke. In total, 39% (95% CI 18%-66%) of recurrent strokes and 10% (95% CI 0%-91%) of post-stroke dementia cases were attributable to pre-stroke cardiovascular risk factors. These percentages were similar for first-ever stroke and dementia in the matched stroke-free population.

Conclusions
Long-term consequences of stroke are substantially influenced by pre-stroke risk factors, emphasizing the need for optimizing primary prevention. The search for other modifiable factors also needs to be continued.

Introduction

Survival after stroke has improved over the last decades thanks to the implementation of thrombolysis, specialized stroke care units, and better secondary prevention.[1-4] However, stroke is still notorious for its high risk of disability,[5] and prolonged survival will lead to more people suffering from long-term hazard of stroke, such as recurrent stroke and dementia.[6-8] In the first year after stroke, approximately 11.1% suffers a recurrent stroke and 7.4% suffers from dementia.[7,9] Yet, the long-term risks of recurrent stroke and post-stroke dementia are not well documented, nor are the factors that determine this risk.

So far, studies investigating risk factors of stroke outcomes have established that the risk of recurrent stroke is strongly determined by cardiovascular risk factors measured at time of stroke, whilst the risk of post-stroke dementia is driven by characteristics of the stroke itself (i.e. location and severity).[9,10] Yet, it is important to note that risk factors that led to the initial stroke may also predispose to clinical adverse events occurring after stroke. Indeed, we previously showed that 27% of all deaths after stroke can be attributed to risk factors already present before stroke, i.e. hypertension and diabetes mellitus.[11] A key consideration here is that measuring these factors after stroke can suffer from reverse causality, i.e. the levels of these factors may have changed due to the stroke and hence not reflect their long-term pre-stroke level.[12-14] Therefore, studying the true contribution of pre-stroke pathology in post-stroke prognosis requires a population-based study with information on risk factors available prior to the stroke. This contribution can then be quantified using the population attributable risk, which informs about the proportion of incident outcome events that can be attributed to the risk factors under investigation.[15]

Our study had two aims. First, to detect the excess risk of a new stroke and dementia in people with stroke compared to those without. Second, to investigate the role of pre-stroke cardiovascular risk factors on the occurrence of those diseases.

Materials and methods

Setting

This study was conducted within the prospective population-based Rotterdam Study. Details regarding the objectives and design of the study have been described elsewhere.[16]

The original cohort consisted of 7,983 participants aged 55 years and older. These participants were invited for a visit in 1990-1993, 1993-1995, 1997-1999, 2002-2004, and 2009-2011. The cohort was extended with 3,011 participants in 2000. These participants were invited for a visit in 2000-2001, 2004-2005, and 2011-2012. In 2006 another 3,932 subjects were included, aged 45 years and older. They visited the center in 2006-2008. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the "Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)". All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

Study design

Within the original cohort, we first defined an exposed group, including all participants who suffered an incident stroke. For this exposed group, the at risk time for a recurrent stroke and dementia started at the time of stroke. Then, we defined a non-exposed or stroke-free group, consisting of 4 participants per stroke patient, randomly matched to this patient on age (± 1 year), sex, and visiting the same examination round, using an incidence density sampling approach.[17] This non-exposed group was free of stroke at the stroke date of their matched stroke patient (index date) and their time at risk of stroke and dementia was from this index date onwards. Persons could be included as a non-exposed match for multiple stroke patients, and could become exposed themselves later on. Of note, this design is not a case-control study, but is actually a cohort study in which exposure is defined by incident stroke status, and matched individuals are subsequently followed in time for the outcome.[11]

Study population

After excluding participants with prevalent stroke (n=453) and those who refused informed consent for collection of follow-up information (n=238), 14,235 participants were followed for occurrence of stroke. After a mean (±SD) follow-up of 9.6 (±6.0) years, 1,325 participants suffered a stroke. We excluded 82 stroke patients because they lacked any pre-stroke center visit. Remaining 1,243 participants were eligible for the exposed group and matched to people who were non-exposed at their index date. Due to old age, 6 stroke patients could not be matched and 8 could only be matched to 1 or 2 stroke-free participants. Consequently, 1,237 participants with stroke and 4,928 stroke-free participants were eligible for analysis. These non-exposed participants consisted out of 3,377 study participants. 2,079 were included once, 1,298 were included multiple times, of whom 378 became exposed later in time.[11]

For the dementia analyses, we additionally excluded the participants with insufficient baseline or follow-up information (n=188), or with prevalent dementia (n=621). In the people with stroke 186 (15.8%) had prevalent dementia, in the people without stroke 435 (9.1%). In total, 993 dementia-free participants with stroke and 4,363 without stroke were eligible for analysis.

Assessment of stroke

History of stroke at baseline was assessed during the home interview and confirmed by reviewing medical records.[18] From baseline onwards, participants were continuously followed-up for occurrence of first-ever and recurrent stroke through automatic linkage between general practitioners' medical records and the study database. In addition, nursing home physicians' medical records and medical records from participants who moved out of the study district were checked on a regular basis. Additional information was obtained from hospital records. Potential strokes as identified in medical records were checked by research physicians and verified by an experienced vascular neurologist, in accordance with World Health Organization criteria.[18,19] Follow-up for stroke was complete until January 1st 2012 for 96.3% of potential person-years.

Assessment of dementia

For the detection of dementia we used the following 3-step protocol. Screening was done using the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level. Screen-positives (MMSE<26 or GMS organic level >0) underwent an examination and informant interview with the Cambridge Examination for Mental Disorders in the Elderly. Participants suspected of dementia underwent further neuropsychological testing if necessary. Additionally, all participants were continuously followed for dementia, similar to the follow-up for stroke, by automatic linkage of the study database with medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. When clinical neuroimaging was available, it was used for decision-making on the diagnosis.[20] The final diagnosis was determined by a consensus panel, led by a neurologist, in accordance with international criteria.[21,22] Follow-up for dementia was complete until January 1st 2012 for 98.6% of potential person-years.

Assessment of cardiovascular risk factors

Pre-stroke cardiovascular risk factors were obtained from the patient's most recent center date preceding the index date, with the use of interview, physical examinations, and laboratory examinations.[23] For the matched stroke-free participants, these were obtained from the same center visit as their matched stroke patient. Information on medication use and smoking was assessed during the home interview. Body mass index was calculated as weight divided by length squared and categorized in underweight (<18.5 kg/m^2), normal weight (18.5-25 kg/m^2), and overweight (>25 kg/m^2). Hypertension was defined as a blood pressure ≥140/90 mmHg and/or the use of blood pressure-lowering medication with the indication hypertension. Hypercholesterolemia was defined as a total cholesterol >6.2 mmol/L.[24] A low high-density lipoprotein (HDL)-cholesterol was defined as a HDL-cholesterol ≤1.0 mmol/L.[24] Diabetes mellitus was defined as a fasting glucose level ≥7.0 mmol/L, non-fasting glucose level ≥11.0 mmol/L, or use of antidiabetic medication. In the second center visit (1993-1995) of the first cohort, we did not have information on cholesterol and diabetes mellitus status. In the fifth visit of the first cohort and the third visit of the second cohort (2011-2012) we did not have information about hypertension. Therefore, information on these missing values was carried forward from the preceding center visit.

Occurrence of transient ischemic attacks (TIAs) and atrial fibrillation was assessed through active follow-up and verified by standardized definitions similar to the follow-up of stroke.[25,26] Therefore, presence of TIA or atrial fibrillation at any time before stroke was considered a pre-stroke cardiovascular risk factor. However, follow-up for atrial fibrillation was only complete until 2010, which means that we missed people with atrial fibrillation after 2010.

Statistical analysis

The methods for statistical analysis used for this study were described in detail previously.[11]

We used Poisson regression adjusted for age and sex to determine incidence rates of a new stroke and dementia in the group with stroke patients and the stroke-free group. Follow-up started at the index date.

Population attributable risks (PARs) with 95% confidence interval (CI) were calculated using the Interactive Risk Attributable Program (IRAP).[27] A PAR adjusted for confounding is estimated by the following:

$$\text{PAR} = 1 - \sum_{i=1}^{I} \sum_{j=1}^{J} \rho_{ij} \, R_{i|j}^{-1}$$

where

$$R_{i|j} = \frac{\Pr(D=1|X=x_i, C=c_j)}{\Pr(D=1|X=x_1, C=c_j)}$$

and

$$\rho_{ij} = \Pr\left(X = x_i, C = c_j \middle| D = 1\right)$$

given $D = 1$ denoting presence of disease, X denoting exposure with i levels and C denoting a confounder with j levels. The hazard ratio's (HRs) with 95% CI are based on a Poisson model. The proportional hazards assumption was checked by inspecting log minus log plots. We calculated the PAR for each risk factor separately and calculated the combined PAR. The combined PAR included the PARs of modifiable risk factors which were associated with an increased risk of recurrent stroke or dementia.[28] We provide logit transformed 95% CI for the PARs as they are more easily interpretable and more stringent with regard to our combined PAR estimate.[29] Because of small numbers, we did not explore the PAR for subtypes of stroke and dementia. Differences in prevalence of risk factors between participants with stroke and stroke-free participants and between men and women were calculated using the Chi-Square test. We explored potential effect modification by presence of stroke or sex by using an interaction term of the stroke or sex status with the potential risk factor.

Information on cardiovascular risk factors was missing for up to 9.7% of cases, and imputed based on the other covariates using multiple imputation with 5 imputation sets. The mean of these 5 sets was used to implement in the Interactive Risk Attributable Program. In each model, we adjusted for age, sex, time between center date and index date, and for the categories of hypertension, cholesterol, HDL cholesterol, BMI, diabetes, smoking, and atrial fibrillation, if appropriate.

All analyses were performed using IRAP version 2.2 (US National Cancer Institute), IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY) and SAS version 9.3 (SAS Institute Inc., Cary, NC).

Results

Characteristics of the study population are reported in Table 1.

The participants with stroke had a mean age of 79.9 (±8.7) years, 60.4% was female and cardiovascular risk factors were measured at a mean of 3.7 (±3.2) years before the stroke. Due to matching, these characteristics were similar in participants without stroke.

Table 1. Baseline characteristics

	People with stroke N=1237	Matched people without stroke N=4928
Age at index date (years)	79.9 (8.7)	79.8 (8.7)
Time-interval between center visit and index date (years)	3.7 (3.2)	3.7 (3.4)
Female	747 (60.4%)	2978 (60.4%)
Hypertension	1007 (85.2%)	3653 (76.2%)
Blood pressure-lowering medication	575 (47.7%)	1989 (41.3%)
Cholesterol		
Hypercholesterolemia	443 (39.6%)	1913 (41.6%)
Lipid-lowering medication	107 (9.6%)	486 (10.6%)
Low HDL cholesterol	225 (19.9%)	771 (16.7%)
Body mass index		
<18.5 kg/m^2	9 (0.8%)	35 (0.8%)
18.5-25 kg/m^2	327 (30.1%)	1455 (32.5%)
>=25 kg/m^2	751 (69.1%)	2992 (66.8%)
Diabetes mellitus	212 (18.7%)	525 (11.4%)
Smoking		
Never	328 (29.3%)	1651 (36.3%)
Past	515 (46.0%)	2197 (48.3%)
Current	276 (24.7%)	698 (15.4%)
TIA	194 (15.7%)	407 (8.3%)
Atrial fibrillation	203 (18.4%)	484 (10.6%)

Abbreviations: N = number of persons at risk; HDL = high-density lipoprotein; TIA = transient ischemic attack.
Percentages are calculated without missing values.
Data are presented as mean (standard deviations) or counts (percentages).

Incidence rates

A recurrent stroke occurred in 233 stroke patients during 4,085 person-years of follow-up (median [interquartile range]: 1.68 [0.12-5.00] years), and 520 stroke-free participants suffered a first-ever stroke during 30,710 person-years (median 5.22 [2.47-9.08] years). Of the stroke patients, 7.0% suffered a recurrent stroke in the first year, and 18% in the first ten years after stroke. Corresponding incidence rates, adjusted for age and sex, were 0.11 (95% CI 0.09; 0.13) per person-year for the first year and 0.07 (95% CI 0.06; 0.08) per person-year for the first ten years. Compared to the people without stroke, incidence rates in stroke patients were 5.67 (95% CI 4.23; 7.61) times higher in the first year, particularly due to high rates in the first month, and subsequently declined. In year 1 to 10 incidence rates were 3.16 (95% CI 2.59; 3.85) times higher, but the difference between people with and without stroke was only significant in year 1 to 5 (Figure 1).

During 3,859 person-years of follow-up (median 2.41 [0.18-6.05] years), 146 stroke patients developed dementia, and during 27,670 person-years of follow-up (median 5.43 [2.59-9.14] years) 653 participants without stroke developed dementia. Of the stroke patients, 3.9% developed dementia in the first year after stroke, and 14.4% in the first 10 years after stroke. Corresponding incidence rates, adjusted for age and sex, were 0.05 (95% CI 0.04; 0.07) per

person-year in the first year and 0.04 (95% CI 0.03; 0.04) per person-year in the first ten years. Compared to people without stroke, incidence rates in stroke patients were 3.32 (95% CI 2.26; 4.87) times higher in the first year, particularly due to high rates in the first month. Following, incidence rates declined, but remained 1.73 (95% CI 1.38; 2.17) times increased in stroke patients versus those without in year 1 to 10 after stroke. This difference was only significant in year 1 to 5 (Figure 1).
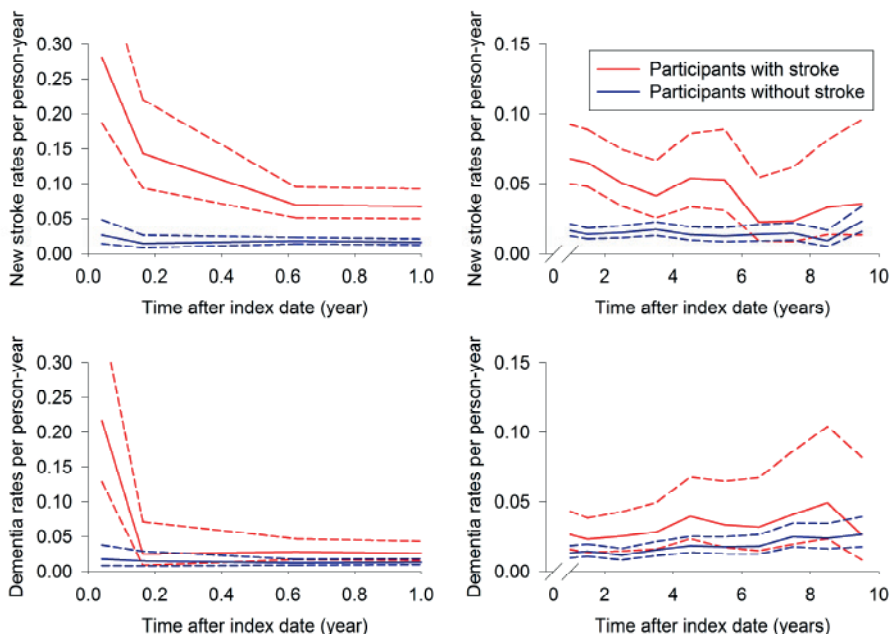
Figure 1. Rates of new stroke and dementia in participants with and without stroke at baseline
Age- and sex adjusted incidence rates for a new stroke (upper panels) or dementia (lower panels), in the first year (left) or starting one year after baseline (right). Dotted lines represent 95% confidence intervals.

Population attributable risk for recurrent stroke
Participants with stroke had a worse cardiovascular profile than the participants without stroke, more people had hypertension, diabetes, a previous TIA, atrial fibrillation, and more people were current smokers (Table 2).

Only underweight, diabetes mellitus, and a previous TIA conferred a significantly increased risk of recurrent stroke in stroke patients. In persons without stroke at the index date, hypertension, low HDL cholesterol, current smoking, TIA, and atrial fibrillation were associated with an increased risk of stroke. The total PAR for all risk factors combined was similar in people with stroke (0.39, 95% CI 0.18; 0.66) compared to those without (0.39, 95% CI 0.24; 0.57). In people with stroke main factors attributable to a new stroke were hypertension (PAR 0.22, 95% CI 0.06; 0.55), low HDL cholesterol (PAR 0.05, 95% CI 0.01; 0.20), diabetes (PAR 0.06, 95% CI 0.02;

0.16), smoking (PAR 0.08, 95% CI 0.004; 0.65) and a previous TIA (PAR 0.06, 95% CI 0.02; 0.16). In people without stroke the PARs of a low HDL cholesterol and a previous TIA were similar, the PARs of smoking and diabetes were lower, and the PARs of hypertension (PAR 0.30, 95% CI 0.18; 0.45)) and atrial fibrillation (PAR 0.03, 95% CI 0.01; 0.09) were higher (Table 2).

Stratified for sex, we found that pre-stroke cardiovascular risk factors had a stronger effect on recurrent strokes in women than in men. Diabetes had a significantly stronger effect, and hypertension and atrial fibrillation a borderline significantly stronger effect in women than men. For diabetes we also found an interaction with stroke in women: diabetes conferred a higher risk of a new stroke in women with stroke compared to those without. In total, women with stroke had a PAR of 0.59 (95% CI 0.33; 0.81), women without stroke of 0.43 (95% CI 0.27; 0.60), men with stroke of 0.14 (95% CI 0.04; 0.40) and men without stroke 0.40 (95% CI 0.21; 0.62) (Supplementary Table I and II).

