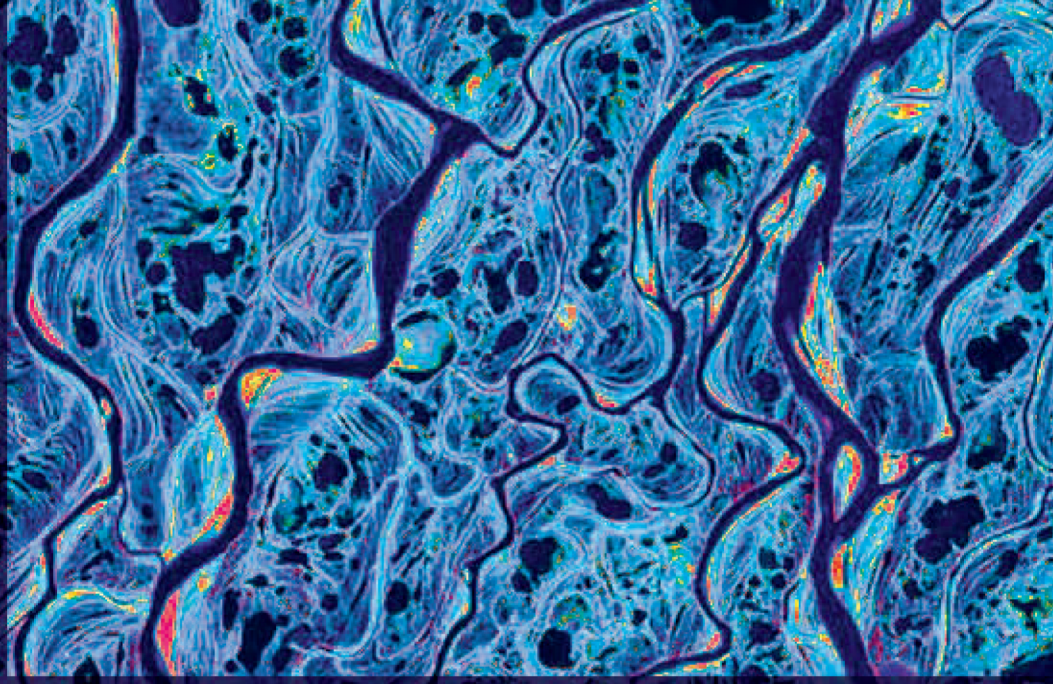
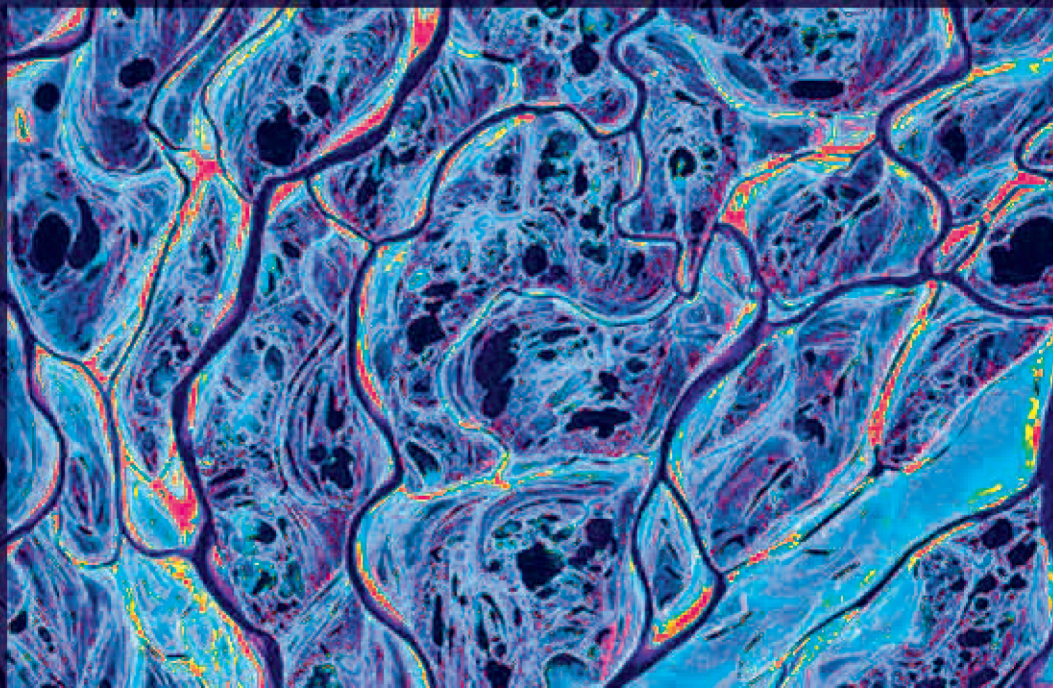


Causes of cerebral small vessel disease

A prospective population-based MRI study

Ewoud van Dijk



Causes of cerebral small vessel disease

A prospective population-based MRI study

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Causes of cerebral small vessel disease

A prospective population-based MRI study

Oorzaken van cerebrale microangiopathie

Een prospectieve MRI studie in de algemene bevolking

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
Rector Magnificus

Prof.dr. S.W.J. Lamberts

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 26 mei 2004 om 11:45 uur
door

Ewoud Jan van Dijk

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Contents

Manuscripts based on the studies described in this thesis

Chapter 2
Van Dijk EJ, Prins ND, Vermeer SE, Koudstaal PJ, Breteler MM. Frequency of white matter lesions and silent lacunar infarcts. *J Neural Transm Suppl.* 2002:25-39.

Chapter 3.1
Van Dijk EJ, Breteler MMB, Schmidt R, Berger K, Nilsson LG, Oudkerk M, Pajak A, Sans S, de Ridder M, Dufouil C, Fuhrer R, Giampaoli S, Launer LJ, Hofman A. The association between blood pressure, hypertension and cerebral white matter lesions: the CASCADE study. (Submitted)

Chapter 3.2
Vermeer SE, van Dijk EJ, Koudstaal PJ, Oudkerk M, Hofman A, Clarke R, Breteler MM. Homocysteine, silent brain infarcts, and white matter lesions: The Rotterdam Scan Study. *Ann Neurol.* 2002;51:285-9

Chapter 3.3
Van Dijk EJ, Vermeer SE, De Groot JC, van de Minkelis J, Prins ND, Oudkerk M, Hofman A, Koudstaal PJ, Breteler MMB. Arterial oxygen saturation, COPD and cerebral small vessel disease. *J Neurol Neurosurg Psych* (in press).