Population attributable risk for dementia

We found no significant associations of pre-stroke cardiovascular risk factors with dementia (Table 3). Additionally, the attributable risk of cardiovascular risk factors was low, namely 0.10 (95% CI 0.001; 0.91) in people with stroke versus 0.09 (95% CI 0.02; 0.37) in those without. Although we did not find any statistically significant differences in hazard ratio's between men and women for the risk of dementia, the attributable risk in men with stroke was 0.36 (95% CI 0.05; 0.84), in men without stroke 0.47 (95% CI 0.19; 0.76), in women with stroke 0.10 (95% CI 0.00; 0.74) and in women without stroke 0.10 (95% CI 0.01; 0.54). The higher attributable risk in men was particularly driven by hypertension (Supplementary Table III and IV).

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Table 2. Cardiovascular risk factors and risk of a new stroke

Risk factors	People with stroke n/N = 233/1237			People without stroke n/N = 520/4928		
	Prevalence (%)	HR (95% CI)	PAR (95% CI)	Prevalence (%)	HR (95% CI)	PAR (95% CI)
Hypertension	84.8%[a]	1.34 (0.91; 1.96)	0.22 (0.06; 0.55)	76.2%[a]	1.56 (1.23; 1.97)	0.30 (0.18; 0.45)
Cholesterol						
Hypercholesterolemia	39.9%	0.71 (0.54; 0.94)	NA	41.8%	0.92 (0.76; 1.10)	NA
Treated	8.6%	0.67 (0.40; 1.14)		9.9%	0.72 (0.49; 1.05)	
Low HDL cholesterol	18.5%[a]	1.28 (0.92; 1.78)	0.05 (0.01; 0.20)	15.9%[a]	1.43 (1.15; 1.77)	0.06 (0.03; 0.12)
BMI						
<18.5 kg/m^2	0.7%	3.98 (1.41; 11.20)	NA	0.7%	0.71 (0.23; 2.23)	0.003 (0.00; 1.00)
>=25 kg/m^2	70.7%	0.70 (0.53; 0.94)[a]		68.5%	1.01 (0.83; 1.22)[a]	
Underweight	0.7%	4.99 (1.80; 13.85)[a]	0.01 (0.004; 0.05)	0.7%	0.71 (0.23; 2.21)[a]	NA
Diabetes mellitus	17.3%[a]	1.47 (1.05; 2.06)	0.06 (0.02; 0.16)	10.8%[a]	1.12 (0.85; 1.49)	0.01 (0.00; 0.13)
Smoking						
Past	47.7%	1.09 (0.77; 1.55)	0.08 (0.004; 0.65)	49.1%	0.94 (0.76; 1.15)	0.02 (0.00; 0.87)
Current	22.5%[a]	1.17 (0.79; 1.75)		14.4%[a]	1.48 (1.12; 1.95)	
TIA	15.7%[a]	1.47 (1.06; 2.05)	0.06 (0.02; 0.16)	8.3%[a]	1.93 (1.51; 2.47)	0.07 (0.05; 0.12)
Atrial fibrillation	16.8%[a]	0.89 (0.60; 1.33)	NA	10.1%[a]	1.32 (1.01; 1.72)	0.03 (0.01; 0.09)
Total			**0.39 (0.18; 0.66)**			**0.39 (0.24; 0.57)**

Abbreviations: n = number of new strokes, N = number at risk; HR = hazard ratio; PAR = population attributable risk; HDL = high-density lipoprotein; BMI = body mass index; TIA = transient ischemic attack; NA = not applicable because the HR is smaller than 1.

Values are hazard ratios (95% confidence intervals) or population attributable risks (95% confidence intervals).

Percentages are calculated including imputed values.

Adjusted for age, sex, time between center visit and index date, and for the categories of hypertension, cholesterol, HDL cholesterol, BMI, diabetes mellitus, smoking, and atrial fibrillation, if appropriate.

[a]Statistically different between people with and without stroke (p<0.05).

Table 3. Cardiovascular risk factors and risk of dementia

Risk factors	People with stroke n/N = 146/993			People without stroke n/N = 653/4363		
	Prevalence (%)	HR (95% CI)	PAR (95% CI)	Prevalence (%)	HR (95% CI)	PAR (95% CI)
Hypertension	85.3%[a]	1.09 (0.66; 1.80)	0.07 (0.00; 0.98)	75.3%[a]	1.06 (0.88; 1.29)	0.05 (0.002; 0.57)
Cholesterol						
Hypercholesterolemia	39.5%	0.98 (0.69; 1.41)	NA	41.5%	0.98 (0.83; 1.15)	NA
Treated	9.0%	1.01 (0.56; 1.82)		10.5%	0.70 (0.49; 0.99)	
Low HDL cholesterol	19.8%[a]	0.96 (0.61; 1.50)	NA	16.2%[a]	0.90 (0.72; 1.12)	NA
BMI						
<18.5 kg/m^2	0.4%	-	NA	0.7%	0.76 (0.28; 2.06)	NA
>=25 kg/m^2	71.5%[a]	0.85 (0.59; 1.23)		68.2%[a]	0.97 (0.82; 1.14)	
Diabetes mellitus	16.7%[a]	1.19 (0.75; 1.87)	0.02 (0.002; 0.31)	10.4%[a]	1.16 (0.90; 1.50)	0.01 (0.002; 0.08)
Smoking						
Past	49.5%	1.08 (0.71; 1.64)	NA	50.5%	0.97 (0.81; 1.16)	NA
Current	22.6%[a]	0.57 (0.32; 1.03)		14.8%[a]	0.91 (0.69; 1.20)	
TIA	15.9%[a]	1.06 (0.68; 1.64)	0.01 (0.00; 0.97)	8.0%[a]	1.27 (0.98; 1.66)	0.02 (0.01; 0.07)
Atrial fibrillation	16.6%[a]	0.68 (0.39; 1.16)	NA	9.6%[a]	1.26 (0.97; 1.62)	0.02 (0.01; 0.07)
Total			**0.10 (0.001; 0.91)**			**0.09 (0.02; 0.37)**

Abbreviations: n = number of dementia cases, N = number at risk; HR = hazard ratio; PAR = population attributable risk; HDL = high-density lipoprotein; BMI = body mass index; TIA = transient ischemic attack; NA = not applicable because the HR is smaller than 1.
Values are hazard ratios (95% confidence intervals) or population attributable risks (95% confidence intervals).
Percentages are calculated including imputed values.
Adjusted for age, sex, time between center visit and index date, and for the categories of hypertension, cholesterol, HDL cholesterol, BMI, diabetes mellitus, smoking, and atrial fibrillation, if appropriate.
[a]Statistically different between people with and without stroke (p<0.05).

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Discussion

We found that stroke patients remain on an increased risk of a new stroke and dementia up to at least 5 years after the stroke compared to people without stroke. Pre-stroke cardiovascular risk factors accounted for a large proportion of recurrent strokes (39%), driven by a particularly high proportion in women. In addition, they contributed to 10% of post-stroke dementia cases. These proportions were similar for first-ever stroke and dementia in stroke-free participants.

Established cardiovascular risk factors for recurrent stroke are hypertension, previous symptomatic vascular disease, hypercholesterolemia, diabetes mellitus, smoking, and atrial fibrillation.[10,30,31] Correspondingly, secondary prevention with blood pressure lowering, lipid lowering and antithrombotic therapy has shown a beneficial effect in clinical trials.[4] Improved secondary prevention may explain why the long-term increased risk for a new stroke in stroke patients compared to stroke-free participants was only three-times increased in our study, compared to four to nine times in studies performed ten to twenty years earlier in time.[32,33] A three-times increased risk is substantial nevertheless. Previously, we showed that pre-stroke cardiovascular risk factors contributed to 27% of deaths after stroke, suggesting that pre-stroke pathology plays a large role in the prognosis after stroke.[11] In this study, we further expand on those findings by showing that pre-stroke cardiovascular risk factors contributed to 39% of recurrent strokes. These factors may have caused irreversible damage before the initial stroke and thus before secondary prevention started. However, it seems secondary prevention does have an effect in men, since the contribution of pre-stroke cardiovascular risk factors in men with stroke was smaller compared to those without. So another explanation may be that secondary prevention in women is not optimal yet.[34] The attributable risk in women with stroke was 59% and higher than in stroke-free women. Factors that appeared to be particularly important in women were hypertension, smoking, and diabetes mellitus. The PAR is estimated based on a combination of prevalence and effect size. Women with stroke had a higher prevalence of those factors compared to women without. Additionally, diabetes mellitus even conferred a higher risk of a new stroke in women with stroke compared to those without. Possible explanations are that diabetes leads to more severe strokes[35] or that diabetes leads to more lacunar strokes, which are known for their high recurrence rates.[36] However, this requires further exploration.

Regarding dementia, incidence rates of previous studies were heterogeneous, due to differences in study design, inclusion-, and exclusion criteria.[9] Moreover, many studies on post-stroke dementia were hospital-based and likely to be subject to attrition and selection bias.[37,38] The few available population-based studies determined that stroke increases the risk of dementia two-fold.[9] We extended these findings by showing that this increased risk remained up to five years after stroke. Although post-stroke dementia often has a vascular component,[39] previous studies only identified diabetes mellitus and atrial fibrillation as risk factors.[9,40] A bigger role was determined for stroke severity and subclinical cerebrovascular disease.[9,40] Although we expected that those factors might be mediators in the association between pre-stroke risk factors and post-stroke dementia, this was unlikely considering the low PAR for pre-stroke cardiovascular risk factors we observed. The low PAR might be the consequence of competing

risks. Dementia has a long preclinical phase and since pre-stroke cardiovascular risk factors are also related to a high risk of dying, people with many risk factors may not survive long enough to develop dementia.

Our findings also underline the importance of the identification of novel modifiable factors for both recurrent stroke and dementia. These may include genetic factors, novel biomarkers, presence of pre-stroke subclinical vascular disease, or response to treatment.[40,41]

Strengths of our study are the population-based setting, the long follow-up, the availability of cardiovascular risk factors measured before time of stroke, and the thorough follow-up for first-ever stroke, recurrent stroke and dementia. We also acknowledge that several considerations need to be taken into account for a proper interpretation of the PAR in our study. The PAR is often interpreted as the percentage of cases that can be prevented if risk factors under study were to be eliminated. On the one hand, the PAR assumes that risk factors are entirely modifiable and preventable and that elimination of the risk factors will completely reduce the harmful effect. However, in the real world risk factors may have caused irreversible damage over the course of years before start of any preventive treatment, which may thus lead to an overestimation of the PAR.[42,43] On the other hand, in our study we only estimated the effect that removal of a risk factor will have on clinical events after the stroke, while assuming that the stroke will still occur. However, in practice removal of a risk factor may also prevent stroke itself, irrespective of any clinical events after the stroke.[42] In turn, prevention of stroke will additionally reduce the burden of post-stroke events. Therefore, the PAR in our study may be an underestimate of the actual percentage that can be prevented. A limitation of our study is that we were unable to implement the long-term effect of risk factors. This could have led to an underestimation of the true attributable risk. Moreover, 6.2% of the population did not visit the research center before the stroke, this could have led to selection bias if they were less healthy.

In conclusion, patients with stroke suffer at least five years a three-times increased risk of a new stroke and an almost doubled risk of dementia compared to people without stroke. Pre-stroke cardiovascular risk factors contribute to 39% of recurrent strokes, mainly apparent in women. Additionally, they contribute to 10% of post-stroke dementia cases. This emphasizes the need of optimizing primary prevention. At the same time, our results encourage exploration of other potentially modifiable factors.

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Supplementary information

Supplementary Table I. Cardiovascular risk factors and risk of a new stroke in men

Risk factors	Men with stroke n/N = 100/490			Men without stroke n/N = 180/1950		
	Prevalence (%)	HR (95% CI)	PAR (95% CI)	Prevalence (%)	HR (95% CI)	PAR (95% CI)
Hypertension	82.4%[a]	1.00 (0.59; 1.68)	0.00 (0.00; 1.00)	71.8%[ab]	1.37 (0.95; 1.98)	0.21 (0.06; 0.52)
Cholesterol						
Hypercholesterolemia	26.9%[b]	0.61 (0.39; 0.95)	NA	29.4%[b]	1.19 (0.86; 1.63)	0.03 (0.00; 0.74)
Treated	9.8%	0.43 (0.17; 1.08)		12.6%[b]	0.69 (0.37; 1.29)	
Low HDL cholesterol	29.2%[ab]	1.17 (0.76; 1.80)	0.05 (0.002; 0.54)	24.7%[ab]	1.54 (1.12; 2.10)	0.12 (0.05; 0.25)
BMI						
<18.5 kg/m^2	0.4%	2.59 (0.35; 19.27)	NA	0.5%	0.70 (0.10; 5.18)	0.07 (0.003; 0.66)
>=25 kg/m^2	70.0%	0.83 (0.53; 1.31)		67.0%	1.12 (0.80; 1.55)	
Underweight	0.4%	2.82 (0.38; 20.83)	0.01 (0.00; 0.12)	0.5%	0.67 (0.09; 4.88)	NA
Diabetes mellitus	17.3%[a]	0.75 (0.41; 1.39)[b]	NA	10.5%[a]	1.06 (0.64; 1.77)	0.01 (0.00; 0.96)
Smoking						
Past	64.5%[ab]	1.06 (0.38; 2.92)	0.002 (0.00; 1.00)	69.6%[ab]	0.79 (0.49; 1.28)	NA
Current	30.2%[ab]	0.90 (0.31; 2.58)		20.4%[ab]	1.33 (0.78; 2.29)	
TIA	16.7%[a]	1.73 (1.05; 2.86)	0.09 (0.03; 0.25)	7.3%[a]	2.66 (1.79; 3.95)	0.11 (0.06; 0.19)
Atrial fibrillation	15.3%[a]	0.50 (0.22; 1.15)	NA	11.0%[a]	0.93 (0.58; 1.50)	NA
Total			0.14 (0.04; 0.40)			0.40 (0.21; 0.62)

Abbreviations: n = number of new strokes, N = number at risk; HR = hazard ratio; PAR = population attributable risk; HDL = high-density lipoprotein; BMI = body mass index; TIA = transient ischemic attack; NA = not applicable because the HR is smaller than 1.

Values are hazard ratios (95% confidence intervals) or population attributable risks (95% confidence intervals).

Adjusted for age, sex, time between center visit and index date, and for the categories of hypertension, cholesterol, HDL cholesterol, BMI, diabetes mellitus, smoking, and atrial fibrillation, if appropriate.

[a]Statistically different between people with and without stroke (p<0.05).

[b]Statistically different between men and women (p<0.05).

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Supplementary Table II. Cardiovascular risk factors and risk of a new stroke in women

Risk factors	Women with stroke n/N = 133/747			Women without stroke n/N = 340/2978		
	Prevalence (%)	HR (95% CI)	PAR (95% CI)	Prevalence (%)	HR (95% CI)	PAR (95% CI)
Hypertension	86.3%[a]	1.84 (0.98; 3.47)	0.42 (0.15; 0.75)	79.0%[ab]	1.66 (1.21; 2.27)	0.34 (0.19; 0.53)
Cholesterol						
Hypercholesterolemia	48.3%[b]	0.85 (0.59; 1.22)	NA	50.0%[b]	0.83 (0.67; 1.04)	NA
Treated	7.9%	0.96 (0.50; 1.83)		8.1%[b]	0.76 (0.46; 1.24)	
Low HDL cholesterol	11.5%[b]	1.48 (0.90; 2.42)	0.05 (0.01; 0.19)	10.1%[b]	1.36 (1.00; 1.85)	0.04 (0.01; 0.11)
BMI						
<18.5 kg/m^2	0.9%	3.23 (0.92; 11.28)	NA	0.9%	0.70 (0.17; 2.83)	NA
>=25 kg/m^2	71.2%	0.65 (0.45; 0.95)		69.5%	0.96 (0.75; 1.21)	
Underweight	0.9%	4.30 (1.26; 14.69)	0.02 (0.00; 0.07)	0.9%	0.72 (0.18; 2.89)	NA
Diabetes mellitus	17.3%[a]	2.25 (1.49; 3.41)[ab]	0.13 (0.07; 0.24)	11.0%[a]	1.14 (0.81; 1.60)[a]	0.01 (0.00; 0.18)
Smoking						
Past	36.7%[b]	0.98 (0.66; 1.47)	0.06 (0.00; 0.60)	35.7%[b]	0.97 (0.76; 1.23)	0.02 (0.00; 0.57)
Current	17.4%[ab]	1.45 (0.98; 2.35)		10.4%[ab]	1.43 (1.00; 2.05)	
TIA	15.0%[a]	1.41 (0.89; 2.23)	0.05 (0.01; 0.21)	8.9%[a]	1.60 (1.17; 2.20)	0.05 (0.02; 0.11)
Atrial fibrillation	17.8%[a]	1.15 (0.72; 1.83)	0.02 (0.00; 0.46)	9.5%[a]	1.56 (1.13; 2.15)	0.05 (0.02; 0.11)
Total			**0.59 (0.33; 0.81)**			**0.43 (0.27; 0.60)**

Abbreviations: n = number of new strokes, N = number at risk; HR = hazard ratio; PAR = population attributable risk; HDL = high-density lipoprotein; BMI = body mass index; TIA = transient ischemic attack; NA = not applicable because the HR is smaller than 1.

Values are hazard ratios (95% confidence intervals) or population attributable risks (95% confidence intervals).

Adjusted for age, sex, time between center visit and index date, and for the categories of hypertension, cholesterol, HDL cholesterol, BMI, diabetes mellitus, smoking, and atrial fibrillation, if appropriate.

[a]Statistically different between people with and without stroke (p<0.05).
[b]Statistically different between men and women (p<0.05).

Supplementary Table III. Cardiovascular risk factors and risk of dementia in men

Risk factors	Men with stroke n/N = 59/425			Men without stroke n/N = 186/1816		
	Prevalence (%)	HR (95% CI)	PAR (95% CI)	Prevalence (%)	HR (95% CI)	PAR (95% CI)
Hypertension	82.8%[a]	1.53 (0.66; 3.57)	0.31 (0.03; 0.85)	71.4%[ab]	1.07 (0.76; 1.51)	0.05 (0.00; 0.91)
Cholesterol						
Hypercholesterolemia	28.0%[b]	0.85 (0.47; 1.55)	NA	29.7%[b]	0.80 (0.58; 1.10)	NA
Treated	9.4%[a]	0.85 (0.35; 2.05)		12.9%[ab]	0.48 (0.26; 0.90)	
Low HDL cholesterol	29.6%[ab]	1.04 (0.59; 1.85)	0.01 (0.00; 1.00)	24.8%[ab]	0.87 (0.62; 1.22)	NA
BMI						
<18.5 kg/m^2	0.2%	-	NA	0.4%	-	0.09 (0.01; 0.56)
>=25 kg/m^2	70.6%	0.70 (0.40; 1.25)		66.9%	1.14 (0.82; 1.57)	
Diabetes mellitus	17.4%[a]	1.17 (0.58; 2.37)	0.03 (0.00; 0.74)	10.7%[a]	1.06 (0.64; 1.76)	0.005 (0.00; 0.98)
Smoking						
Past	65.9%[b]	0.87 (0.27; 2.84)	NA	69.4%[b]	1.64 (0.93; 2.91)	0.36 (0.12; 0.71)
Current	28.9%[ab]	0.47 (0.13; 1.73)		20.1%[ab]	1.60 (0.83; 3.07)	
TIA	16.7%[a]	1.19 (0.60; 2.36)	0.03 (0.00; 0.70)	7.4%[a]	0.84 (0.47; 1.50)	NA
Atrial fibrillation	15.1%[a]	0.77 (0.33; 1.80)	NA	11.1%[ab]	1.39 (0.91; 2.12)	0.04 (0.01; 0.16)
Total			**0.36 (0.05; 0.84)**			**0.47 (0.19; 0.76)**

Abbreviations: n = number of dementia cases, N = number at risk; HR = hazard ratio; PAR = population attributable risk; HDL = high-density lipoprotein; BMI = body mass index; TIA = transient ischemic attack; NA = not applicable because the HR is smaller than 1.
Values are hazard ratios (95% confidence intervals) or population attributable risks (95% confidence intervals).
Adjusted for age, sex, time between center visit and index date, and for the categories of hypertension, cholesterol, HDL cholesterol, BMI, diabetes mellitus, smoking, and atrial fibrillation, if appropriate.
[a]Statistically different between people with and without stroke (p<0.05).
[b]Statistically different between men and women (p<0.05).

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Supplementary Table IV. Cardiovascular risk factors and risk of dementia in women

Risk factors	Women with stroke n/N = 87/568			Women without stroke n/N = 467/2547		
	Prevalence (%)	HR (95% CI)	PAR (95% CI)	Prevalence (%)	HR (95% CI)	PAR (95% CI)
Hypertension	87.1%[a]	0.87 (0.47; 1.64)	NA	78.1%[ab]	1.06 (0.84; 1.33)	0.04 (0.00; 0.79)
Cholesterol						
Hypercholesterolemia	48.1%[b]	1.10 (0.69; 1.76)	0.07 (0.00; 0.85)	49.8%[b]	1.04 (0.86; 1.26)	0.01 (0.00; 0.99)
Treated	8.6%	1.20 (0.54; 2.65)		8.8%[b]	0.83 (0.54; 1.26)	NA
Low HDL cholesterol	12.5%[b]	0.89 (0.42; 1.86)	NA	10.1%[b]	0.90 (0.66; 1.21)	NA
BMI						
<18.5 kg/m^2	0.5%	-	NA	0.9%	1.00 (0.37; 2.70)	NA
>=25 kg/m^2	72.2%	0.97 (0.60; 1.58)		69.1%	0.90 (0.74; 1.10)	
Diabetes mellitus	16.2%[a]	1.23 (0.67; 2.25)	0.03 (0.00; 0.41)	10.2%[a]	1.21 (0.89; 1.63)	0.02 (0.00; 0.09)
Smoking						
Past	37.3%[b]	1.05 (0.66; 1.67)	NA	37.0%[b]	0.90 (0.74; 1.10)	NA
Current	17.8%[ab]	0.53 (0.25; 1.13)		11.0%[ab]	0.83 (0.58; 1.18)	
TIA	15.3%[a]	1.04 (0.58; 1.86)	0.01 (0.00; 1.00)	8.3%[a]	1.46 (1.08; 1.96)	0.03 (0.01; 0.09)
Atrial fibrillation	17.8%[a]	0.60 (0.30; 1.20)	NA	8.5%[ab]	1.20 (0.87; 1.65)	0.01 (0.00; 0.09)
Total			**0.10 (0.00; 0.74)**			**0.10 (0.01; 0.54)**

Abbreviations: n = number of dementia cases, N = number at risk; HR = hazard ratio; PAR = population attributable risk; HDL = high-density lipoprotein; BMI = body mass index; TIA = transient ischemic attack; NA = not applicable because the HR is smaller than 1.

Values are hazard ratios (95% confidence intervals) or population attributable risks (95% confidence intervals).

Adjusted for age, sex, time between center visit and index date, and for the categories of hypertension, cholesterol, HDL cholesterol, BMI, diabetes mellitus, smoking, and atrial fibrillation, if appropriate.

[a]Statistically different between people with and without stroke (p<0.05).
[b]Statistically different between men and women (p<0.05).

Chapter 5.4

Higher education is associated with a lower risk of dementia after a stroke or TIA

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* These authors contributed equally to the manuscript

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Abstract

Background and purpose
Higher education is associated with a lower risk of dementia, possibly because of a higher tolerance to subclinical neurodegenerative pathology. Whether higher education also protects against dementia after clinical stroke or transient ischemic attack (TIA) remains unknown.

Methods
Within the population-based Rotterdam Study, 12,561 participants free of stroke, TIA, and dementia were followed for occurrence of stroke, TIA and dementia. Across the levels of education, associations of incident stroke or TIA with subsequent development of dementia, and differences in cognitive decline following stroke or TIA, were investigated.

Results
During 124,862 person-years, 1,463 persons suffered a stroke or TIA, 1,158 persons developed dementia, of whom 186 developed dementia after stroke or TIA. Risk of dementia after a stroke or TIA compared to no stroke or TIA, was highest in the low education category, HR 1.46 (95% CI 1.18; 1.81), followed by intermediate education, HR 1.36 (95% CI 1.03; 1.81). No significant association was observed in high education category, HR 0.62 (95% CI 0.25; 1.54). In gender stratified analyses, decrease in risk of dementia with increasing education was significant only in men.

Conclusion
Higher education is associated with a lower risk of dementia after stroke or TIA, particularly in men, which might be explained by a higher cognitive reserve.

Introduction

Higher education is associated with a lower risk of dementia, which is thought to be explained by a higher cognitive reserve.[1,2] The cognitive reserve hypothesis postulates that people with a higher reserve can tolerate more neurodegenerative pathology and maintain brain function for longer than people with low reserve, before the damage manifests clinically as dementia.[3-5] In the context of Alzheimer's disease, people with higher cognitive reserve can have more senile plaques and amyloid- β deposits before the disease manifests clinically.[6-9]

Although vascular disease is also an important risk factor for dementia including Alzheimer's disease,[10] it is less known if people with higher education, and thus more cognitive reserve, can also tolerate more cerebrovascular damage before dementia occurs. Evidence does suggest, however, that subclinical vascular lesions such as white matter lesions[11] or silent brain infarcts[12] result in less cognitive decline in people with higher education. Since clinical cerebrovascular events such as stroke and transient ischemic attack (TIA) are related to more tissue damage than silent lesions,[13] it remains to be established whether higher education also protects against dementia after clinical events. More importantly, stroke and TIA have a high clinical impact and may lead to considerable morbidity, which is further aggravated by sequelae like dementia.[14,15] Hence, identification of (modifiable) factors that act as protectors against dementia following a stroke or TIA is of major public health and clinical importance. Finally, given the differences in risk factors,[16] education categories,[17] and incidence of stroke and dementia in men and women,[18,19] the association between stroke or TIA and consequent dementia across education categories needs to be explored for sex differences.

Therefore, in this population-based study we investigated whether educational level used as a marker of cognitive reserve, is associated with a lower risk of dementia after a stroke or TIA. We also examined differences in cognitive decline after a stroke or TIA across levels of education.

Materials and Methods

Setting and study population
This study was part of the population-based Rotterdam Study.[20] In 1990, 7,983 persons aged 55 years and older were recruited. In 2000, the cohort was expanded by 3,011 persons aged 55 and older, and in 2006 in a second expansion wave, an additional 3,932 persons aged 45 and older were added. Of these total 14,926 participants, 527 with prevalent dementia, and 902 with insufficient baseline information for dementia or no consent for the follow-up of stroke, TIA or dementia were excluded. We also excluded 354 persons with prevalent stroke, 324 with prevalent TIA, and 258 with missing education information, resulting in 12,561 participants eligible for the analyses of incident dementia.

For the analyses of cognition, only participants who suffered a stroke or TIA between the two follow-up rounds in 2004-2008 and 2009-2013 were eligible, as these rounds comprised the most comprehensive cognitive test battery. In these examination rounds, 7,039 persons participated, of which 34 participants with no consent for collection of follow-up data, 435

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participants with prevalent stroke or TIA at the first test date, 3 participants with prevalent dementia, 80 participants with insufficient information for dementia at the first test date, and 83 with missing data on education were excluded. Of the remaining 6,404 participants, 5,588 had at least one cognitive test in both follow-up rounds. 205 of these participants suffered a stroke or TIA between the two follow-up rounds and were therefore eligible for analyses.

The Rotterdam Study is approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the "Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)". A written informed consent was obtained from all participants.

Educational level as a measure of cognitive reserve

Educational level as marker of cognitive reserve can be measured based on years of education or degree of literacy. We used the latter since it measures educational attainment more directly and might therefore be a better marker of cognitive reserve.[21] Participants were classified as having low (primary, unfinished secondary, and lower vocational), intermediate (secondary or intermediate vocational), or high education (higher vocational or university).

Assessment of stroke and TIA

At baseline, history of stroke and TIA was assessed using home interviews and verified using medical records. From baseline onwards, participants were continuously followed-up for occurrence of stroke and TIA through a computerized linkage between the study database and medical records of general practitioners (GPs). This data linkage system is highly efficient in the Dutch situation where the GPs receive all medical information about their patients if they contact any medical caregiver or professional, including specialists. Additionally, nursing home physicians' files and files from GPs of participants that moved out of the study area were checked on a regular basis. Information from GPs and hospital records was collected from participants with a potential stroke or TIA. Research physicians reviewed the information and an experienced vascular neurologist verified the strokes according to World Health Organization criteria.[22,23] We defined TIAs as temporary attacks with presence of focal symptoms, which are attributable to dysfunction of one arterial territory of the brain.[24] Follow-up for stroke and TIA was complete until 2013 for 98.5% of potential person-years.[25]

Assessment of dementia

Participants were screened for dementia at baseline and follow-up examinations using a three-step protocol.[26] First, screening was done using the Mini-Mental State Examination (MMSE)[27] and the Geriatric Mental Schedule (GMS) organic level. Second, screen-positives (MMSE < 26 or GMS organic level > 0) subsequently underwent an examination and informant interview with the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX). Participants who were suspected of having dementia, underwent if necessary, further neuropsychological testing. Additionally, the total cohort was continuously monitored for dementia through computerized linkage between the study database and digitized medical records from GPs and the Regional Institute for Outpatient Mental Health Care. Third, a consensus panel led by a

neurologist decided on the final diagnosis in accordance with standard criteria using the DSM-III-R criteria for dementia, the NINCDS-ADRDA for Alzheimer disease,[28] and the NINCDS-AIREN criteria for vascular dementia.[29] Follow-up for dementia was complete until 2013 for 98.4% of potential person-years.

Cognitive tests

From 2004-2008 and 2009-2013, participants underwent extensive cognitive testing. Executive function was assessed by the Stroop test (interference task) which tests attention and concentration, Letter-Digit Substitution Task which tests processing speed, and Verbal Fluency Test which assesses verbal fluency. Memory was assessed by the 15-Word verbal Learning test including both immediate and delayed recall. Fine motor skills and coordination was assessed by the Purdue pegboard test for both hands.[30] A higher score indicates a better cognitive performance for all tests, except the Stroop test in which a higher score indicates a worse performance as it measures time to complete the task.

Covariates

Smoking habits and medication use were assessed during the home interview. Participants were categorized into current, former and never smokers. Body mass index was calculated as weight in kilograms/height in meters squared. Total cholesterol and high-density lipoprotein cholesterol were measured in serum in mmol/L. Blood pressure was measured twice at the right arm in sitting position at the research center and average of two blood pressure readings was used. Diabetes mellitus type 2 was diagnosed as fasting blood glucose ≥ 7.00 mmol/L, or use of anti-diabetic medication evaluated by interview and pharmacy records.[31] Cognitive score was assessed using the Mini-Mental State Examination.[27] Since the study population included 3 cohorts from the Rotterdam Study, cohort was also used as a covariate.

Statistical analyses

We examined the risk of dementia in people with stroke or TIA as compared to people without stroke or TIA using Cox proportional hazard models. Adherence to the proportional hazards assumption was tested by plotting smoothed Schoenfeld residuals against time; no violations of the assumption were identified. Stroke or TIA was used as time varying exposure, which took into account the incident cases of stroke or TIA as they occurred during follow-up. Participants were censored at date of dementia, date of death, or last date of follow-up, whichever came first. Subsequently, we examined this association across levels of education by stratifying on educational level as well as by including an interaction term. In secondary analyses, we examined this association in men and women separately since levels of education differ between older men and women, as well as their risk factors for stroke.[16]

Subsequently, we tested whether education is associated with change in cognitive test scores after a stroke or TIA using linear regression models. This was conducted in a subgroup of participants with stroke or TIA, for which cognitive test scores were available both before and after the stroke or TIA. Using low education level as the reference, we first examined the association of education with cognitive test scores before stroke or TIA. Second, we examined

the association of education with cognitive test scores after stroke or TIA. Third, the association of education with the change in cognitive tests scores after stroke or TIA was examined. This was tested by performing linear regression of education with cognitive test scores after stroke or TIA, adjusting for the test score before stroke or TIA. In these analyses, an interaction between cognitive performance and sex was also tested.

Finally, we tested cognitive decline in persons with a stroke or TIA altogether, as compared to persons without a stroke or TIA, across levels of education.

As sensitivity analyses, we repeated the dementia analyses combining the intermediate and high education categories.

For all analyses, two models were fitted. Model 1 was adjusted for age and sex only (where applicable). Model 2 was additionally adjusted for education level (where applicable), body mass index, smoking, total and high-density lipoprotein cholesterol, systolic and diastolic blood pressure, lipid- and blood pressure-lowering medication, diabetes mellitus type 2, MMSE, and study cohort. The analyses of change in cognition were additionally adjusted for time between the two cognitive examinations. The dementia analyses were adjusted for covariates using their baseline values, while cognition analyses were adjusted using the values of covariates from the first visit of cognition assessment. Missing values on covariates ($< 6\%$) were handled by multiple imputations.

Data were analyzed using the Stata Software Version 13 (StataCorp, College Station, TX, USA) and IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY).

Results

This study included 12,561 dementia-, TIA-, and stroke-free participants at baseline. Table 1 summarizes baseline characteristics of the study population. People with higher education were younger and more frequently men. During a mean follow-up of 9.9 ± 5.2 years, 1,463 persons suffered a stroke or TIA and 1,158 persons were diagnosed with dementia. Of these 1,158, 186 persons developed dementia after a stroke or TIA.

Differences in baseline characteristics of persons excluded and those included in the analyses are summarized in Supplementary Table I.

Of the total 1,463 stroke cases or TIAs, the low education category had 449 (8.5%) stroke cases and 300 (5.7%) TIAs. The intermediate education category suffered 331 (6.2%) strokes and 245 (4.6%) TIAs, whereas the high education category had 64 (3.3%) stroke cases and 74 (3.8%) TIAs.

Table 1. Baseline characteristics of the study population

	Levels of education		
Characteristics	Low N=5,299	Intermediate N=5,342	High N=1,920
Age, years	67.8 (9.9)[bc]	63.3 (8.8)[ac]	60.0 (8.2)[ab]
Women	3754 (70.8)[bc]	2838 (53.1)[ac]	735 (38.3)[ab]
Study cohort			
First cohort	3463 (65.4)[bc]	2402 (45.0)[ac]	532 (27.7)[ab]
Second cohort	910 (17.2)[bc]	1344 (25.2)[ac]	470 (24.5)[ab]
Third cohort	926 (17.5)[bc]	1596 (29.9)[ac]	918 (47.8)[ab]
Body mass index, kg/m^2	27.2 (4.2)[bc]	26.8 (4.1)[ac]	26.5 (3.9)[ab]
Smoking			
Never	1,993 (37.9)[c]	1,558 (29.3)[c]	539 (28.1)[ab]
Former	2,018 (38.4)[bc]	2,459 (46.2)[a]	950 (49.6)[a]
Current	1,247 (23.7)[bc]	1,301 (24.5)[ac]	428 (22.3)[ab]
Total cholesterol, mmol/L	6.4 (1.3)[bc]	6.1 (1.2)[ac]	5.8 (1.2)[ab]
High-density lipoprotein cholesterol, mmol/L	1.4 (0.4)[bc]	1.4 (0.4)[ac]	1.4 (0.4)[ab]
Lipid lowering medication	409 (7.7)	556 (10.4)	222 (11.6)
Systolic blood pressure, mmHg	139.8 (22.2)	137.3 (20.9)	135.3 (21.1)
Diastolic blood pressure, mmHg	76.0 (12.0)[bc]	78.0 (11.6)[ac]	79.8 (11.7)[ab]
Blood pressure lowering medication	1,313 (24.8)[c]	1,131 (21.2)	350 (18.3)[a]
Mini Mental State Examination, points	27.2 (2.1)[bc]	28.2 (1.5)[ac]	28.6 (1.4)[ab]
Diabetes mellitus type 2	417 (8.6)	390 (7.7)	107 (5.8)

Abbreviations: N = number of persons included in study.
Values are means (standard deviation) or counts (percentage).
[a] Significantly different from people with low education (p<0.05), after age and sex adjustment - if applicable.
[b] Significantly different from people with intermediate education (p<0.05), after age and sex adjustment - if applicable.
[c] Significantly different from people with high education (p<0.05), after age and sex adjustment - if applicable.