Chapter 3.4.1
Van Dijk EJ, Prins ND, Vermeer SE, Hofman A, Van Duijn C, Koudstaal PJ, Breteler MMB. Plasma Amyloid ß, APOE, lacunar brain infarcts, and white matter lesions. *Ann Neurol* (in press)

Chapter 3.4.2
Van Dijk EJ, Prins ND, Hofman A, Van Duijn C, Koudstaal PJ, Breteler MMB. Plasma Amyloid ß, APOE and impaired vasomotor reactivity. (Submitted)

Chapter 4.1
Van Dijk EJ, Prins ND, Vrooman HA, HofmanA, Oudkerk M, Koudstaal PJ, Breteler MMB. Progression of cerebral white matter lesions in the population-based Rotterdam Scan Study. (Submitted)

Chapter 4.2
Van Dijk EJ, Prins ND, Vermeer SE, Vrooman HA, HofmanA, Koudstaal PJ, Breteler MMB. C-reactive protein and progression of cerebral small vessel disease: The Rotterdam Scan Study. (Submitted)

Chapter 5
Vermeer SE, Hollander M, van Dijk EJ, Hofman A, Koudstaal PJ, Breteler MMB. Silent brain infarcts, white matter lesions and the risk of stroke. *Stroke* 2003;34:1126-1129.

Voor mijn familie

In the field of observation,
chance favours only the prepared mind.
Louis Pasteur, lecture 1854

1 Introduction

In the 1970s, the introduction of computed tomography (CT) and magnetic resonance imaging (MRI) in medicine, made it possible to image the living brain. These images showed that cerebral white matter lesions and asymptomatic lacunar brain infarcts are extremely common in elderly people.[1,2] Evidence is accumulating that these lesions are associated with an increased risk of late-life depression, gait disturbances, and cognitive decline.[3-5] Eventually, they may either by themselves or in combination with Alzheimer's pathology and other late life degenerative changes, lead to dementia.[6-8] In addition to clinically manifest infarcts and hemorrhages, white matter lesions and lacunar infarcts are frequently observed on brain images, suggesting a common etiology.[9]

Atherosclerosis-like changes and wall thickening of deep cerebral arterioles are observed on autopsy of elderly people in areas with white matter lesions and lacunar infarcts.[10,11] Hence, these lesions are referred to as cerebral small vessel disease. The exact etiology of white matter lesions in elderly people is unknown and a wide variety of rare causes have been described.[7,12-14] Nevertheless, almost all studies point to increasing age, arterial hypertension, and indicators of systemic atherosclerosis as the main risk factors.[1,15,16] Post-mortem and blood-flow studies show ischemia and hypoperfusion in brain areas with large white matter lesions.[7,12] Reports on the evaluation of these brain lesions over time are scarce.[17] A better understanding of the etiology of cerebral small vessel disease could contribute to the prevention of stroke, dementia and depression, as well as more subtle cognitive decline, depressive symptoms and gait disturbances in a rapidly increasing elderly population.[14]

The objective of this thesis was to gain more insight into the etiology of cerebral small vessel disease. To obtain this goal, we assessed the presence, severity and progression of cerebral white matter lesions and lacunar brain infarcts and studied the relationship with their potential causes. This was done within the Rotterdam Scan Study, a prospective population-based cohort study that included 1,077 non-demented people aged 60 to 90 years. All participants underwent brain MRI in 1995 to 1996, 668 of whom underwent a second MRI more than three years later. In chapter 2, we review studies on the frequency of cerebral small vessel disease in the general population, and in patients with stroke and dementia. In chapter 3, we describe the relation between several risk factors and cerebral small vessel disease. First, in chapter 3.1, we focus on the risk of blood pressure, change in blood pressure, hypertension and treatment of hypertension within a large European collaborative study. In chapter 3.2, we examine the relationship between homocysteine and cerebral small vessel disease. Chapter 3.3 deals with the relation between cerebral small vessel disease and low arterial oxygen saturation and chronic obstructive pulmonary disease. Chapter 3.4 concentrates on the role of plasma amyloid ß and APOE ε4 in relation to lacunar infarcts and white matter lesions (3.4.1) and impaired cerebral autoregulation (3.4.2). In chapter 4.1, we describe the progression of white matter lesions and the risk factors associated with this progression. In chapter 4.2, the relation between the inflammation marker, C-reactive protein, and

progression of white matter lesions and incident lacunar infarcts is explored. Finally, we compare the risk of stroke between people with and without cerebral small vessel disease on MRI in chapter 5. In chapter 6, I reflect on the main findings in the context of current knowledge and give suggestions for future research.

References

1. de Leeuw FE, de Groot JC, Achten E, Oudkerk M, Ramos LM, Heijboer R, Hofman A, Jolles J, van Gijn J, Breteler MM. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. J Neurol Neurosurg Psychiatry. 2001;70:9-14.
2. Vermeer SE, Koudstaal PJ, Oudkerk M, Hofman A, Breteler MM. Prevalence and risk factors of silent brain infarcts in the population-based Rotterdam Scan Study. Stroke. 2002;33:21-5.
3. de Groot JC, de Leeuw FE, Oudkerk M, Hofman A, Jolles J, Breteler MM. Cerebral white matter lesions and depressive symptoms in elderly adults. Arch Gen Psychiatry. 2000;57:1071-6.
4. Whitman GT, Tang Y, Lin A, Baloh RW, Tang T. A prospective study of cerebral white matter abnormalities in older people with gait dysfunction. Neurology. 2001;57:990-4.
5. de Groot JC, de Leeuw FE, Oudkerk M, van Gijn J, Hofman A, Jolles J, Breteler MM. Cerebral white matter lesions and cognitive function: the Rotterdam Scan Study. Ann Neurol. 2000;47:145-51.
6. Pathological correlates of late-onset dementia in a multicentre, community-based population in England and Wales. Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS). Lancet. 2001;357:169-75.
7. Roman GC, Erkinjuntti T, Wallin A, Pantoni L, Chui HC. Subcortical ischaemic vascular dementia. Lancet Neurol. 2002;1:426-36.
8. Snowdon DA, Greiner LH, Mortimer JA, Riley KP, Greiner PA, Markesbery WR. Brain infarction and the clinical expression of Alzheimer disease. The Nun Study. Jama. 1997;277:813-7.
9. Leys D, Englund E, Del Ser T, Inzitari D, Fazekas F, Bornstein N, Erkinjuntti T, Bowler JV, Pantoni L, Parnetti L, De Reuck J, Ferro J, Bogousslavsky J. White matter changes in stroke patients. Relationship with stroke subtype and outcome. Eur Neurol. 1999;42:67-75.
10. Fisher CM. Lacunes: small, deep cerebral infarcts. Neurology. 1965;15:774-784.
11. van Swieten JC, van den Hout JH, van Ketel BA, Hijdra A, Wokke JH, van Gijn J. Periventricular lesions in the white matter on magnetic resonance imaging in the elderly. A morphometric correlation with arteriolosclerosis and dilated perivascular spaces. Brain. 1991;114 (Pt 2):761-74.
12. Pantoni L, Garcia JH. Pathogenesis of leukoaraiosis: a review. Stroke. 1997;28:652-9.
13. Barkhof F, Scheltens P. Imaging of white matter lesions. Cerebrovasc Dis. 2002;13 Suppl 2:21-30.
14. van Gijn J. Leukoaraiosis and vascular dementia. Neurology. 1998;51:S3-8.
15. Bots ML, van Swieten JC, Breteler MM, de Jong PT, van Gijn J, Hofman A, Grobbee DE. Cerebral white matter lesions and atherosclerosis in the Rotterdam Study.