People with a stroke or TIA had an increased risk of dementia compared to participants without stroke or TIA (multivariable adjusted hazard ratio (HR) 1.42 (95% CI 1.20; 1.67)). In analyses stratified for education, this risk was highest in persons with low education, HR 1.46 (95% CI 1.18; 1.81), followed by those with intermediate education, HR 1.36 (95% CI 1.03; 1.81). In the high education group, people with a stroke or TIA did not have an increased risk of dementia compared to people without a stroke or TIA, HR 0.62 (95% CI 0.25; 1.54) (Table 2). Stratification by gender showed a similar pattern of associations, which was more pronounced in men than in women (Table 3). Interaction testing of educational level with stroke or TIA on the risk of dementia yielded p-value 0.65 in the overall population, 0.05 in men, and 0.82 in women.

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Table 2. Risk of dementia after stroke or TIA by levels of education

		Dementia		
			Model 1	Model 2
	n/N[a]	n/N[b]	HR (95% CI)	HR (95% CI)
Total population	1,158/12,561	186/1,463	1.40 (1.19; 1.65)	1.42 (1.20; 1.67)
Strata of education				
Low education	732/5,299	112/749[c]	1.47 (1.19; 1.81)	1.46 (1.18; 1.81)
Intermediate education	364/5,342	68/576[c]	1.38 (1.04; 1.82)	1.36 (1.03; 1.81)
High education	62/1,920	6/138[c]	0.74 (0.31; 1.77)	0.62 (0.25; 1.54)
p-value for interaction[d]			0.65	0.65

Abbreviations: TIA = transient ischemic attack.
Values are hazard ratios with 95% confidence intervals.
Model 1: adjusted for age and sex.
Model 2: adjusted for age, sex, education (where applicable), study cohort, Mini-Mental state Examination score, body mass index, smoking, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication use, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication use, and diabetes mellitus type 2.
[a] n/N = number of dementia cases/total number of participants at risk for dementia.
[b] n/N = number of dementia cases after stroke or TIA/total number of stroke or TIA.
[c] Low education = 300 TIAs, 449 strokes; Intermediate education = 245 TIAs, 331 strokes; High education = 64 TIAs, 74 strokes.
[d] Interaction between presence of stroke or TIA and educational level for the risk of dementia.

Table 3. Risk of dementia after a stroke or TIA by levels of education in men and women

	Dementia							
	Men				Women			
			Model 1	Model 2			Model 1	Model 2
	n/N[a]	n/N[b]	HR (95% CI)	HR (95% CI)	n/N[a]	n/N[b]	HR (95% CI)	HR (95% CI)
Total population	360/5,234	70/618	1.56 (1.18; 2.06)	1.66 (1.25; 2.20)	798/7327	116/845	1.33 (1.08; 1.63)	1.32 (1.08; 1.62)
Strata of education								
Low education	147/1,545	29/202	2.10 (1.36; 3.23)	2.21 (1.43; 3.43)	585/3,754	83/547	1.33 (1.04; 1.70)	1.32 (1.03; 1.69)
Intermediate education	172/2,504	36/320	1.36 (0.91; 2.03)	1.45 (0.97; 2.17)	192/2838	32/256	1.41 (0.95; 2.09)	1.40 (0.94; 2.08)
High education	41/1,185	5/96	0.87 (0.33; 2.30)	0.72 (0.25; 2.06)	21/735	1/42	0.33 (0.04; 2.61)	0.24 (0.03; 2.37)
p-value for interaction[c]			0.08	0.05			0.85	0.82

Abbreviations: TIA = transient ischemic attack.

Model 1: adjusted for age.

Model 2: adjusted for age, education (where applicable), study cohort, Mini-Mental state Examination score, body mass index, smoking, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication use, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication use, and diabetes mellitus type 2.

[a] n/N: number of dementia cases/total number of participants at risk for dementia.

[b] n/N: number of dementia cases after stroke or TIA/total number of stroke or TIA.

[c] Interaction between presence of stroke or TIA and educational level for the risk of dementia.

People with high education scored better on 15-Word verbal learning test (both immediate and delayed recall), Verbal fluency test, and Letter Digit Substitution task both before and after stroke or TIA (Table 4). When we studied the change in cognitive test scores from before to after stroke or TIA, we found that that people with high education declined less in delayed recall compared to people with low education, β 1.33 (95% CI 0.24; 2.43). Effect sizes of the Stroop interference task and Verbal fluency test also suggested a lower decline in the higher education group, although only borderline significant. We did not observe an interaction between cognitive performance and sex, which is why we did not stratify on sex in these analyses.

When comparing cognitive decline in those with a stroke or TIA to those without, we found that people with a stroke or TIA had a stronger decline on the Stroop test, immediate recall, and delayed recall than people without stroke or TIA in low and intermediate education categories (Supplementary Table II). This difference was not observed in the high education category. However, interaction testing of educational level with stroke or TIA on cognitive decline only gave a borderline significant interaction for the Stroop test (p=0.08).

In the sensitivity analyses for risk of dementia, our results did not change after combining the intermediate and high education categories (data not shown).

Table 4. Change in cognitive test scores after stroke or TIA by levels of education

	Nª	Stroop interference task (seconds)	LDST (correct answers)	VFT (animal names)	15-WLT-Immediate recall (correct answers)	15-WLT-Delayed recall (correct answers)	Purdue Pegboard (number of pins placed)
				Difference (95% confidence intervals)			
Before stroke or TIA							
Low education	73	Ref	Ref	Ref	Ref	Ref	Ref
Intermediate education	89	-0.12 (-6.54; 6.30)	3.88 (1.62; 6.15)	1.32 (-0.41; 3.04)	2.52 (0.42; 4.62)	1.33 (0.32; 2.35)	0.55 (0.01; 1.08)
High education	43	-3.93 (-11.88; 4.02)	5.77 (2.98; 8.57)	5.11 (3.01; 7.21)	4.61 (2.07; 7.16)	2.03 (0.80; 3.25)	0.32 (-0.33; 0.97)
After stroke or TIA							
Low education	73	Ref	Ref	Ref	Ref	Ref	Ref
Intermediate education	89	-3.04 (-11.19; 5.11)	3.28 (1.07; 5.48)	1.60 (-0.21; 3.41)	3.46 (1.33; 5.60)	1.30 (0.30; 2.29)	0.18 (-0.42; 0.78)
High education	43	-11.40 (-21.51; -1.30)	4.82 (2.10; 7.55)	4.72 (2.51; 6.92)	4.87 (2.29; 7.44)	2.39 (1.19; 3.60)	-0.09 (-0.82; 0.64)
				Difference in change in cognition (95% confidence intervals)			
Decline after stroke or TIA							
Low education	73	Ref	Ref	Ref	Ref	Ref	Ref
Intermediate education	89	-3.07 (-9.84; 3.70)	0.64 (-1.01; 2.29)	0.88 (-0.68; 2.45)	2.06 (0.20; 3.92)	0.63 (-0.26; 1.52)	0.01 (-0.57; 0.59)
High education	43	-9.13 (-17.64; -0.63)	0.93 (-1.14; 3.01)	1.94 (-0.09; 3.96)	2.13 (-0.20; 4.46)	1.33 (0.24; 2.43)	-0.22 (-0.92; 0.48)

Abbreviations: LDST = Letter-Digit Substitution Task; VFT = Verbal Fluency Test; 15-WLT = 15-Word Learning Test; N = number of persons with at least one cognitive test.

Estimates represent differences in test score, and differences in change in cognitive test scores as compared to the low education category, with 95% confidence intervals. A higher score indicates a better cognitive performance for all tests (scores), except the Stroop test (time taken to finish the task, in seconds) in which a higher score indicates a worse performance.

Change in cognition is defined as cognition after stroke or TIA, adjusted for cognition before stroke or TIA.

Estimates are adjusted for age, sex, study cohort, body mass index, smoking, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication use, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication use, and diabetes mellitus type 2. Estimates for change in cognition are additionally adjusted for time between the two examination dates.

ª Low education: 42 TIAs, 26 strokes, 5 both stroke and TIA; Intermediate education 50 TIAs, 35 strokes, 4 both stroke and TIA; high education: 26 TIA, 16 strokes, 1 both stroke and TIA.

Discussion

This population-based study showed that stroke or TIA increased the risk of subsequent dementia in persons with low and intermediate education, but not in persons with high education. Additionally, as compared to people with low education, those with high education not only scored better on cognitive tests both before and after stroke or TIA, but also declined less in memory and executive function after a stroke or TIA.

It is known that people with stroke or TIA have an increased risk of dementia compared to those without stroke or TIA,[24,32] but we showed that this effect was dependent upon the level of education.

Previously, clinical studies have identified low education as a risk factor for dementia in patients with stroke, but because of the clinical setting, a comparison to risk of dementia in persons without stroke was lacking.[15,33-35] It is specifically this comparison with persons without stroke that provides evidence for higher education to be protective against post-stroke dementia. Therefore, a major novelty of our study is that we were able to make this comparison, and importantly showed that in persons with high education, stroke or TIA did not increase the risk of dementia. This differing effect across levels of education has two major probable explanations.

One likely explanation is the cognitive reserve, which is an established concept and has been shown to protect against Alzheimer pathology. Cognitive reserve is thought to be built by cognitively enduring activities such as education and occupation complexity. Our results are novel as they demonstrate the protective role of cognitive reserve against clinical cerebrovascular pathology. Perhaps people with higher education, and consequently higher cognitive reserve are more resilient to the damage caused by a stroke or TIA either due to better efficiency, or more capacity or flexibility of brain networks already present before the damage (neural reserve), or because of better compensation for the damage (neural compensation). Neural compensation pertains to the ability of persons with higher cognitive reserve, to form collateral networks in the brain, when the usual neuronal networks are compromised by the vascular damage.[4,36,37] Studies have suggested that cognitively stimulating activities, which are mostly experienced during education, not only promote neurogenesis, but also upregulate Brain Derived Neurotrophic Factor (BDNF) which in turn promotes plasticity.[3,37]

However, an alternative explanation is that people with higher education have a more favorable environment including a healthier lifestyle, better compliance to treatment, and better access to healthcare. Such a favorable environment might lead to less severe strokes, perhaps better detection of less severe strokes, and more importantly, early hospitalization and thus fewer complications after stroke. In our study, we adjusted for the potential cardiovascular risk factors. Although associations did not change meaningfully, these adjustments might not fully address every aspect of a favorable environment as pointed out above. This was also reflected in our data, as we found relatively fewer strokes compared to TIAs with increasing educational level.

In analyses stratified on gender, we found that the decrease in the risk of dementia after a stroke or TIA with increasing education was only significant in men. This suggests a stronger

protective effect of cognitive reserve against dementia following stroke in men. However, only few women had dementia after a stroke or TIA in the high education group, which could have affected our power, and might explain the findings. For both men and women, we did not observe a higher risk in the high education category. Alternatively, perhaps in women from older generation birth cohorts, education is less representative of their cognitive reserve than in men, particularly for West-European populations. In our study, many women were born in a period when girls were not equally encouraged for education as boys, and often only completed limited years of education, which was not reflective of their potential. Instead, they quit school to work at home. Therefore, in this group, educational level might not be the best proxy for cognitive reserve and thus obscured any associations in women. The time spent on leisure activities including social, physical and recreational activities might have been a better proxy for cognitive reserve, but we did not have that information in our study.

The finding in our study of less cognitive decline in the high education category in people with stroke or TIA further supports the role of cognitive reserve. Unlike previous studies which only had information on cognitive decline after stroke or TIA,[34,35] we had cognition assessments both before and after the stroke or TIA. This allowed to demonstrate that the impact of a stroke or TIA on executive function and memory was smaller in people with high education, suggesting that persons with higher education not only have a better cognition in the first place, they can also adapt better to the cerebrovascular damage, indicating cognitive reserve.

Strengths of this study include a large population-based sample representative of different levels of education, a long and robust follow-up of incident TIA, stroke, and dementia, and the availability of cognitive tests before and after stroke or TIA. However there are certain limitations. First, only education as a measure of cognitive reserve was available. Activities in later life, such as occupational complexity or leisure activities including recreation, physical and social engagements could not be taken into account, which might be important particularly in older adults. Education might also reflect a better socio-economic status and thus a better access to healthcare. Nevertheless, these other markers of cognitive reserve might have the same limitations as the educational level, and education remains the most used measure of cognitive reserve in existing literature.[36,38] Second, we could not adjust for brain reserve in our study, since brain volumes were not available in this population. This might have led to an overestimation of results, as the observed associations could partly be explained by brain reserve. Another limitation is that we did not have information about the severity of stroke. It is possible that people with higher education had less severe strokes leading to less brain damage and therefore a smaller risk of dementia. Third, we did not have enough cases of dementia in the high education category for women and therefore the effect estimates might be underpowered. Finally, complete cognitive testing was available in a subgroup of our study population only, therefore the results might be influenced by selection. Since people with severe strokes primarily stop attending the research center, our results are only applicable to those with less severe stroke or TIA.

In conclusion, these results suggest that higher education is associated with a lower risk of dementia after cerebrovascular events, particularly in men. Future studies should explore the mechanisms underlying this protective effect as well as investigate whether improvement of

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cognitive reserve later in life, for instance using cognitively stimulating activities, might delay or prevent dementia in stroke patients.

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Supplementary information

Supplementary Table I. Comparison of baseline characteristics of participants included and not included in the analyses

Characteristics	Excluded N=2,364	Included in dementia analyses N=12,561	Included in cognition analyses 5,588	Not included in cognition analyses 6,973
		Participants		
Age, years	72.8 (12.3)	64.7 (9.6)[a]	63.1 (8.8)[b]	72.6 (11.0)
Women	1,496 (63.3)	7,327 (58.3)[a]	3,219 (57.6)	4,108 (58.9)
Body mass index, kg/m^2	26.6 (4.2)	26.9 (4.1)[a]	27.7 (4.2)	27.6 (4.5)
Smoking				
Never	730 (36.1)	4,090 (32.7)[a]	1,614 (29.2)	943 (27.7)
Former	745 (36.9)	5,428 (43.4)[a]	2,851 (51.6)	1,753 (51.5)
Current	545 (27.0)	2,976 (23.8)[a]	1,064 (19.2)	705 (20.7)
Total cholesterol, mmol/L	6.0 (1.3)	6.2 (1.2)[a]	5.6 (1.0)[b]	5.5 (1.0)
High-density lipoprotein cholesterol, mmol/L	1.3 (0.4)	1.4 (0.4)[a]	1.4 (0.4)	1.4 (0.4)
Lipid lowering medication	245 (10.5)	1,187 (9.4)[a]	1,185 (21.3)	765 (22.1)
Systolic blood pressure, mmHg	141.6 (23.3)	138.0 (21.5)[a]	139.6 (20.6)[b]	149.2 (23.7)
Diastolic blood pressure, mmHg	75.8 (13.1)	77.4 (11.8)[a]	81.4 (10.7)[b]	80.1 (11.9)
Blood pressure lowering medication	609 (25.9)	2,794 (22.3)[a]	1,426 (25.5)[b]	1,293 (18.5)
Mini Mental State Examination, points	24.1 (5.8)	27.8 (1.8)[a]	28.1 (1.7)[b]	26.9 (3.1)
Diabetes mellitus type 2	236 (11.4)	753 (6.0)[a]	525 (9.5)[b]	1,191 (30.1)

Values are means (standard deviation) or counts (percentage).
[a] Significantly different (P-value<0.05) from persons in the excluded group.
[b] Significantly different from the group not included in the cognition analysis.