Lancet. 1993;341:1232-7.

16. de Leeuw FE, de Groot JC, Oudkerk M, Witteman JC, Hofman A, van Gijn J, Breteler MM. Hypertension and cerebral white matter lesions in a prospective cohort study. Brain. 2002;125:765-72.
17. Schmidt R, Fazekas F, Kapeller P, Schmidt H, Hartung HP. MRI white matter hyperintensities: three-year follow-up of the Austrian Stroke Prevention Study. Neurology. 1999;53:132-9.

2 Frequency of white matter lesions and silent lacunar infarcts

2

Frequency of white matter lesions and silent lacunar infarcts

Abstract

White matter lesions and silent lacunar infarcts are related to and may result from cerebral small vessel disease. Reported frequencies of these lesions vary largely among studies. Differences in imaging techniques, rating scales, cut-off points in lesion severity grading and study populations contribute to the variation, in addition to differences in risk factor profiles across studies.

In this paper, we will firstly discuss general methodological issues that may influence reported frequencies of white matter lesions and silent lacunar infarctions, and then review published data. We will focus on the results from population-based studies and only briefly comment on patient series of stroke and dementia.

INTRODUCTION

White matter lesions and silent lacunar infarcts are frequently observed on CT and MRI scans of healthy elderly, as well as in patients with dementia and stroke. The pathogenesis of these lesions is not yet fully understood, although findings from epidemiological, clinical and neuropathological studies suggest that small vessel disease is a major cause. Arteriolosclerosis (micro-atheromatosis and hyalinosis) causes obstruction and endothelial dysfunction of cerebral small vessels, resulting in ischemic damage.[1-4]

Although white matter lesions and silent lacunar infarcts do not result in acute symptoms, they are associated with dementia, stroke, depression and gait disturbances.[5-8] Reported prevalences of white matter lesions and silent lacunar infarcts vary largely among studies, whereas data on incidence and consequences of these lesions are scarce. In this review, we will address the methodological issues that underlie the differences in reported frequencies of these small vessel disease related cerebral lesions, and provide an overview of the frequencies reported in population-based studies, and in series of dementia and stroke patients.

METHODOLOGICAL ISSUES IN COMPARING FREQUENCIES

Imaging

White matter lesions are visible on both MRI and CT images as patchy lesions in the white matter of the centrum semi-ovale or directly adjacent to the ventricles. On MRI, white matter lesions are seen as hyperintense lesions on Proton Density, T2-weighted or FLAIR images, without prominent hypodensity on T1-weighted images. On CT, white matter lesions are poorly delineated hypodense areas.[9,10] Fisher defined lacunar infarcts according to pathological observations as deep sharply margined focal lesions with a diameter ranging from 3 to 20 mm.[11] Both on CT and MRI lacunar infarcts have approximately the same intensity as cerebrospinal fluid. However, small and incomplete infarctions located in the basal ganglia are often visible on T2-weighted MR images only. Differences in the definition of these lesions can result in variation in the reported frequencies. Furthermore, misclassification of white matter lesions and lacunar infarcts may influence frequencies in CT and MRI studies. Widened perivascular spaces can mimic lacunar infarctions.[12,13] The sensitivity of MRI for white matter lesions is superior to CT, especially for small white matter lesions and white matter lesions in the parieto-occipital region.[9] The innovation in MR scanning techniques, i.e. higher field strength magnets, thinner slices and new pulse sequences, has resulted in even larger differences with CT studies and also in differences between MRI studies.[10,14]

Rating scales

For the evaluation of the white matter lesions, numerous semi-quantitative rating scales are available. These rating scales differ in whether and to what extent they focus on size, number or configuration of the lesions, and whether they distinguish between lesions located in left or right hemisphere, in different lobes, and in periventricular or subcortical regions. The different rating scales are suitable for different study goals, albeit at the cost of their comparability.[15,16] The moderate reproducibility of some scales is another source for variation. In addition to the semi-quantitative rating scales, white matter lesions can be assessed volumetrically. Semi- and full-automatic operating methods based on different principles are in use for this purpose.[17,18] Further development of

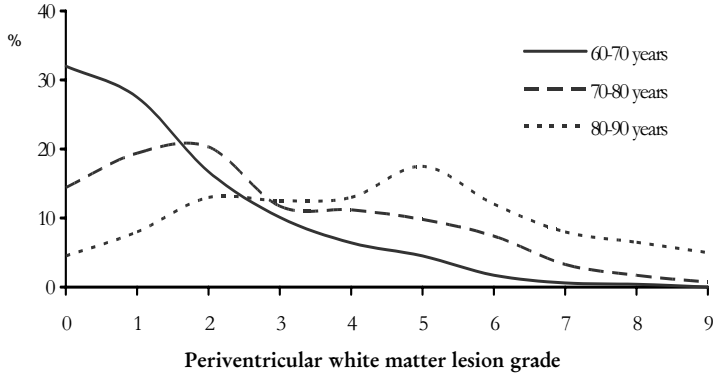

Figure 1a. Distribution of periventricular white matter lesions by 10 years age category. Reprinted with permission.[19]

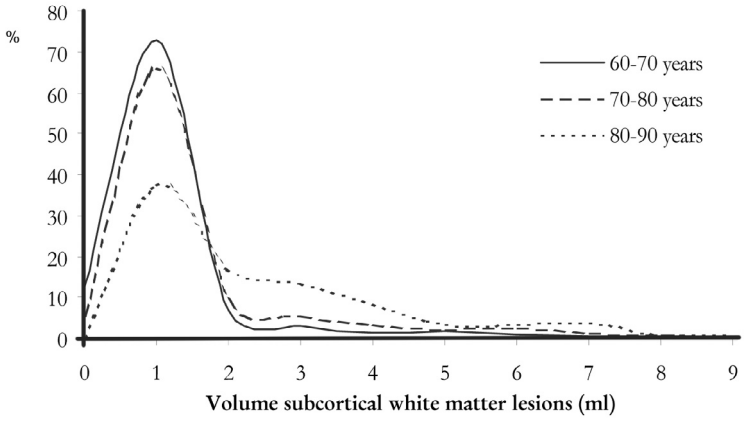

Figure 1b. Distribution of subcortical white matter lesions by 10 years age category. Reprinted with permission.[19]

these methods will hopefully lead to more uniform and interpretable measures of white matter lesions.

Cut-offs of white matter lesions distribution

Most studies, even if they rate lesions (semi-) quantitatively, use a cut-off point when they report prevalences of white matter lesions. This implies that lesion severity is dichotomised somewhere along its spectrum.

Figure 1 shows age-specific distributions of periventricular and subcortical white matter lesion severity for the population based Rotterdam Scan Study and may illustrate the effect of dichotomisation at different cut-off points on the prevalence of white matter lesions.[19] The most simple cut-off point for the dichotomisation is presence or absence of any white matter lesions. With this cut-off the prevalence of subcortical white matter lesions for people age 60 to 70 years is 87% (figure 1b). If however a cut-off at 2 ml was chosen, to indicate more severe white matter lesions, the reported prevalence would be less than 10%.

Study population

The frequency of lesions will also depend on the definition of the study population. Some studies are based on healthy volunteers, whereas others took representative samples from the general population, or only included dementia and stroke patients. Additional selection criteria that may have a major impact on frequency of lesions are a.o. exclusion of high-risk patients, co-morbidity, disease subgroups or exclusion based on age, sex or ethnicity.

Selective overrepresentation of subgroups with a different risk profile influences the overall frequency. This effect can be extensive in hospital-based studies among patients with heterogeneous diseases like dementia and stroke. Furthermore, referral patterns, disease severity and disease subgroups will influence the frequency in these hospital-based populations as well.

Response rate and chance

Lower response rates in high-risk groups will decrease the overall frequency in population-based studies. In the Rotterdam Scan Study, response rate dropped from 74% in the 60- to 70-year-old age group to 41% in the 85-year-old and over age group.[19] A Swedish study (Lund Study) reported a drop from 77% in the 45- to 55-year-old age group to 19% in the 85-year-old and over age group.[20] In the Cardiovascular Health Study (CHS) respondents in the MRI subcohort were not only significantly younger and healthier, but also higher educated and more likely to have never smoked than the original cohort.[21]

Differences in frequencies can also be caused just by sample variation. In particular, in studies with a small sample size chance can result in extreme estimates.

Table 1. Characteristics of population-based studies on white matter lesions and silent lacunar infarcts with MR imaging

Study	N	Age (mean, range)	Women (%)	Response Rate	Selection
Rotterdam Study[52]	111	74 (65-84)	54	87	Random sample from ongoing population-based study, no dementia, age and sex stratified
Lund Study[20]	77	65 (36-95)	49	44	No cerebrovascular disease
Helsinki Ageing Study[53]	128	72 (56-88)	54	38	No neuropsychiatric disease, age stratified
Cardiovascular Health Study[21]	3301	75 (65-97)	58	62	Random sample from ongoing population-based study, African-Americans oversampled (16%), not institutionalised, no cerebrovascular disease
Atherosclerosis Risk In Communities Study[26]	1920	62 (55-72)	60	68	Random sample from ongoing population-based study, African-Americans oversampled (50%)
Austrian Stroke Prevention Study[54]	355	59 (45-75)	48	28	No neuropsychiatric disease, normal neurological exam, age and sex stratified
Rotterdam Scan Study[19]	1077	72 (60-90)	52	63	Random sample from ongoing population-based study, no dementia, age and sex stratified

FREQUENCIES IN DIFFERENT STUDY POPULATIONS

Prevalence in population-based studies

We used Medline database to identify studies that were population-based, published in English, had substantial data available and used MRI to assess the prevalences of white matter lesions and silent lacunar infarcts. Table 1 shows the characteristics of the 7 studies included. The largest difference among studies is the age distribution in combination with sample size and response rate. Only the CHS and the Rotterdam Scan Study had a substantial amount of participants aged over 75 years. Furthermore, studies differed in respect that some excluded persons with neurodegenerative and cerebrovascular diseases.

Table 2 shows the overall prevalences of any white matter lesions, severe white

Table 2. Prevalence of white matter lesions and silent lacunar infarcts in population-based studies with MR imaging

Study	White matter lesions		Silent Lacunes (%)	Field strength MRI	Rating Scale white matter lesions and cut-off for severe lesions
	Any (%)	Severe (%)			
RS*	n.s.	10	n.s.	1.5T	Van Swieten scale (=Fazekas scale‡‡, without early confluent lesions rated as severe)
Lund†	62	23	8	0.2T	Fazekas scale‡‡
HAS‡	39/22††	16/16††	8	0.02T	Fazekas scale‡‡
CHS§	96	33	20	1.5T/0.35T	Combined PVWML and SCWML score on a 10 point scale (cut-off at 3)
ARIC||	86	12	9	1.5T	Combined PVWML and SCWML score on a 10 point scale (cut-off at 3)
ASPS¶	45	16	6	1.5T	Fazekas scale‡‡
RSS**	95 80/92††	28 6/28††	19	1.5T	PVWML on a 9 point scale (cut-off conform Fazekas scale‡‡) and SC WML approximated volumes based on the lesion diameter (cut-off at diameter >10mm)

n.s. = not specified; * Rotterdam Study[52]; † Lund Study[20]; ‡ Helsinki Ageing Study[53]; § Cardiovascular Health Study[21,25]; || Atherosclerosis Risk In Communities Study[22,26]; ¶ Austrian Stroke Prevention Study[24,54]; ** Rotterdam Scan study[19,23]; †† Prevalence of periventricular white matter lesions (PVWML) and subcortical white matter lesions (SCWML), respectively; ‡‡ PVWML and SCWML rated separately on a 3 point scale (cut-off for PVWML extending into the subcortical white matter, and for SCWML confluent and early confluent lesions)

matter lesions and silent lacunar infarcts. A brief description of the used white matter lesions rating scale and MR scanner modalities is given. The prevalence of any white matter lesions ranges from 39 to 96%. The Helsinki Ageing Study and Austrian Stroke Prevention Study had relatively low prevalences. In the Helsinki Ageing Study, all central nervous system and major psychiatric disease were excluded, a low field strength MR scanner was used, and periventricular and subcortical white matter lesions were only rated separately. In the Austrian Stroke Prevention Study, people with neurological or psychiatric disease and those with an abnormal neurological examination were excluded. The prevalence of any white matter lesions in the CHS and Rotterdam Scan Study is almost 100%. In contrast with the other studies no participants below the age of 60 years were included.