5

Supplementary Table II. Change in cognitive test scores in people with a stroke or TIA versus people without a stroke or TIA by levels of education

	n/N	Stroop interference task (seconds)	LDST (correct answers)	VFT (animal names)	15-WLT-Immediate recall (correct answers)	15-WLT-Delayed recall (correct answers)	Purdue Pegboard (number of pins placed)
			Difference in change in cognition (95% confidence intervals)				
Low education	73/1687	3.52 (-0.56; 7.61)	-0.40 (-1.39; 0.58)	-0.55 (-1.57; 0.47)	-1.73 (-3.05; -0.41)	-0.56 (-1.14; 0.01)	-0.05 (0.81; -0.40)
Intermediate education	89/2673	3.30 (0.49; 6.11)	-0.77 (-1.62; 0.09)	-0.77 (-1.70; 0.17)	-0.55 (-1.64; 0.55)	-0.58 (-1.09; -0.08)	-1.02 (0.31; -0.49)
High education	43/1228	-0.49 (-3.23; 2.25)	-1.28 (-2.53; -0.03)	0.13 (-1.22; 1.48)	-0.38 (-1.20; 0.44)	0.00 (-0.38; 0.37)	-0.33 (0.13; -0.77)
p-value for interaction[a]		0.08	0.37	0.60	0.28	0.35	0.29

Abbreviations: LDST = Letter-Digit Substitution Task; VFT = Verbal Fluency Test; 15-WLT = 15-Word Learning Test; n = number of people with a stroke or TIA; N = total number of persons with at least one cognitive test.
Estimates represent differences in change in cognitive test scores in people with a stroke or TIA as compared to people without stroke or TIA, with 95% confidence intervals. Change in cognition is defined as cognition at follow-up, adjusted for baseline cognition. A higher score indicates a better cognitive performance for all tests (scores), except the Stroop test (time taken to finish the task, in seconds) in which a higher score indicates a worse performance.
Estimates are adjusted for age, sex, study cohort, time between the two examination dates, body mass index, smoking, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication use, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication use, and diabetes mellitus type 2.
[a] interaction between presence of stroke or TIA and educational level for the risk of dementia.
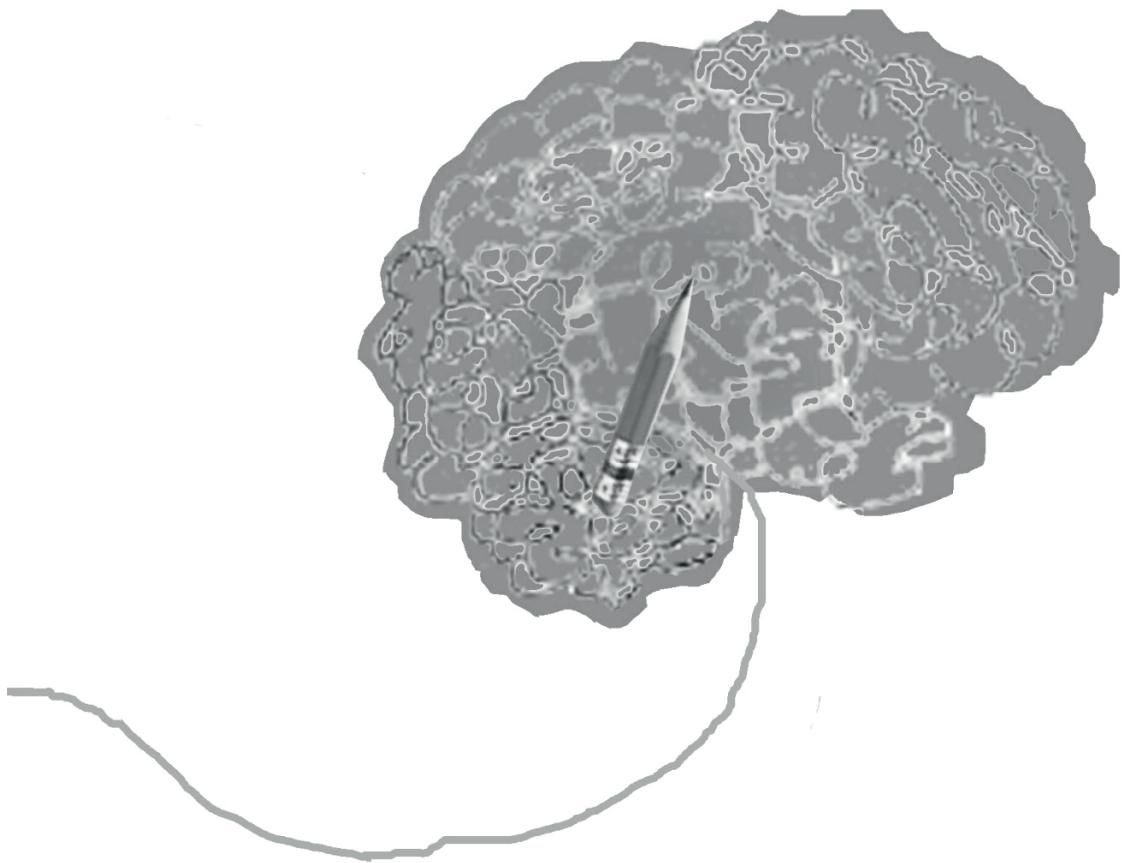
Chapter 6

General discussion

It has been estimated that 50-90% of strokes are attributable to known risk factors.[1,2] Despite implementation of such risk factors in prevention guidelines,[3] the incidence and mortality of stroke remains high.[4,5] The search for new factors and a better understanding of the pathophysiological pathways is therefore still of major importance. This thesis combined studies into determinants of stroke and stroke outcomes, focusing on emerging markers within known pathophysiological pathways. All studies were performed within the population-based Rotterdam Study.[6]

In the following chapter I will first discuss the main findings of my thesis in the context of the current literature, before discussing some methodological considerations. Finally, I will translate the results into clinical practice and will provide suggestions for future research within the field of stroke.

Main findings

Subclinical cardiac disease increases the risk of stroke
One major aim of my thesis was to examine early risk factors or markers for stroke, within categories of known pathophysiology. I first examined markers of subclinical cardiac disease. The clinical cardiac diseases atrial fibrillation, heart failure, and myocardial infarction have all been related to stroke.[7-9] However, abnormalities in the heart can be present before they become clinically apparent.[10-12] Previous echocardiographic studies detected structural abnormalities, e.g. left ventricular hypertrophy or atrial enlargement,[13-16] or a decreased cardiac function in people free of clinical cardiac disease.[10-12] Many structural measures of the heart have been related to stroke before, of which left ventricular hypertrophy appeared to be most important.[13-16] However, cardiac function may provide additional information.[17] In **Chapter 2.1**, I showed that in people without clinical cardiac disease, a diminished systolic and diastolic function of the heart as measured by echocardiography was related to an increased risk of stroke. These associations were independent of atrial diameter and left ventricular mass, a marker of left ventricular hypertrophy (data not shown), and suggest that detecting early cardiac dysfunction may add to the identification of people at high risk of stroke. Future studies should investigate whether presence of a diminished cardiac function contributes to stroke prediction.

Since echocardiography is time-consuming, this may not be the best screening method for cardiac function in prediction studies, particularly if population-based. This raised my interest in the association between a laboratory marker of cardiac dysfunction and the risk of stroke. N-terminal pro-B-type natriuretic peptide NT-proBNP is a cardiac marker that is excreted in response of ventricular wall stress.[18,19] Similar to echocardiographic values, it may reveal cardiac dysfunction even in the absence of clinical cardiac disease.[20] In **Chapter 2.2**, I examined the association of N-terminal pro-B-type natriuretic peptide and the risk of stroke. NT-proBNP related to an increased risk of both ischemic and hemorrhagic stroke. Even after censoring for incident cardiac disease, the associations remained. The association between NT-proBNP and stroke was known from previous studies.[21-25] Our results further enhance this relation by

showing that it can serve as a marker for stroke risk even in a very early phase, namely in people without clinical cardiac disease.

I hypothesized that associations described above may be the consequence of cardioembolism[26] or cerebral hypoperfusion.[27] However, part of the association may also be driven by shared etiology, which I will discuss later in further detail.

Novel markers of large and small vessel disease and the risk of stroke
The search for preclinical markers was continued in **Chapter 3**. In this chapter, I focused on markers of the two other major etiologies of stroke besides cardiac disease, i.e. large and small vessel disease. Large vessel disease, or atherosclerosis, starts with lipid depositions in the artery wall, followed by an inflammatory response, accumulation of fibrous elements and arterial calcification.[28,29] Small vessel disease has a similar pathophysiology. Infiltration of inflammatory cells and lipid depositions damage the smaller arterioles, also known as arteriolosclerosis. In addition, small vessels can get damaged due to cerebral amyloid angiopathy (CAA).[30,31]

Atherosclerosis can be detected from the aortic arch up to the brain using CT, MRI, or ultrasonography[32] and the burden may differ across vessel beds.[33] For stroke, presence of asymptomatic extracranial carotid stenosis has gained a lot of attention as risk factor,[3,34,35] but studies in Asian and African populations revealed that intracranial atherosclerosis, even closer to the brain, might be more useful in the preclinical phase.[36] I explored in **Chapter 3.1** whether these results can be extrapolated to a white population, especially as Asian and African populations have disproportionally higher rates of intracranial atherosclerosis than white populations.[36] Intracranial carotid artery calcification on CT was used as proxy for intracranial atherosclerosis. Around 80% of the participants in our study had some calcification in the intracranial carotid artery. This increased the risk of stroke four-fold and played a role in up to 75% of all strokes. Importantly, this study underlines the value of the location of atherosclerosis for the risk of stroke. As expected, atherosclerosis closer to the brain was associated with a higher risk of stroke. Yet, this does not necessarily imply that markers close to the brain provide less information about systemic vascular damage. For example, in **Chapter 3.2** I found that vasomotor reactivity, a marker for cerebral autoregulation measured in the medial cerebral artery,[37] was associated with an increased risk of mortality independent from stroke. This suggests that an impaired vasomotor reactivity reflects a more systemic dysfunction of the vascular system rather than a local dysfunction in the brain.

Subclinical small vessel disease can be visible on MRI as white matter lesions, lacunar infarcts, and microbleeds.[30,31] Silent lacunar infarcts and white matter lesions are known to increase the risk of stroke.[38-41] Microbleeds increased the risk of a recurrent stroke in people who already suffered a stroke,[42] but whether these results can be extended to the general population was still unclear. **Chapter 3.3** showed that presence of microbleeds increased the risk of both ischemic and hemorrhagic stroke, even independent of white matter lesions and lacunes. This means these lesions might also be a good marker for subclinical cerebrovascular pathology in the general population.

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Together, these results show that emerging markers of large and small vessel disease may help to identify people at high risk of stroke. Another question is whether large and small vessel disease are independent pathways leading to stroke. Given that large and small vessel disease are associated with different subtypes of stroke, i.e. cortical versus lacunar, large and small vessel disease are currently considered to be separate etiological mechanisms.[43] However, in **Chapter 3.4** I showed that different markers of vessel disease may interact in their risk of stroke. For example, I found that the risk of stroke and TIA was only increased in people with a combination of impaired brain perfusion and large retinal venules. Large retinal vessels reflect small vessel disease,[44,45] whereas a low cerebral blood flow may reflect large vessel disease.[46] This suggests that instead of focusing on one etiological cause, the possibility of overlapping causes should be considered. I hypothesized that cerebral autoregulation may compensate for a diminished perfusion. In people with small vessel disease this mechanism may be impaired. In reverse, a high perfusion may compensate for a diminished autoregulation. This may also explain why I did not observe an association between vasomotor reactivity and stroke in **Chapter 3.2**. Vasomotor reactivity may only associate with stroke in combination with a low perfusion. Another explanation for the interaction I observed may be that small vessel disease only relates to stroke if it is severe enough to lead to a lower blood flow demand.[47]

The last paragraph of this chapter focused on hypertension, which is a major modifiable vascular risk factor for stroke,[1,2] possibly because it relates to both large and small vessel disease.[31,36] It is known that treatment of hypertension has had a major preventative effect on stroke,[3,48] but this only targets high levels of blood pressure.[49-52] Yet, in **Chapter 3.5** I showed that the risk of stroke may be further influenced by long-term trajectories of blood pressure. Of particular interest was the finding that the trajectory with a normal blood pressure at middle age, but a steep increase afterwards, had a high risk of stroke and death compared to the trajectory with normal blood pressure at middle age and a more gradual increase. This risk was equally high compared to the trajectory characterized by a high blood pressure at middle age and beyond. The trajectory characterized by a moderate blood pressure at middle age and beyond only had an increased risk of stroke. This raises the question whether the temporal course of blood pressure should also be taken into account when deciding about treatment, especially since the trajectory with a steep increase appeared to be undertreated. Limitations in the use of trajectories, e.g. concomitant time frames, hampered me in the ability to answer this causal and treatment question. However, the results do emphasize that future studies examining the impact of increasing blood pressure are of major interest.

Non-cardiovascular disorders and their risk of stroke

In **Chapter 4**, I moved from markers that reflect the pathophysiology of stroke towards non-cardiovascular diseases for which recently it has been suggested they might influence the cardiovascular system. Specifically, I focused on the relation of COPD and anxiety with stroke.[54,55]

COPD possibly relates to stroke through systemic inflammation resulting in atherosclerosis.[53] COPD is characterized by an inflammatory response of the lungs to noxious

particles or gases,[54] which may extend systemically.[53] Some previous studies did find associations with stroke,[55-59] but were hampered by a cross-sectional design and limited assessment of confounders such as smoking. In **Chapter 4.1**, I observed associations between COPD and both ischemic and hemorrhagic stroke, which strongly attenuated after adjustment for smoking. However, risks remained for certain subtypes of stroke, namely the cortical and atherosclerotic strokes. Adjustment for CRP did not have an effect, which suggests a minor role for inflammation. Furthermore, I found an increased risk of stroke following severe exacerbations, which could be the consequence of acute inflammation or hypoperfusion.[60] I therefore concluded that smoking particularly explains the association between COPD and stroke, but people with COPD may be extra vulnerable for certain subtypes of stroke and as well as for stroke following exacerbations.

Anxiety activates the hypothalamic-pituitary-adrenal axis and sympathetic nervous system.[61] This could damage the endothelial system and contribute to stroke. However, previous studies were inconsistent on whether anxiety relates to stroke.[62,63] I therefore used measures of both anxiety symptoms and anxiety disorders to relate to stroke. In **chapter 4.2**, I found no association between anxiety and stroke. Discrepancies between mine and other studies may be explained by different scores to assess anxiety. It is difficult to disentangle anxiety symptoms from depressive and stress symptoms, which are both disorders that have previously been related to stroke.[64,65] Furthermore, our study pointed towards the importance of a long follow-up. I did find an effect in the short-term, but this is likely to be explained by reversed causality. People might become anxious when they notice their health is deteriorating. The short-term increased risk of stroke is a spurious effect due to the increased health.

The studies described above underline the importance of a complete set of confounders, a large follow-up, and a detailed exposure assessment to explore the risk of non-cardiovascular disorders with stroke. Spurious effects may occur due to confounding, reverse causality, or misclassification.

Challenges after stroke

Having examined the determinants of stroke, I continued studying problems in stroke recognition and determinants of stroke outcome. When a stroke occurs, patients need to be hospitalized immediately for a rapid assessment of the main subtype, i.e. ischemic or hemorrhagic. Ischemic strokes are treated with intravenous thrombolysis and, in case of a proximal artery occlusion, by intra-arterial mechanical thrombectomy.[66-68] Recognizing a stroke is therefore of utmost importance. Elderly themselves, however, often experience difficulty recognizing symptoms of stroke.[69,70] This might be even more difficult for some right hemispheric strokes, as these can lead to vague symptoms such as neglect, spatial disorientation or anosognosia.[71] Previous studies noticed fewer right-sided than left-sided strokes and suggested this could either be due to the aforementioned recognition problems or due to an actual lower frequency of right-sided strokes, for instance as a consequence of differences in anatomy.[72-74] To explain this difference, I compared in **Chapter 5.1** clinical strokes to infarcts on MRI. I detected more left-sided than right-sided clinical strokes, but an equal

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distribution of MRI infarcts in both hemispheres, suggesting that the difference has to do with recognition: right-sided strokes are more prone to be overlooked.

As soon as the stroke is recognized, specified, and treated, patients are admitted to the stroke unit.[66] Although improvement in stroke treatment has strongly improved functional outcome, improved stroke fatality and ageing of the population have increased the number of people who live with the long-term consequences after stroke, such as recurrent stroke, dementia, and death.[4,67,68,75-78] The current approach to reduce these events is aggressive cardiovascular risk management, initiated directly after the diagnosis of stroke.[79] However, in **Chapter 5.2** and **5.3**, I observed that pre-stroke cardiovascular risk factors contributed to 27% of deaths, 39% of recurrent strokes, and 10% of dementia cases after stroke. This suggests that prevention of consequences after stroke should preferably start before stroke. At time of secondary prevention, irreversible damage contributing to the consequences may have already occurred. Compared to previous studies on stroke prognosis,[77,80-84] I had the advantage of being able to compare the results in the stroke patients with those in a stroke-free reference population. Risks of death, recurrent stroke, and dementia were 1.7 to 3 times higher in the stroke patients compared with the reference population. In the reference population, however, cardiovascular risk factors contributed to a similar amount of adverse events, suggesting that these factors cannot fully explain the increased risk in stroke patients. Therefore, further research into other factors is required. Subclinical large or small vessel disease may play a role, or inflammation, thrombosis, or cardiac dysfunction,[85,86] but I did not yet have enough power to reliably investigate this.

Education was not included in **Chapter 5.2** and **5.3**, since it is a non-cardiovascular marker and the potential to modify its effect is still unclear. Education may however have an additional effect on dementia after stroke, being a proposed marker of cognitive reserve.[87] Cognitive reserve represents the ability of the brain to tolerate brain damage, in which people with higher reserve can handle more damage before it becomes apparent as dementia.[88,89] In **Chapter 5.4**, I detected that higher education was associated with a lower risk of dementia after a stroke or TIA, particularly in men. This suggests that cognitive reserve might protect against dementia after a stroke or TIA.

Methodological considerations

My study was conducted within the framework of the prospective population-based Rotterdam Study.[6] Benefits of this study are the large study population, currently consisting of 14,926 participants aged 45 years and over, the availability of a large variety of exposures and the thorough collection of follow-up information. The Rotterdam Study has a thorough case finding for many diseases, including stroke and TIA, but also heart diseases, COPD, and COPD exacerbations.[90-94] This allowed me to examine the association of cardiac dysfunction and stroke independent from heart disease, and even to examine the risk of stroke after a COPD exacerbation.