Most studies had data on severe white matter lesions defined as early confluent or confluent subcortical white matter lesions or periventricular white matter lesions extending to the subcortical regions. This cut-off was based on a pathological study, which found that punctate subcortical white matter lesions and pencil-thin periventricular lining in contrary to the above mentioned lesions correlate badly with ischemic damage.[1] We used this cut-off to compare prevalences of more severe white matter lesions.

The highest prevalences of severe white matter lesions and silent lacunar infarcts were observed in the CHS and the Rotterdam Scan Study. The ARIC and the Rotterdam Scan Study did not exclude participants with a history of stroke or TIA. In the Rotterdam Scan Study, the prevalence of silent lacunar infarcts was similar in people with and without such a history.[22,23] The combined prevalence of severe white matter lesions and silent lacunar infarcts in the Austrian Stroke Prevention Study was 20%.[24] After exclusion of participants with a history of stroke or TIA, the prevalence of this combination in the Rotterdam Scan Study was 33%.

Age – All studies consistently report that the prevalence and severity of both white matter lesions and silent lacunar infarcts increase with age. Age specific data were given for three studies (figure 2). Comparing data from Rotterdam Scan Study and ARIC suggest that the age distribution explains a major part of the differences in observed prevalences. The Lund Study shows the marked increase in prevalence from middle to older age. The lower prevalence in older age in this study may be explained by exclusion of people with cardiovascular disease and the low response rate, especially in the highest age stratum. Prevalences of infarct like lesions (of which approximately 80% are lacunar infarcts) ranged from 8% in 55- to 59-year-old age group to 23% in 65- to 72 year-old age group in the ARIC study, from 22% in 65- to 69-year-old age group to 43% in the age group over 85 years in the CHS, and from 8% in 60- to 64-year-old age group to 35% in 85- to 90-year-old age group in the Rotterdam Scan Study.[22,23,25] This suggests that also differences in prevalence of lacunar infarcts across studies largely result from differences in age distribution.

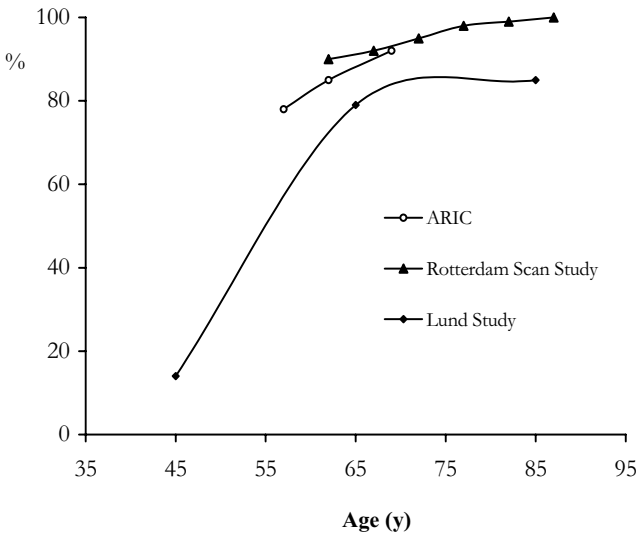

Figure 2. Prevalence of any white matter lesion by age.

Sex – Sex differences in prevalence of white matter lesions were inconsistently found over studies, with higher frequencies and more severe lesions in women. However, much of the sex differences disappeared after adjusting for age. In the Rotterdam Scan Study women had significantly more frontal periventricular white matter lesions than men in all age strata.[19] In the CHS the sex differences remained significant after adjusting for age. However, in the ARIC no significant sex difference was found.

Silent lacunar infarcts were significantly more often seen in women in the CHS and Rotterdam Scan Study, adjusted for age.

Ethnicity – Reports on ethnic differences in white matter lesions and silent lacunar infarcts prevalences are scarce and equivocal. Prevalence of any white matter lesions was significantly lower in Afro-Americans (81%) than in Caucasians (90%) in the ARIC study, adjusted for age and sex. However, the prevalence of severe white matter lesions was significantly higher in Afro-Americans (14 versus 11%). This difference was largely determined by a stronger effect of blood pressure in this group and not by differences in socio-economic status.[26]

Prevalence of infarct like lesions was doubled in middle-aged African-Americans compared to Caucasians of the same age in the ARIC. No such difference was found in the older cohort of the CHS.[25]

There are no data on prevalence of white matter lesions in population-based studies in Asia. A Japanese study that included over 900 healthy volunteers without a history of cerebrovascular disease, found a prevalence of any subcortical white matter lesions of 6% and of moderate to severe periventricular white matter lesions of 20%.[27] Possible

explanations for these relatively low prevalences include the selection of healthy people and the large proportion participants younger than 60 years of age. The prevalence of silent lacunar infarcts in Japanese studies in healthy volunteers ranges from 11 to 47%.[27,28]

Location – Subcortical white matter lesions are not equally distributed over the cerebral lobes. The highest prevalence is in the frontal lobe, followed by the parietal lobe.[19] The larger volume of these lobes or overrepresentation of these regions on the scans can possible explain this finding. Differences in vascular architecture or a different susceptibility for arteriolosclerosis of medulary arteries in the frontal and parietal lobe probably play a role as well.[29]

The vast majority of silent lacunar infarcts are located in the lentiform nucleus, internal capsule (lenticulostriatal arteries) and thalamus (thalamoperforating arteries). Approximately two-third of the participants with silent lacunar infarcts has a single lesion.[22,23,25]

Prevalence in dementia patients

White matter lesions are associated with cognitive dysfunction in the general population.[30] A large number of studies reported the frequency, risk factors and clinical significance of these lesions in dementia. The reported prevalence of white matter lesions in dementia ranges from 44 to 100% for periventricular white matter lesions and from 60 to 100% for subcortical white matter lesions.[6] Heterogeneity in dementia populations plays an important additional role in the difference of reported frequencies of white matter lesions and silent lacunar infarctions. Different clinical criteria are used for both vascular dementia and Alzheimer dementia.[31,32] This variation will influence frequencies in the dementia subgroups. The implementation of the NINDS-AIREN criteria will have contributed to differences between studies before and after 1993. According to these criteria, white matter lesions alone may be sufficient to diagnose vascular dementia when 25% or more of the white matter is involved.[33]

Furthermore, selection on disease severity by referral patterns (patients from nursing homes, memory clinics, neurological outpatient's clinics, tertiary centres, etc.) and history of the disease (early or late diagnosis in relation to disease onset) will cause incomparability. A case-control study by Barber et al. gives an overview of the prevalence of white matter lesions in the three main groups of dementia compared to age matched controls. The prevalence of any periventricular white matter lesions was 100% in vascular dementia, Alzheimer dementia and Lewy body dementia compared to 92% in controls. The prevalence of subcortical white matter lesions or hyperintensities in the basal ganglia was 89% in Alzheimer dementia, 96% in vascular dementia and 85% in Lewy body dementia, compared to 73% in controls. Both periventricular white matter lesions and subcortical white matter lesions were significantly more frequent and more severe

in demented of all three subtypes than in controls. Subcortical white matter lesions and hyperintensities in the basal ganglia were most frequent and most severe in vascular dementia compared to Alzheimer and Lewy body dementia.[6]

In other studies only periventricular white matter lesions were associated with Alzheimer dementia.[7,34,35] However others confirm the association of Alzheimer dementia with subcortical white matter lesions.[36] Fazekas et al. proposed that the greater extent of periventricular white matter lesions in Alzheimer disease patients compared to controls is an epiphenominon of subcortical brain atrophy.[34] Future longitudinal data should give clarity on this issue.

Prevalence in stroke

In 10 to 27% of stroke patients additional silent infarcts are observed on CT.[37,38] About 80% of these lesions are small lacunar infarcts, predominantly located in the basal ganglia or thalamus.[37,39]

Table 3 shows the prevalences of white matter lesions in stroke patients from studies that used CT imaging. The prevalence ranges from 3 to 44%. This variation in prevalence was largely based on differences in rating white matter lesions. One study rated white matter lesions only if the hypointensity involved both the periventricular white matter and the core of the centrum semi-ovale,[40] where another already considered a hypointensity that only affected the ventricular margins as white matter lesions.[5]

Furthermore the prevalence of white matter lesions and silent lacunar infarcts within stroke subtypes varies. Clinically manifest lacunar infarcts and deep intracerebral hemorrhage were associated with higher prevalence of silent lacunar infarcts and white matter lesions on CT within the studies. The highest prevalence of white matter lesions was found in the study that only included lacunar infarcts.[41]

MRI studies confirm the higher prevalence of severe subcortical white matter lesions (diameter lesions >10mm) in persons with lacunar infarcts compared to those with non-lacunar infarcts (53 versus 29%). They also show higher prevalences of moderate to severe periventricular white matter lesions (diameter lesions >5mm) in persons with border-zone infarcts compared to those with non border-zone infarcts.[42] However others find no difference in prevalence or severity of white matter lesions on MRI in infarct subtypes.[43]

In patients with primary intracerebral hematoma the prevalence of early confluent and confluent white matter lesions on MRI is 45% and of silent lacunar infarcts is 51%.[44] Severe white matter lesions and silent lacunar infarcts were more frequent in patients with deep (basal ganglionic and thalamic) primary intracerebral hematoma compared to hemorrhages involving the lobar regions.[44,45] In patients with clinical manifest lacunar infarction, the presence of multiple small lacunar infarcts was associated with white matter lesions.[46] Progression of white matter lesions and small lacunar infarcts was associated with symptomatic lacunar stroke compared to territorial stroke at study entry.[47]

Table 3. Characteristics of studies in stroke patients and the prevalence of any white matter lesions on CT.

Study	Population					
	N	Age (mean)	Women (%)	Inclusion criteria	Lacunar infarct (%)†	White matter lesions (%)
Bogousslavsky et al. (1987)[40]	1000	68	n.s.	Ischemic stroke	n.s.	3
Inzitari et al. (1990)[45]	116	63	39	Intracerebral hemorrhage	0	18
Hijdra et al. (1990)[55]	367	64	47	Stroke	≥26	38
Leys et al. (1992)[5]	322	68	51	TIA / Stroke	n.s.	43
Van Swieten et al. (1992)[56]	3017	65	35	TIA / minor stroke	28	11
Miyao et al. (1992)[41]	215	68	55	Lacunar stroke	100	44
Jorgensen et al. (1995)[57]	1084	73	52	TIA / Stroke	n.s.	15
Van Zagten et al. (1996)[47]	107	58*	35	Ischemic stroke	59	15
Wiszniewska et al. (2000)[58]	2289	63	38	Ischemic stroke	≥16	7

n.s. = not specified; * median; † symptomatic lacunar infarcts

Incidence in population based studies

Very little data are available on incidence or progression of lesions over time. The Austrian Stroke Prevention Study found progression within 3 years in 17.9% of the 273 participants, of which 9.9% was minor (less than 4 new punctate lesions) and 8.1% was marked (more than 4 punctate lesions or a transition to early confluent or confluent lesions). Age was not associated with lesion progression.[48] The main determinant of progression was the lesion load at baseline. A longitudinal study on gait disturbances and white matter lesions found a significant overall increase of white matter lesions volume from 3.1 to 4.2 ml in a mean follow-up of 4 years. The periventricular white matter lesions showed the most marked increase.[8] Progression may be underestimated as a result of selective survival of the less severely affected.[41,49-51] In the Austrian Stroke Prevention Study this selection effect will be even larger since people with an incident stroke, which was an end point in that study, were excluded.

SUMMARY AND CONCLUSIONS

Reported prevalence of white matter lesions and silent lacunar infarcts varies largely among studies. Differences in study design and assessment of lesions may account for the large variation in the frequencies reported.

Any white matter lesions are seen in almost all persons over the age of 70 years and studied with modern MRI techniques. Severe white matter lesions are seen in 10 to 33% and silent lacunar infarcts in 6 to 20%. Higher age is associated with higher prevalences of any white matter lesions, severe white matter lesions and silent lacunar infarcts. Women and Afro-Americans tend to have more severe white matter lesions and silent lacunar infarcts in some studies.

Because of heterogeneity in dementia and stroke populations it is difficult to compare frequencies across populations. White matter lesions are more often seen in dementia, especially in vascular dementia but also in Alzheimer dementia and Lewy body dementia. White matter lesions and silent lacunar infarcts are associated with clinical manifest lacunar infarcts and deep hemorrhagic stroke.

Harmonisation of methodological issues and assessment of lesions is needed for future studies on small vessel disease related cerebral lesions. Also there is a need for more data on incidence and progression of lesions in both population-based studies and patient series.