Common biases in observational studies are selection bias, information bias, and confounding. These have been minimized by limiting the loss-to-follow-up, a thorough search

for outcomes blinded from the exposure, and adjustment for many known confounders. Still, some biases may have affected results of studies presented in this thesis to some extent as described in the following paragraphs.

Healthy volunteer effect

The healthy volunteer effect is a form of selection bias that may have affected my results since participation in the Rotterdam Study is voluntary.[95,96] In order to assess many of the markers used in my thesis, subjects needed to attend the research center. People who were older, less educated, and had more cardiovascular risk factors were less likely to attend.[95] Participation rates were, however, still high and as non-participants were often people at highest risk, the healthy volunteer effect was likely to result only in a slight underestimation of the effect if it had any effect at all.

In the studies that required post-stroke visits, the healthy volunteer effect was stronger. After all, people with severe stroke are unlikely to visit the study center. My finding that cognitive decline after stroke or TIA is smaller in those with high compared to low education in the cognitive reserve study, can therefore only be generalized to people with minor strokes or TIA. Similarly, it has to be considered that in the study about recognition of left- and right-sided strokes, people with severe stroke did not undergo an MRI scan. If left-sided strokes are more severe, this may explain why I found fewer left-sided infarcts on MRI than left-sided strokes in the total population. However, symptomatic infarcts were also more often left-sided compared to the silent infarcts in the MRI population, although not significantly, suggesting that results were robust. Yet, also these results may be less generalizable to people with severe stroke.

The healthy volunteer effect is unlikely to have affected the results of the studies on stroke prognosis. The ascertainment of follow-up information through medical records continued also in participants who terminated their visits to the study center. My results even seem to be more valid and generalizable than those of previous studies, especially with respect to post-stroke dementia. The population-based design and the continuous follow-up through medical records avoided selection bias and attrition that may have been present in the previous hospital-based studies that often did require a visit after stroke.[77,97,98]

Misclassification of stroke subtypes

Within the Rotterdam Study, participants are continuously monitored for occurrence of stroke using automated data-linkage of general practitioner files with the stroke database.[90] Additionally, medical records from people who moved out of the study area or moved to nursing homes are checked on a regular basis. Further data is obtained from hospital records. Potential events identified by medical records are reviewed by research physicians. The diagnosis is based on WHO criteria,[99] and an experienced vascular neurologist verifies each diagnosis.[90] This approach does not rely on participants' memory and therefore prevents recall bias. Additionally, the availability of hospital records limits misclassification. Yet, some information bias is still present in the subclassification of stroke. Classification into ischemic and hemorrhagic is based on neuroimaging reports. Strokes are classified as unspecified if no

neuroimaging is performed.[90] Since we also collected stroke data of people who were not admitted to the hospital, our study population contains a relatively large percentage of unspecified strokes (33%). People with unspecified strokes were on average older and had more risk factors and comorbidities compared to people with ischemic or hemorrhagic stroke. The consequences of this misclassification have been extensively described in a previous thesis.[100] Misclassification may be differential, which means it can lead to both over and underestimation of the estimates of ischemic and hemorrhagic stroke. In the studies about echocardiography and anxiety and stroke, associations with unspecified strokes were in general stronger compared to the other subtypes. This suggests that this misclassification led to underestimation of the effects with ischemic and hemorrhagic stroke. In contrast, NT-proBNP and COPD had a weaker association with unspecified strokes compared to other subtypes, suggesting that this misclassification led to an overestimation of the result with ischemic stroke. This implies that subtype analyses have to be interpreted with caution. However, in all studies even reclassifying all unspecified strokes as ischemic did not materially change the effects with ischemic stroke, suggesting our results were robust. Moreover, since an increasing number of people is now referred, this source of misclassification has reduced over time. Over the past 20 years it dropped from 40.5% in 1994 to just 8.0% in 2013 (Figure 1).
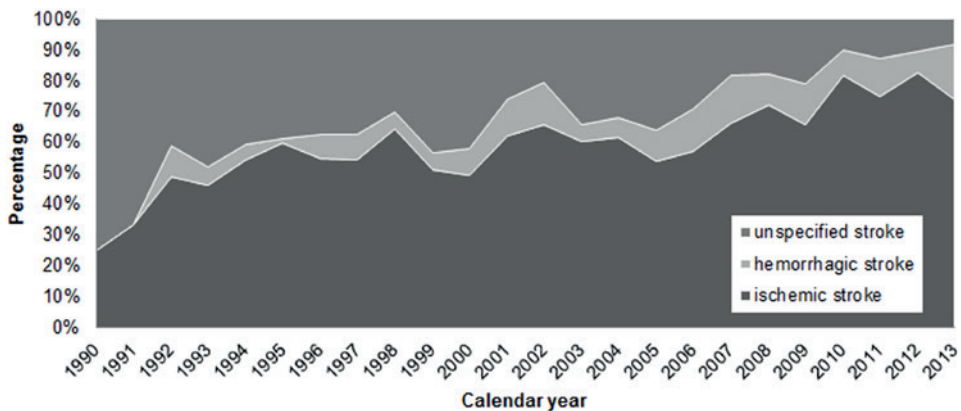

Figure 1. Trends in stroke subtypes per calendar year

Even at a high age (90+) the frequency of unspecified strokes decreased from 75.0% in 1994-1999 to 34.5% in 2009-2014 (Figure 2). Therefore, the misclassification had a smaller role in studies with a later baseline visit; e.g. the studies in which I related ICAC and microbleeds to stroke.
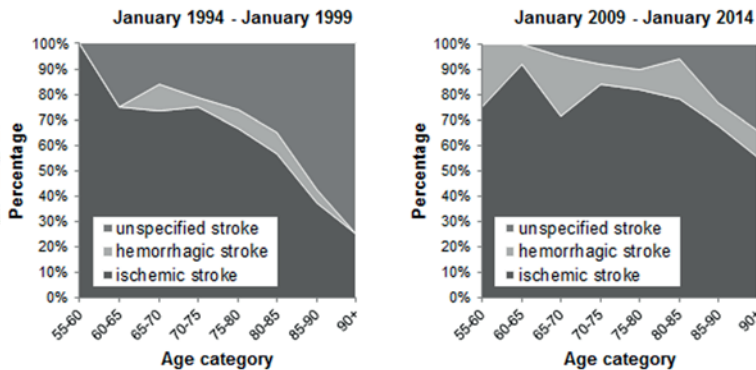
Figure 2. Trends in stroke subtypes per age category
Trends in 1994-1999 (left) and 2009-2014 (right).

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Misclassification between TIA and stroke
The definition of TIA suggests it leaves no permanent damage in the form of an infarct.[101,102] However, evidence is increasing that some TIA do leave (subtle) neurological damage.[103] Furthermore, studies using MRI revealed that many events classified as TIA do appear as infarct on later scans.[104] They are actually strokes misclassified as TIA. To measure the extent of this misclassification in our study, I compared the results of clinical TIA to MRI infarcts (Box 1). My results suggest that approximately 15% of TIA is misclassified. This misclassification may be differential if people with more risk factors are more prone to get an MRI after their TIA. However, in most cases a CT was performed that showed no abnormalities. Misclassification is therefore likely to be non-differential. Even if this had any effect, it would be a dilution.

Box 1. Misclassification between TIA and stroke

Of the 5081 participants with an MRI scan,[105] 225 had one or more prevalent TIA. Participants who also experienced a stroke before the scan date (n=35) or who only had vertebrobasilar TIA or a TIA with unknown location, were excluded (n=57). This left 115 participants with left- or right-sided TIA for analysis. 64 people had one or more left-sided TIA, 57 one or more right-sided TIA.

Of the 64 participants with a left-sided TIA, eight (12.5%) had a left-sided infarct on the MRI scan, and of the 57 participants with a right-sided TIA, eleven (19.3%) had a right-sided infarct on the MRI scan. Assuming that the infarct on MRI coincides temporarily with the TIA, this suggests that approximately 15% of TIA is misclassified. This percentage is lower than what was found in previous studies. Those studies found that 44% of TIAs lead to diffusion abnormalities.[106] This may be explained by a previous finding that only half of TIAs with diffusion abnormalities appear as infarcts on later scans.[107]

Immortal time bias
Immortal time is defined as a period of follow-up during which the outcome cannot occur.[108] This is present in studies that relate incident events to an outcome, in this thesis the study that related COPD to stroke and the studies that related pre-stroke risk factors to consequences after stroke. In the first study, the time between baseline and COPD was immortal time, in the last studies the time between baseline and stroke. People could not get a stroke before start of COPD and could not suffer from stroke consequences before they had the stroke. When

comparing exposed people (with incident event) to unexposed people (without incident event), it has to be considered that people were unexposed during this immortal time. Immortal time bias is introduced if this period is misclassified as exposed (information bias), or if it is excluded (selection bias) (Figure 3). In both cases, this will lead to an underestimation of the effect. It results in a spuriously high rate of cases among the unexposed, since a large period of unexposed time is missed or even misclassified.[108,109]

This form of bias can be solved in several ways (Figure 4). The first is to use a time-dependent analysis. This analysis attributes the unexposed time to the unexposed. I used this analysis in the study on COPD and stroke. A limitation is that COPD does not have a clear start. Using this method, the latent period will be classified as unexposed.
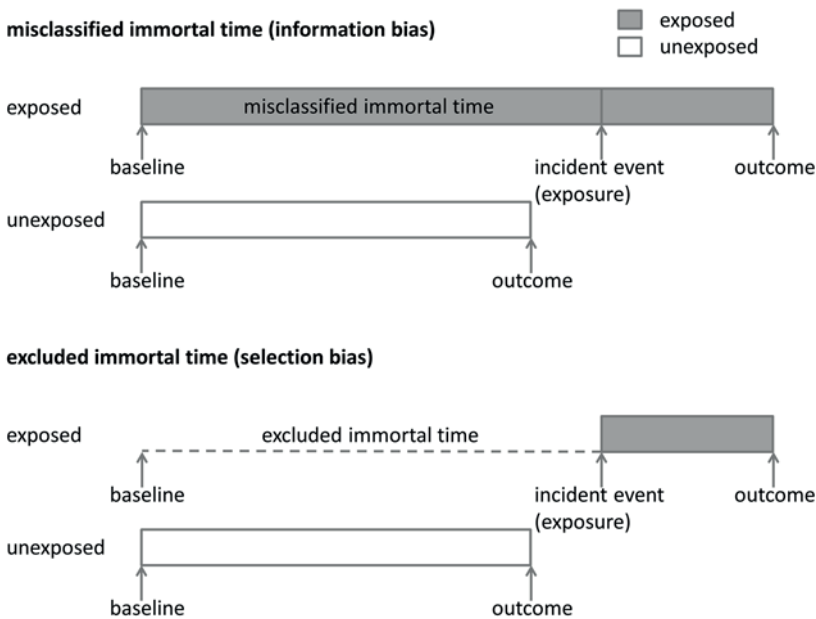
Figure 3. Bias due to immortal time
Immortal time bias introduced when the immortal time is misclassified (top) or when the immortal time is excluded (bottom). Adapted from Levesque et al.[108]

A time-dependent analysis was unfeasible for our analysis on stroke prognosis, since my interest was not in the effect of stroke itself, but in the effect of pre-stroke factors. I therefore used a second solution, which is to include only survivors of the immortal person-time in both the exposed and unexposed groups.[108] This was done by randomly matching unexposed stroke-free participants to the stroke cases at the date of the incident stroke (index date). It prevents immortal person-time bias by taking only survivors until the index date.[108] This approach allowed me to use cardiovascular risk factors measured at approximately the same time before the index date in the exposed and unexposed.
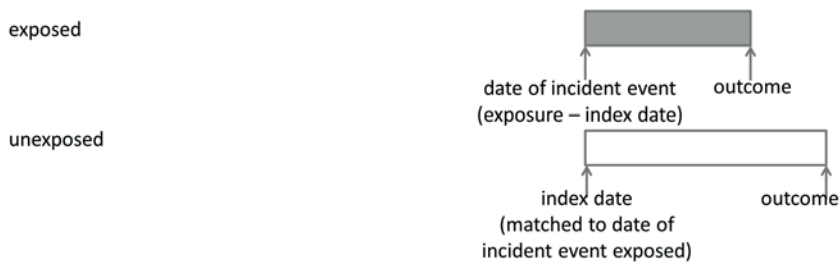
Figure 4. Solutions to immortal time bias
Top: use of a time-dependent analysis in which immortal time is classified as unexposed (as in actual situation). Bottom: exclude immortal time in both exposed and unexposed, for instance by using a nested matched cohort. Adapted from Levesque et al.[108]

Residual confounding

To prevent confounding, all analyses described in this thesis have been adjusted for a set of known confounding factors. However, some considerations need to be taken into account. I mostly adjusted for confounders that were measured once (at baseline), and which might not be representative for the life-long exposure. The markers for subclinical cardiovascular disease tested in this thesis may be better markers of life-long exposure to cardiovascular risk factors than the single measured risk factors I adjusted for. Therefore, although adjustment for cardiovascular risk factors did not change the effect, a shared etiology may still be a plausible explanation for their association with stroke. On the other hand, the markers may be mediators in the association between cardiovascular risk factors and stroke. Additionally, in the analysis with COPD and stroke and in the analysis with stroke prognosis, changes in therapy during follow-up may have affected the result. At time of COPD and at time of stroke, participants are expected to be more intensively treated. This may have given an underestimation of the effect.

Another issue is that adjustment leads to overadjustment if the factors are in the causal pathway. Therefore, I always compared multivariable adjusted results to a basic model, which only adjusts for age and sex. Improving methods of mediation analysis may facilitate detecting whether factors function as confounders or mediators of an association.[110]

Population attributable risk

In the study relating ICAC to stroke and in the studies that related pre-stroke cardiovascular risk factors to consequences after stroke, I used the population attributable risk (PAR) to describe what proportion of events is attributable to the exposure or combination of exposures. The PAR requires that associations are causal. A cause is defined as a "*an antecedent event, condition or characteristic that was necessary for the occurrence of the disease at the moment it occurred, given that other conditions are fixed*".[96] In other words, the event would not have occurred if this antecedent, condition, or characteristic was absent.[96] However, most diseases have multiple causes, i.e. they are multifactorial. This includes stroke.[3] The disease would be absent if either one of the causes is absent. This interaction between causes means that just adding up individual PARs would lead to an overestimation of the proportion of events that can be prevented. To reduce this effect we used adjusted PARs in our analyses. Still, some considerations need to be addressed. The PAR assumes that risk factors can be completely avoided and that the harmful effect disappears in an instant after removing the risk factor, which is generally false.[1,111] It is often possible to modify a risk factor, but not to avoid it altogether. For the relationship between ICAC and stroke it is not even clear whether, or to what extent, the calcification is modifiable.[112] Additionally, if one cause is eliminated, another set of causes may result in the event. This may lead to an overestimation of the PAR. In contrast, PARs with respect to pre-stroke risk factors may also be underestimated. I calculated the PAR of consequences after stroke assuming that the stroke would still occur. However, a more aggressive management of pre-stroke risk factors will also prevent the first-ever stroke and additionally reduce the burden of post-stroke consequences. A final consideration is that even if many events can be attributed to a single cause, for example, we estimated that 75% of strokes is attributable to ICAC, 100% of events can be attributable to other, possibly unknown causes. Such causes may interact with the already known factors.[96] Therefore, the search for new factors needs to be continued until all strokes and consequences of stroke are eliminated.

Clinical implications and future directions

Prevention of stroke

In my thesis, I identified several markers of subclinical cardiac dysfunction, large vessel disease, and small vessel disease to stroke. Being able to use this information for prevention strategies is of major clinical interest, since it may reduce the burden of stroke. There are two ways in which the detected factors may improve stroke prevention. Firstly, risk factors may be a direct target for prevention. Secondly, risk factors may identify people at high risk of stroke that benefit from more aggressive preventive management. In the following paragraphs, I will first discuss the potential use of our identified factors for prevention, followed by the use of these factors for stroke prediction.

Potential targets for prevention

Requisites for a marker to be a direct target for stroke prevention are that the factor is causally related to stroke and that it is modifiable. Three factors described in this thesis may fulfill this requirement, namely a steep blood pressure increase, cardiac dysfunction, and ICAC.

The study in which I related blood pressure trajectories to stroke raised the question whether blood pressure management can be improved if people with fast increasing blood pressure are treated irrespective of their blood pressure level. The trajectory with a steep increase was associated with a high risk of stroke and death. Since blood pressure is highly treatable and the class characterized by a steep increase had limited use of blood pressure lowering medication, the occurrence of a steep increase may be a potential target for prevention. However, the increased risk in this trajectory may also be the consequence of several non-causal explanations, e.g. limited access to health care, difference in health behaviors, and competing risks. Future studies should therefore determine the etiologic significance of this finding. This can subsequently allow for examination of the potential for prevention.

The same applies to cardiac dysfunction. Cardiac dysfunction may causally relate to stroke through cardioembolism[26] or cerebral hypoperfusion.[27] However, shared etiology may also play a role. Although associations in our studies remained after adjustment for cardiovascular risk factors, subclinical cardiac dysfunction may better reflect the long-term strain of cardiovascular risk factors on the heart than a single measurement of those risk factors. Before it is possible to examine the preventive potential, we need to be confident about the causal effect. Importantly, if the association is caused by cardioembolism, people may need to be treated with oral anticoagulants.[113]

The finding that 75% of strokes can be attributed to intracranial carotid atherosclerosis arouses interest in whether ICAC can be a target for prevention. It might be that the plaques themselves cause stroke, since they are a source of emboli. On the other hand, ICAC may also reflect atherosclerosis in other vessels of the brain. Furthermore, it is unclear whether this can be a direct target for treatment. So far, intensive medical management with antiplatelet therapy, intensive management of vascular risk factors, and life-style modification appear to outweigh the effect of stenting in symptomatic intracranial stenosis.[112,114] Whether similar aggressive management can be beneficial in an asymptomatic population with ICAC needs to be further explored.