References

1. Fazekas F, Kleinert R, Offenbacher H, Schmidt R, Kleinert G, Payer F, Radner H, Lechner H. Pathologic correlates of incidental MRI white matter signal hyperintensities. Neurology. 1993;43:1683-9.
2. van Swieten JC, van den Hout JH, van Ketel BA, Hijdra A, Wokke JH, van Gijn J. Periventricular lesions in the white matter on magnetic resonance imaging in the elderly. A morphometric correlation with arteriolosclerosis and dilated perivascular spaces. Brain. 1991;114 (Pt 2):761-74.
3. Lammie GA, Brannan F, Slattery J, Warlow C. Nonhypertensive cerebral small-vessel disease. An autopsy study. Stroke. 1997;28:2222-9.
4. Pantoni L, Garcia JH. Pathogenesis of leukoaraiosis: a review. Stroke. 1997;28: 652-9.
5. Leys D, Pruvo JP, Scheltens P, Rondepierre P, Godefroy O, Leclerc X. Leukoaraiosis. Relationship with the types of focal lesions occuring in acute cerebrovascular disorders. Cerebrovasc Dis. 1992;2: 169-176.
6. Barber R, Scheltens P, Gholkar A, Ballard C, McKeith I, Ince P, Perry R, O'Brien J. White matter lesions on magnetic resonance imaging in dementia with Lewy bodies, Alzheimer's disease, vascular dementia, and normal aging. J Neurol Neurosurg Psychiatry. 1999;67:66-72.
7. O'Brien J, Desmond P, Ames D, Schweitzer I, Harrigan S, Tress B. A magnetic resonance imaging study of white matter lesions in depression and Alzheimer's disease. Br J Psychiatry. 1996;168:477-85.
8. Whitman GT, Tang Y, Lin A, Baloh RW, Tang T. A prospective study of cerebral white matter abnormalities in older people with gait dysfunction. Neurology. 2001;57: 990-4.
9. Wahlund LO, Barkhof F, Fazekas F, Bronge L, Augustin M, Sjogren M, Wallin A, Ader H, Leys D, Pantoni L, Pasquier F, Erkinjuntti T, Scheltens P. A new rating scale for age-related white matter changes applicable to MRI and CT. Stroke. 2001;32: 1318-22.
10. Mantyla R, Aronen HJ, Salonen O, Korpelainen M, Peltonen T, Standertskjold-Nordenstam C, Erkinjuntti T. The prevalence and distribution of white-matter changes on different MRI pulse sequences in a post-stroke cohort. Neuroradiology. 1999;41:657-65.
11. Fisher CM. Lacunar strokes and infarcts: a review. Neurology. 1982;32:871-6.
12. Jungreis CA, Kanal E, Hirsch WL, Martinez AJ, Moossy J. Normal perivascular spaces mimicking lacunar infarction: MR imaging. Radiology. 1988;169:101-4.
13. Bokura H, Kobayashi S, Yamaguchi S. Distinguishing silent lacunar infarction from enlarged Virchow-Robin spaces: a magnetic resonance imaging and pathological study. J Neurol. 1998;245:116-22.
14. Pantoni L, Garcia JH. The significance of cerebral white matter abnormalities 100 years after Binswanger's report. A review. Stroke. 1995;26:1293-301.
15. Scheltens P, Erkinjunti T, Leys D, Wahlund LO, Inzitari D, del Ser T, Pasquier F, Barkhof F, Mantyla R, Bowler J, Wallin A, Ghika J, Fazekas F, Pantoni L. White matter changes on CT and MRI: an overview of visual rating scales. European Task Force on Age-Related White Matter Changes. Eur Neurol. 1998;39:80-9.
16. Mantyla R, Erkinjuntti T, Salonen O, Aronen HJ, Peltonen T, Pohjasvaara T, Standertskjold-Nordenstam CG. Variable agreement between visual rating scales for white matter hyperintensities on MRI. Comparison of 13 rating scales in a post-stroke cohort. Stroke. 1997;28:1614-23.
17. Hirono N, Kitagaki H, Kazui H, Hashimoto M, Mori E. Impact of white mat-

ter changes on clinical manifestation of Alzheimer's disease: A quantitative study. Stroke. 2000;31:2182-8.
18. DeCarli C, Miller BL, Swan GE, Reed T, Wolf PA, Garner J, Jack L, Carmelli D. Predictors of brain morphology for the men of the NHLBI twin study. Stroke. 1999;30: 529-36.
19. de Leeuw FE, de Groot JC, Achten E, Oudkerk M, Ramos LM, Heijboer R, Hofman A, Jolles J, van Gijn J, Breteler MM. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. J Neurol Neurosurg Psychiatry. 2001;70:9-14.
20. Lindgren A, Roijer A, Rudling O, Norrving B, Larsson EM, Eskilsson J, Wallin L, Olsson B, Johansson BB. Cerebral lesions on magnetic resonance imaging, heart disease, and vascular risk factors in subjects without stroke. A population-based study. Stroke. 1994;25:929-34.
21. Longstreth WT, Jr., Manolio TA, Arnold A, Burke GL, Bryan N, Jungreis CA, Enright PL, O'Leary D, Fried L. Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people. The Cardiovascular Health Study. Stroke. 1996;27:1274-82.
22. Bryan RN, Cai J, Burke G, Hutchinson RG, Liao D, Toole JF, Dagher AP, Cooper L. Prevalence and anatomic characteristics of infarct-like lesions on MR images of middle-aged adults: the atherosclerosis risk in communities study. AJNR Am J Neuroradiol. 1999;20:1273-80.
23. Vermeer SE, Koudstaal PJ, Oudkerk M, Hofman A, Breteler MM. Prevalence and risk factors of silent brain infarcts in the population-based Rotterdam Scan Study. Stroke. 2002;33:21-5.
24. Schmidt R, Fazekas F, Hayn M, Schmidt H, Kapeller P, Roob G, Offenbacher H, Schumacher M, Eber B, Weinrauch V, Kostner GM, Esterbauer H. Risk factors for microangiopathy-related cerebral damage in the Austrian stroke prevention study. J Neurol Sci. 1997;152:15-21.
25. Bryan RN, Wells SW, Miller TJ, Elster AD, Jungreis CA, Poirier VC, Lind BK, Manolio TA. Infarctlike lesions in the brain: prevalence and anatomic characteristics at MR imaging of the elderly--data from the Cardiovascular Health Study. Radiology. 1997;202:47-54.
26. Liao D, Cooper L, Cai J, Toole JF, Bryan NR, Hutchinson RG, Tyroler HA. Presence and severity of cerebral white matter lesions and hypertension, its treatment, and its control. The ARIC Study. Atherosclerosis Risk in Communities Study. Stroke. 1996;27:2262-70.
27. Kobayashi S, Okada K, Koide H, Bokura H, Yamaguchi S. Subcortical silent brain infarction as a risk factor for clinical stroke. Stroke. 1997;28:1932-9.
28. Shimada K, Kawamoto A, Matsubayashi K, Ozawa T. Silent cerebrovascular disease in the elderly. Correlation with ambulatory pressure. Hypertension. 1990;16: 692-9.
29. Furuta A, Ishii N, Nishihara Y, Horie A. Medullary arteries in aging and dementia. Stroke. 1991;22:442-6.
30. de Groot JC, de Leeuw FE, Oudkerk M, van Gijn J, Hofman A, Jolles J, Breteler MM. Cerebral white matter lesions and cognitive function: the Rotterdam Scan Study. Ann Neurol. 2000;47:145-51.
31. Pohjasvaara T, Mantyla R, Ylikoski R, Kaste M, Erkinjuntti T. Comparison of different clinical criteria (DSM-III, ADDTC, ICD-10, NINDS-AIREN, DSM-IV) for the diagnosis of vascular dementia. National Institute of Neurological Disorders and Stroke-Association Internationale pour la Recherche et l'Enseignement en Neurosciences. Stroke. 2000;31:2952-7.
32. Erkinjuntti T, Ostbye T, Steenhuis R, Hachinski V. The effect of different diagnostic criteria on the prevalence of dementia. N Engl J Med. 1997;337:1667-74.
33. Roman GC, Tatemichi TK, Erkinjuntti