Potential markers for stroke prediction

Markers that cannot be a direct target for prevention, may still be helpful for stroke prediction. To determine whether a marker is useful for stroke prediction, the incremental predictive value of this new marker above known markers needs to be investigated. The detected markers of cardiac disease, large vessel disease, and small vessel disease should for instance be added to risk assessment tools such as the Framingham Stroke Profile, Qstroke, or the Stroke Riskometer™.[115-117] Even if they improve prediction, it remains to be seen if CT, echocardiography, and MRI are feasible screening tools. Their costs are high and CT gives radiation exposure. A laboratory marker, such as NT-proBNP, is easier to use for screening

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purposes and has therefore been tested. It appears to improve prediction of stroke and coronary heart disease.[24,118] Another question is what therapy should be initiated in people at high risk of stroke. Detection of a high risk population is only useful if there are methods to reduce their risk. Treatment of risk factors such as hypertension, hypercholesterolemia, and diabetes is well accepted and has proven to be effective.[3] However, the treatment with aspirin in people without a cardiovascular event remains subject of discussion.[3,119,120] So far, trials with people without cardiovascular disease only showed a modest reduction in total cardiovascular events, but not of stroke or cardiovascular deaths.[3,119,120] Guidelines therefore suggest that use of aspirin may be reasonable in people with sufficiently high risk, but especially for prevention of cardiac disease.[3] However, when prediction of stroke improves, the detection of high risk groups will become more accurate, which could lead to better trial results also for stroke.

Primary prevention to reduce the consequences after stroke
A better prevention of stroke will subsequently prevent its consequences, but in this paragraph I will further discuss the possibilities for prevention by translating our results on stroke prognosis into clinical practice. The three principle strategies in secondary prevention are lowering of blood pressure, statin therapy, and antiplatelet therapy (or oral anticoagulants in selected patients), with the aim to reduce the increased vascular risk after stroke.[121] My main message from **Chapter 5.2** and **5.3** was, however, that secondary prevention may be insufficient to prevent all adverse consequences after stroke, since pre-stroke cardiovascular risk factors already largely contribute to the adverse prognosis after stroke. Primary prevention is necessary to reduce these consequences. Risk factors important for prognosis were hypertension, diabetes, and smoking, and for mortality also atrial fibrillation. Since attacking these factors is already a goal of primary prevention,[3] this raises the question whether and how this can be further improved. Room for further improvement may be in the awareness of risk factors and the adherence to treatment and lifestyle changes. Many people are never tested for cardiovascular risk factors and therefore may be unaware of their cardiovascular risk.[3,122] Furthermore, of those who are aware of their risk factors, only half adheres to the therapy prescribed.[123,124] Poor awareness and adherence is a major risk factor for stroke and possibly of the consequences after stroke.[125] Prevention programs should therefore further improve and test all people above a certain age (e.g. 50 years) for risk factors.[3] Furthermore, more awareness among the general population of their cardiovascular risk and its consequences is necessary.[3] Our finding that these factors also influence the consequences of stroke may be a further incentive for people to keep using their medication.

Cognitive reserve is another factor that may be a target for prevention of dementia after stroke besides the cardiovascular risk factors. Higher education, occupation, learning another language and playing a musical instrument have all been related to a higher cognitive reserve.[126-128] However, people engaging in these activities may also have a healthier lifestyle with fewer risk factors compared to people who do not. This may result in fewer severe strokes and consequently a lower risk of dementia. Future studies should determine whether cognitive reserve can be enhanced and consequently prevent dementia after stroke.

Novel risk factors for the prognosis after stroke

Although 10-39% of the consequences after stroke could be attributed to pre-stroke risk factors, an even larger part of the consequences remained unexplained. This encourages further study of novel risk factors. Other pre-stroke markers that are potentially useful to define the risk of adverse events after stroke are genetic markers, imaging markers of large and small vessel disease[85] and biomarkers of inflammation, thrombosis, or cardiac dysfunction.[86] As described previously, such factors may not be direct targets for prevention, but may identify those people who need more aggressive therapy.

Studying risk factors for stroke severity is another approach to detect factors that relate to adverse events after stroke. Factors that increase the severity of stroke, may subsequently increase the risk of its consequences.[129] Population-based studies are required to properly investigate these risks, since they measure risk factors before stroke occurs. However, these studies often need to assess stroke severity retrospectively based on medical records. Medical records often lack specific information on symptoms, and unfortunately still rarely mention the NIH Stroke Scale (NIHSS) score, a well-validated score for severity.[130,131] Still, since symptoms that are not described are usually absent, the score appears to be reliable even if retrospectively assessed using medical records.[132] The value of the information is therefore likely to outweigh the small risk of misclassification.

Directions for future stroke research

A main question for future stroke research is why even though many of its risk factors are known stroke remains such a major health problem.[1,2] Could it be that prevention starts too late? What part is caused by a poor compliance with preventive measures? Do we require novel preventive therapies? Novel research should define the proportion of events that occurs due to undertreatment, for instance as a consequence of low adherence to or unawareness of the condition or guidelines. Research into risk factors should also be continued. We particularly need to be able to detect abnormalities within the pathophysiological pathway earlier in time. Although factors like NT-proBNP and ICAC are subclinical, they may appear relatively late, reflecting the life-time exposure of risk factors. Knowing the starting point of abnormalities may further inform about the moment prevention should be initiated. To do so, imaging is of value, possibly at a younger starting age. Research into genetics and metabolomics could also add information,[133-135] especially if this further focuses on specific pathways, such as large or small vessel disease.[136] Finally, the use of longitudinal data is likely to be of additional value. Similarly to the long-term pattern of blood pressure, change over time in markers of cardiac dysfunction, cerebral small vessel disease and large vessel disease may enhance the understanding of stroke occurrence.

Concluding remarks

The scientific discoveries described in my thesis provide a useful insight in the relationship between subclinical pathology and stroke and hence may provide the basis for future studies that examine the value of these markers for stroke prediction. Furthermore, they reveal the

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importance of pre-stroke risk factors to reduce the consequences after stroke. Although the knowledge on stroke is rapidly expanding, the solution to prevent all strokes is still a long way off. Hopefully, my results will inspire novel research and bring us a step further towards a better prevention of stroke and its consequences. More research remains necessary to determine when to start with prevention, how to interfere with the subclinical pathways leading to stroke and whether it is possible overturn this subclinical process.

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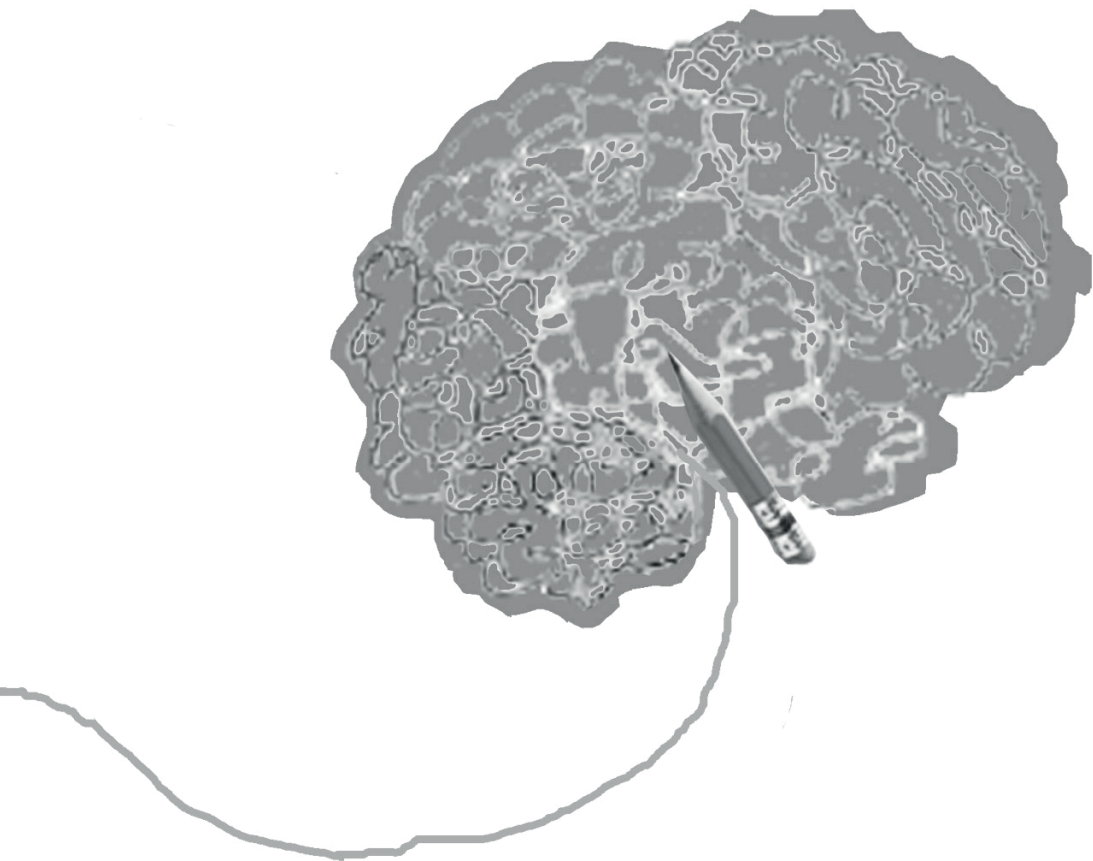
Chapter 7

Summary / Samenvatting

Summary

Despite knowledge on many risk factors, stroke remains the second leading cause of death and a major cause of disability. Further improvement of stroke prevention is therefore essential. This may be achieved by early intervention in the process that leads to stroke, which requires a thorough knowledge on the pathophysiology of stroke. Cardiac abnormalities, as well as large and small vessel disease evolve silently for years before suddenly blocking a vessel to the brain, which causes a stroke. Imaging and laboratory markers that can identify this preclinical damage are emerging. Studying their relation with stroke may help us to understand the pathophysiology of stroke and to detect people at high risk of stroke and may inspire us to find novel options for prevention. Additionally, a better insight in the adverse consequences of stroke is mandatory, since these further increase the burden of stroke. Possible consequences not only include recurrent stroke and dementia, but also death. Since they often have a vascular background and vascular pathology already accumulates years before the stroke, pre-stroke cardiovascular risk factors might predispose to these clinical adverse events after stroke.

Therefore, my thesis had the following aims: First, to detect novel determinants of stroke, mainly focused on the cardiovascular system. Second, to detect pre-stroke risk factors for the long-term consequences after stroke. All studies were embedded in the Rotterdam Study, a population-based cohort study currently consisting of 14,926 participants aged 45 years and over.

Chapter 2 focused on subclinical markers of cardiac disease and their relation with stroke. People without cardiac disease may have abnormalities of the heart. To determine the association between subclinical cardiac disease and stroke, I used echocardiographic values of people without clinical cardiac disease in **Chapter 2.1**. I detected that both a diminished systolic and diastolic function were related to an increased risk of stroke, even though these values were not severe enough to diagnose participants with heart failure. The relation between subclinical cardiac dysfunction and stroke was further emphasized by my findings in **Chapter 2.2**. A higher N-terminal pro-B-type natriuretic peptide (NT-proBNP), which reflects increased ventricular wall stress of the heart, was related to an increased risk of stroke and transient ischemic attack (TIA), even in people without cardiac disease before their stroke or TIA. Results of these two studies suggest that cardiac dysfunction measured by echocardiography and by NT-proBNP can serve as an early marker for stroke.

In **Chapter 3** the search for subclinical markers continued, with focus on two other etiologic pathways for stroke, i.e. large and small vessel disease. Intracranial carotid artery calcification (ICAC) is a large vessel disease marker of interest for stroke, since it is a proxy for atherosclerosis in the intracranial vessels. In **Chapter 3.1**, I used CT-scans to show that presence of ICAC increased the risk of stroke four-fold and contributed to 75% of strokes. Interestingly, ICAC had a stronger association with stroke compared to values of atherosclerosis further from the brain, suggesting that the location of atherosclerosis is important. Another vascular marker close to the brain is vasomotor reactivity. It is measured in the medial cerebral artery and reflects cerebral autoregulation. Despite the close relation to the brain, I observed in **Chapter 3.2** that

this marker was only associated with mortality, and not with stroke, suggesting that a diminished vasomotor reactivity reflects a more systemic instead of local dysfunction of the vascular system.

A small vessel disease marker that raised my interest was presence of microbleeds. Previous studies showed that silent lacunar infarcts and white matter lesions, which are other markers of small vessel disease, relate to an increased risk of ischemic stroke. In **Chapter 3.3**, I showed that microbleeds relate to an increased risk of both ischemic and hemorrhagic stroke. These results were independent of lacunar infarcts and white matter lesions, suggesting microbleeds may be a useful marker of subclinical cerebrovascular pathology in the general population.

Having studied several large and small vessel disease markers, my next step was to examine whether large and small vessel disease reflect separate etiologies of stroke. This is a common assumption since they are associated with different stroke subtypes, namely cortical versus lacunar. In **Chapter 3.4**, I question this assumption, since the risk of stroke and TIA was only increased in people with a combination of impaired brain perfusion and large retinal venules. Since impaired brain perfusion is a possible marker of large vessel disease, and large retinal venules of small vessel disease, this suggests that pathophysiological mechanisms may overlap and interact. This, however, needs further exploration. Severe small-vessel disease can also induce a lower blood flow, which is another explanation for the higher risk of stroke in people who have both.

The final part of this chapter (**Chapter 3.5**) focused on blood pressure, which is a major risk factor for stroke, possibly through its effect on large and small vessel disease. I examined the association of long-term trajectories of systolic blood pressure with stroke and death. Current treatment guidelines only target high levels of blood pressure, whereas trajectories may provide additional information. I observed that not only levels, but also trajectories of blood pressure are associated with the risk of stroke and mortality. Of particular interest was my finding that the trajectory characterized by a steep increase in blood pressure was associated with a high risk of stroke and death. This is potentially important for preventive strategies.

Chapter 4 described the association between two primarily non-cardiovascular diseases and stroke, namely COPD and anxiety. Both diseases may affect the cardiovascular system, which suggests they potentially relate to stroke. A connection was indeed found in **Chapter 4.1**. COPD was associated with an increased risk of stroke, both ischemic and hemorrhagic. This was mainly the consequence of a shared etiology of smoking, but for atherosclerotic subtypes of stroke there may also be a direct causal effect. Furthermore, I detected people were at high risk of stroke following severe exacerbations. In **Chapter 4.2**, I assessed that neither anxiety symptoms nor anxiety disorders were related to stroke. Anxiety symptoms were only related to an increased risk of stroke in the short-term, which may reflect reverse causality. People may experience a deteriorating health in the years preceding stroke and become anxious in response.

Chapter 5 was dedicated to challenges after stroke. **Chapter 5.1** was inspired by studies that noticed a higher frequency of left-sided strokes compared to right-sided strokes. I investigated

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whether this was the consequence of a better recognition of left-sided strokes or whether left-sided infarcts actually occur more often. I compared the left-right sided distribution of clinical strokes and TIA to the distribution of infarcts on MRI. My finding that clinical strokes and TIA are more frequently left-sided than right-sided, and that infarcts on MRI are more evenly distributed, suggests that right-sided strokes are more prone to be overlooked.

In the remaining paragraphs of **Chapter 5**, I aimed to examine the association between pre-stroke risk factors and consequences after stroke. In **Chapter 5.2** and **5.3**, I detected that pre-stroke cardiovascular risk factors contribute to 27% of deaths, 39% of recurrent strokes, and 10% of dementia cases after stroke. This suggests that secondary prevention may be initiated too late to prevent these events and underlines the importance of primary prevention from another perspective. Since pre-stroke cardiovascular risk factors could not fully explain the higher risk of stroke, dementia, and death people in people with stroke, results of this chapter also encourage research into novel risk factors for adverse events after stroke. One novel risk factor for dementia after stroke or TIA may be a low cognitive reserve. Cognitive reserve reflects someone's ability to cope with brain pathology. In **Chapter 5.4**, I related education, a proposed marker of cognitive reserve, with dementia after a stroke or TIA. I detected that people, particularly men, with higher education had a lower risk of dementia after stroke compared to people with low education. I therefore concluded that cognitive reserve might protect against dementia after stroke or TIA.

In **Chapter 6**, I reviewed the main findings of my thesis in light of previous literature, discussed methodological issues, and translated the findings into potential clinical implications. To summarize, I found that subclinical pathology in the heart, large vessels, and small vessels all relate to an increased risk of stroke. This should form the basis for future studies into the predictive value of these markers. Additionally, I underlined the importance of an early start of prevention, since pathology is present early in time and even contributes to the adverse consequences after stroke. Future studies should continue the search for early abnormalities and their change over time, which hopefully will eventually give us the opportunity to slow down or stop their further deterioration towards stroke.

Samenvatting

Ondanks dat veel risicofactoren al bekend zijn, blijft de beroerte de op één na meest voorkomende doodsoorzaak en een belangrijke oorzaak van invaliditeit. Een verdere verbetering van beroertepreventie is dan ook van groot belang. Dit kan bereikt worden door vroeg in te grijpen in het proces dat leidt tot een beroerte, wat een grondige kennis over de pathofysiologie van beroertes vereist. Hartafwijkingen en macro- en microvasculaire schade hebben vaak een lange onopvallende aanloop, voordat ze plots een bloedvat in de hersenen blokkeren en zo een beroerte veroorzaken. Met nieuwe imaging- en laboratoriummarkers kan deze subklinische schade steeds beter in beeld gebracht worden. Het bestuderen van de relatie tussen zulke markers en beroertes kan ons verder helpen de pathofysiologie van beroertes te begrijpen, mensen met een hoog risico op beroertes te detecteren en ideeën voor preventie op te doen. Daarnaast is het van belang om een beter inzicht te krijgen in de schadelijke gevolgen van beroertes. Deze omvatten een recidief beroerte, dementie en overlijden en hebben vaak een vasculaire oorzaak. Gezien de geleidelijke toename van vasculaire pathologie in de periode voor de beroerte, is het mogelijk dat cardiovasculaire risicofactoren die al aanwezig waren voor de beroerte, bijdragen aan deze schadelijke gevolgen van beroertes.

De doelen van mijn proefschrift waren daarom als volgt: ten eerste, het ontdekken van nieuwe determinanten van beroertes, met een focus op het cardiovasculaire systeem. Ten tweede, het ontdekken van risicofactoren aanwezig vóór de beroerte die bijdragen aan de gevolgen na een beroerte. Alle studies waren onderdeel van de Rotterdam Studie (ERGO onderzoek). Dit is een langlopende cohortstudie die inmiddels bestaat uit 14.926 deelnemers van 45 jaar en ouder.

Hoofdstuk 2 is gericht op subklinische markers van hartziekten en hun relatie met beroertes. Mensen zonder hartziekten kunnen namelijk al hartafwijkingen hebben. Om de associatie tussen subklinische hartziekte en beroertes te onderzoeken, gebruikte ik in **Hoofdstuk 2.1** echocardiografische waarden van mensen zonder klinische hartziekten. Ik ontdekte dat zowel een verminderde systolische als een verminderde diastolische functie gerelateerd was aan een verhoogd risico op beroertes, zelfs ondanks dat deze waarden niet ernstig genoeg waren voor de diagnose hartfalen. De relatie tussen een subklinisch verminderde hartfunctie en beroertes werd verder ondersteund door mijn bevindingen in **Hoofdstuk 2.2**. Een hogere NT-proBNP waarde, wat betekent dat het hart zwaarder belast wordt, was gerelateerd aan een verhoogd risico op beroertes en TIA's, zelfs in mensen die nooit een hartziekte hadden gehad. Bovenstaande studies laten zien dat een verminderde hartfunctie gemeten met echocardiografie of met NT-proBNP gebruikt kan worden als vroege marker voor beroertes.

In **Hoofdstuk 3** vervolgde ik de zoektocht naar subklinische markers, met een focus op twee andere etiologische routes voor beroertes, namelijk macro- en microvasculaire schade. Verkalking in de intracraniële arteriën is een macrovasculaire risicofactor die vanwege zijn locatie interessant is voor beroertes. In **Hoofdstuk 3.1** vond ik dat verkalking in de intracraniële arteriën, gemeten op CT-scans, het risico op beroertes verviervoudigde en dat het bijdroeg aan

75% van de beroertes. Een andere interessante bevinding was dat intracraniële atherosclerose een sterkere relatie had met beroertes dan atherosclerose verder weg van de hersenen. Dit suggereert dat de locatie van atherosclerose van belang is. Een andere vasculaire marker dichtbij de hersenen is de reactiviteit van de cerebrale vaten. Dit wordt gemeten in de arteria cerebri media en is een reflectie van de cerebrale autoregulatie. Ondanks dat deze marker vlakbij de hersenen gemeten wordt, vonden we in **Hoofdstuk 3.2** alleen een relatie met overlijden en niet met beroertes. Dit suggereert dat een verminderde reactiviteit van de cerebrale vaten meer een systemisch dan een lokale verminderde functie van het vaatsysteem weergeeft.

De microvasculaire marker waar ik geïnteresseerd in was, was de aanwezigheid van microbloedingen. Het is bekend dat andere markers van microvasculaire schade, zoals stille lacunaire infarcten en witte stof afwijkingen, gerelateerd zijn aan een verhoogd risico op herseninfarcten. In **Hoofdstuk 3.3** liet ik zien dat microbloedingen een verhoogd risico op zowel herseninfarcten als hersenbloedingen geven. Deze resultaten waren onafhankelijk van de aanwezigheid van lacunaire infarcten of witte stof afwijkingen, wat suggereert dat microbloedingen een bruikbare marker voor subklinische microvasculaire schade kunnen zijn in de algemene populatie.

Na het bestuderen van markers voor macro- en microvasculaire schade, was mijn volgende stap om te onderzoeken of macro- en microvasculaire schade elkaars risico op beroertes versterken. Er wordt vaak gedacht dat het verschillende etiologische routes zijn, aangezien ze geassocieerd zijn met verschillende subtypes van beroertes, namelijk corticaal versus lacunair. Ik trek deze assumptie in twijfel in **Hoofdstuk 3.4**, aangezien het risico op beroertes en TIA's alleen verhoogd was in mensen met een combinatie van een verminderde breinperfusie en verwijde retinavenen. Een verminderde breinperfusie is een mogelijke marker van macrovasculaire schade en wijde retinavenen van microvasculaire schade. Dit suggereert dat verschillende pathofysiologische mechanismen elkaar overlappen en versterken. Hier is nog wel extra onderzoek voor nodig. Microvasculaire schade kan namelijk ook een verminderde breinperfusie veroorzaken. Dit is een mogelijke andere verklaring voor waarom mensen met beide afwijkingen een verhoogd risico op beroertes hebben.

Het laatste deel van dit hoofdstuk (**Hoofdstuk 3.5**) richtte zich op bloeddruk, een belangrijke risicofactor voor beroertes, waarschijnlijk door zijn effect op zowel macro- als microvasculaire schade. Ik onderzocht de associatie tussen het lange termijn verloop van systolische bloeddruk en beroertes en overlijden. Huidige richtlijnen voor de behandeling van te hoge bloeddruk richten zich nu alleen nog maar op de hoogte van de bloeddruk, terwijl het verloop in de tijd extra informatie kan geven. Ik vond dat niet alleen de hoogte, maar ook het verloop van bloeddruk in de tijd geassocieerd was met het risico op beroertes en overlijden. Een belangrijke bevinding was dat de groep met een sterke toename in bloeddruk een verhoogd risico op beroertes en overlijden had, zelfs op het moment dat de bloeddruk zelf nog niet sterk verhoogd was. Dit is mogelijk van belang voor preventiestrategieën.

Hoofdstuk 4 beschrijft de associatie tussen twee niet-cardiovasculaire ziekten en beroertes, namelijk COPD en angst. Voor beiden is gesuggereerd dat ze het cardiovasculaire systeem

beïnvloeden, wat mogelijk een verhoogd risico op beroertes geeft. In **Hoofdstuk 4.1** vond ik dat aanwezigheid van COPD inderdaad het risico op beroertes verhoogt, zowel het risico op herseninfarcten als hersenbloedingen. Dit kwam met name door het gedeelde effect van roken op zowel COPD als beroertes, maar mogelijk is er ook een causaal verband tussen COPD en beroertes van het atherosclerotische subtype. Daarnaast vond ik dat COPD patiënten een verhoogd risico op beroertes hadden na een ernstige exacerbatie. In **Hoofdstuk 4.2** stelde ik vast dat angstsymptomen en angststoornissen beiden niet gerelateerd waren aan een verhoogd risico op beroertes. Angstsymptomen waren alleen gerelateerd aan een verhoogd risico op beroertes op de korte termijn. Dit komt mogelijk doordat mensen in de jaren voor hun beroerte achteruitgaan in hun gezondheid en daar angstig van worden.

Hoofdstuk 5 was gericht op problemen na de beroertes. Inspiratie voor **Hoofdstuk 5.1** kwam van studies die opmerkten dat linkszijdige beroertes vaker voorkomen dan rechtszijdige beroertes. Ik onderzocht of dit het gevolg was van een betere herkenning van linkszijdige beroertes of van het werkelijk vaker optreden van linkszijdige infarcten. Ik vergeleek de links-versus rechtszijdige distributie van klinische beroertes en TIA's met de distributie van infarcten op MRI. Ik vond dat klinische beroertes en TIA's vaker linkszijdig waren dan rechtszijdig, maar dat dit verschil voor infarcten op MRI significant kleiner was. Hieruit concludeerde ik dat rechtszijdige beroertes vaker over het hoofd worden gezien.

In de laatste paragrafen van **Hoofdstuk 5** onderzocht ik de associatie tussen risicofactoren aanwezig voor de beroerte en de gevolgen na de beroerte. In **Hoofdstuk 5.2** en **5.3** vond ik dat cardiovasculaire risicofactoren gemeten voor de beroerte bijdroegen aan 27% van de overlijdens, aan 39% van de recidief beroertes en aan 10% van de dementiegevallen na de beroerte. Dit suggereert dat secundaire preventie te laat komt om deze gevolgen te voorkomen en laat vanuit een ander perspectief zien dat primaire preventie zeer belangrijk is. Aangezien de risicofactoren gemeten voor de beroerte niet volledig het verhoogde risico op recidief beroertes, dementie en overlijden in patiënten met beroertes konden verklaren, moedigen de resultaten in deze hoofdstukken ook aan om extra onderzoek te doen naar risicofactoren voor schadelijke gevolgen van beroertes. Eén nieuwe risicofactor voor dementie na een beroerte of TIA is mogelijk een lage cognitieve reserve. Iemands cognitieve reserve weerspiegelt zijn vermogen om hersenschade op te vangen. In **Hoofdstuk 5.4** relateerde ik onderwijs, een mogelijke marker van cognitieve reserve, met dementie na een beroerte of TIA. Ik vond dat mensen, voornamelijk mannen, die hoger onderwijs hadden genoten een lager risico hadden op dementie na een beroerte vergeleken met mensen die lager onderwijs hadden gehad. Ik concludeerde hieruit dat cognitieve reserve mogelijk beschermt tegen dementie na een beroerte of TIA.

In **Hoofdstuk 6** bediscussieerde ik de belangrijkste bevindingen van mijn proefschrift, de methodologische problemen en de potentiële klinische implicaties van mijn bevindingen. Samenvattend vond ik dat zowel subklinische schade in het hart, als in de grote en kleine vaten gerelateerd was aan een verhoogd risico op beroertes. Dit kan de basis vormen voor toekomstige studies naar de voorspellende waarde van deze markers. Daarnaast onderstreepte

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ik het belang van een vroege start van primaire preventie, aangezien pathologie vaak al vroeg aanwezig is en zelfs bijdraagt aan de schadelijke gevolgen na de beroerte. Toekomstige studies zullen de zoektocht naar vroegtijdige afwijkingen en hun verloop in de tijd moeten continueren. Hopelijk geeft dat ons uiteindelijk de mogelijkheid om hun voortschrijding richting een beroerte te voorkomen.
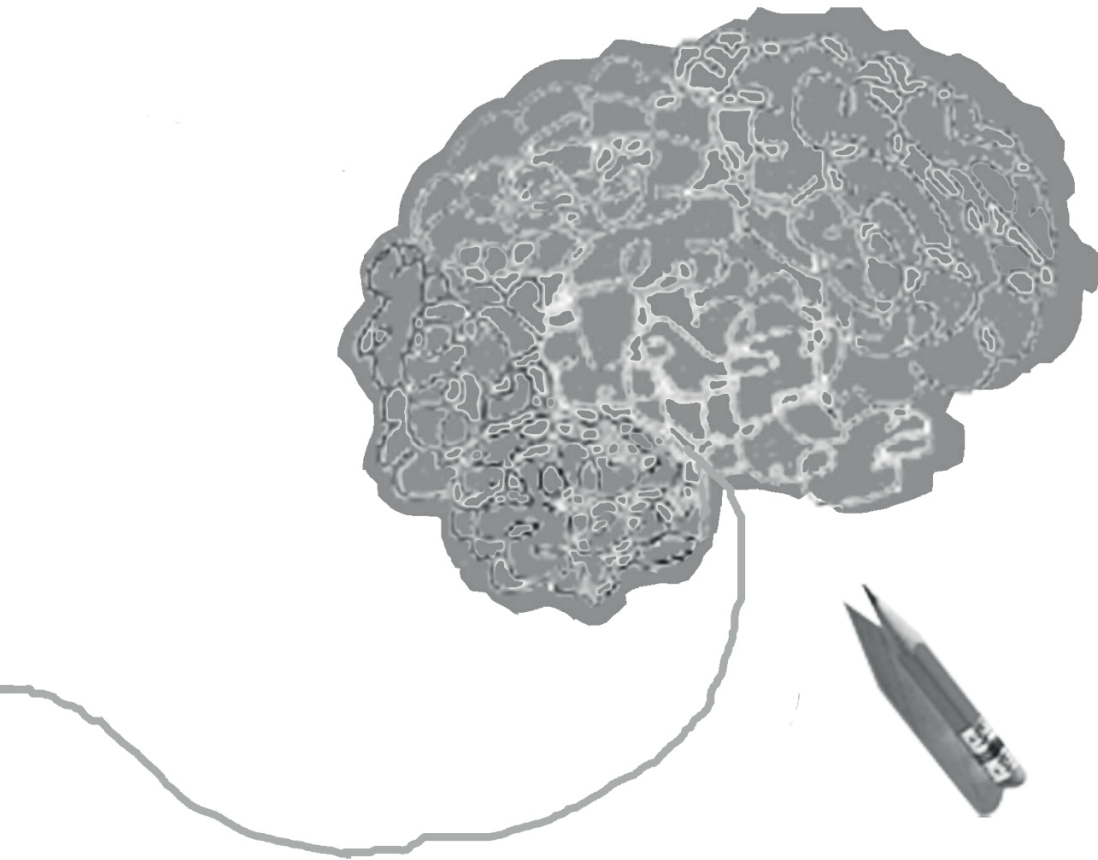
Chapter 8

Dankwoord

PhD Portfolio

List of publications

About the author

Dankwoord

Dit proefschrift is mede tot stand gekomen dankzij de hulp en steun van vele anderen. Ik wil iedereen daar hartelijk voor bedanken. Een aantal mensen wil ik in het bijzonder noemen.

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PhD Portfolio

Name PhD student:	Maria Lena Petronella (Marileen) Portegies
Erasmus MC department:	Epidemiology and Neurology
Research school:	Netherlands Institute for Health Sciences (NIHES)
PhD Period:	August 2012 – March 2016
Promotors:	Prof. dr. A. Hofman, Prof. dr. P.J. Koudstaal
Co-promotor:	Dr. M.A. Ikram

	Year	Workload (ECTS)
1. PhD training		
General courses		
Master of Science in Epidemiology (NIHES)	2012-2014	70
Research Integrity (Erasmus MC)	2014	0.3
International Conferences		
European Stroke Conference, London, UK	2013	1.0
European Stroke Conference, Nice, France	2014	1.0
European Stroke Organisation Conference, Glasgow, Scotland	2015	1.0
Alzheimer's Association International Conference, Washington DC, US		
Session chair in session: *Cognitive reserve, education and long-term effect on cognitive change and dementia incidence*	2015	2.0
Oral presentations		
Vasomotor reactivity and the risk of mortality *European Stroke Conference*	2013	0.6
Cardiac function in clinically asymptomatic persons is associated with stroke *European Stroke Conference*	2014	0.6
Pre-stroke vascular pathology and long-term prognosis after stroke *European Stroke Organisation Conference*	2015	0.6
Cognitive reserve protects against dementia after a stroke or TIA *Alzheimer's Association International Conference*	2015	0.6
Seminars and Workshops		
Research Seminars, department of epidemiology, Erasmus MC	2012-2016	4.0

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	Year	Workload (ECTS)
Other		
Peer review for scientific journals	2014-present	2.0
2. Teaching activities		
Teaching assistant, Principles of research in medicine and epidemiology, Erasmus Summer Programme, NIHES	2014, 2015	2.0
Teaching assistant, Biostatistical Methods I, NIHES	2014, 2015	2.0
Teaching assistant, Biostatistical Methods I: basic principles, NIHES	2014, 2015	2.0

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About the author

Maria Lena Petronella (Marileen) Portegies was born on June 23rd 1987 in Vlissingen, the Netherlands and was raised in Middelburg. After graduating in 2005 at the Christelijke Scholengemeenschap Walcheren in Middelburg, she started her medical education at Utrecht University. During this period, she participated in the Excellent Program, a program focusing on participating in extracurricular research. As part of this program, she conducted research at the department of Neurology under the supervision of prof. dr. G.J. Biessels.

In August 2011, Marileen obtained her medical degree cum laude. Subsequently, she worked as a resident at the department of Neurology at the Tergooi hospital in Blaricum under supervision of dr. J.A. Carpay.

In August 2012, Marileen started working on the projects described in this thesis at the department of Epidemiology of the Erasmus University Medical Center Rotterdam under supervision of prof. dr. A. Hofman (department of Epidemiology), dr. M.A. Ikram (department of Epidemiology), and prof. P.J. Koudstaal (department of Neurology). The abstract "Cerebral vasomotor reactivity and the risk of mortality: the Rotterdam Study" she presented during the European Stroke Conference in London 2013, was awarded the Junior Investigator Award. In 2014 she obtained her Master of Science degree in Health Sciences (specialization: Epidemiology) at the Netherlands Institute of Health Sciences.

In April 2016, Marileen started working as a resident at the department of Neurology at the Erasmus MC, under supervision of prof. dr. P.A.E. Sillevis Smitt.

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