T, Cummings JL, Masdeu JC, Garcia JH, Amaducci L, Orgogozo JM, Brun A, Hofman A, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. Neurology. 1993;43:250-60.

34. Fazekas F, Kapeller P, Schmidt R, Offenbacher H, Payer F, Fazekas G. The relation of cerebral magnetic resonance signal hyperintensities to Alzheimer's disease. J Neurol Sci. 1996;142:121-5.
35. Waldemar G, Christiansen P, Larsson HB, Hogh P, Laursen H, Lassen NA, Paulson OB. White matter magnetic resonance hyperintensities in dementia of the Alzheimer type: morphological and regional cerebral blood flow correlates. J Neurol Neurosurg Psychiatry. 1994;57:1458-65.
36. Scheltens P, Barkhof F, Valk J, Algra PR, van der Hoop RG, Nauta J, Wolters EC. White matter lesions on magnetic resonance imaging in clinically diagnosed Alzheimer's disease. Evidence for heterogeneity. Brain. 1992;115(Pt 3):735-48.
37. Boon A, Lodder J, Heuts-van Raak L, Kessels F. Silent brain infarcts in 755 consecutive patients with a first-ever supratentorial ischemic stroke. Relationship with index-stroke subtype, vascular risk factors, and mortality. Stroke. 1994;25:2384-90.
38. Kase CS, Wolf PA, Chodosh EH, Zacker HB, Kelly-Hayes M, Kannel WB, D'Agostino RB, Scampini L. Prevalence of silent stroke in patients presenting with initial stroke: the Framingham Study. Stroke. 1989;20:850-2.
39. Herderschee D, Hijdra A, Algra A, Koudstaal PJ, Kappelle LJ, van Gijn J. Silent stroke in patients with transient ischemic attack or minor ischemic stroke. The Dutch TIA Trial Study Group. Stroke. 1992;23:1220-4.
40. Bogousslavsky J, Regli F, Uske A. Leukoencephalopathy in patients with ischemic stroke. Stroke. 1987;18:896-9.
41. Miyao S, Takano A, Teramoto J, Takahashi A. Leukoaraiosis in relation to prognosis for patients with lacunar infarction. Stroke. 1992;23:1434-8.
42. Mantyla R, Aronen HJ, Salonen O, Pohjasvaara T, Korpelainen M, Peltonen T, Standertskjold-Nordenstam CG, Kaste M, Erkinjuntti T. Magnetic resonance imaging white matter hyperintensities and mechanism of ischemic stroke. Stroke. 1999;30:2053-8.
43. Schmidt R, Fazekas F, Kleinert G, Offenbacher H, Gindl K, Payer F, Freidl W, Niederkorn K, Lechner H. Magnetic resonance imaging signal hyperintensities in the deep and subcortical white matter. A comparative study between stroke patients and normal volunteers. Arch Neurol. 1992;49:825-7.
44. Offenbacher H, Fazekas F, Schmidt R, Koch M, Fazekas G, Kapeller P. MR of cerebral abnormalities concomitant with primary intracerebral hematomas. AJNR Am J Neuroradiol. 1996;17:573-8.
45. Inzitari D, Giordano GP, Ancona AL, Pracucci G, Mascalchi M, Amaducci L. Leukoaraiosis, intracerebral hemorrhage, and arterial hypertension. Stroke. 1990;21:1419-23.
46. Boiten J, Lodder J, Kessels F. Two clinically distinct lacunar infarct entities? A hypothesis. Stroke. 1993;24:652-6.
47. van Zagten M, Boiten J, Kessels F, Lodder J. Significant progression of white matter lesions and small deep (lacunar) infarcts in patients with stroke. Arch Neurol. 1996;53:650-5.
48. Schmidt R, Fazekas F, Kapeller P, Schmidt H, Hartung HP. MRI white matter hyperintensities: three-year follow-up of the Austrian Stroke Prevention Study. Neurology. 1999;53:132-9.
49. Tarvonen-Schroder S, Raiha I, Kurki T, Rajala T, Sourander L. Clinical characteristics of rapidly progressive leuko-araiosis. Acta Neurol Scand. 1995;91:399-404.
50. Inzitari D, Cadelo M, Marranci ML, Pracucci G, Pantoni L. Vascular deaths in elderly neurological patients with leukoa-

raiosis. J Neurol Neurosurg Psychiatry. 1997;62:177-81.
51. Briley DP, Haroon S, Sergent SM, Thomas S. Does leukoaraiosis predict morbidity and mortality? Neurology. 2000;54:90-4.
52. Breteler MM, van Swieten JC, Bots ML, Grobbee DE, Claus JJ, van den Hout JH, van Harskamp F, Tanghe HL, de Jong PT, van Gijn J, et al. Cerebral white matter lesions, vascular risk factors, and cognitive function in a population-based study: the Rotterdam Study. Neurology. 1994;44: 1246-52.
53. Ylikoski A, Erkinjuntti T, Raininko R, Sarna S, Sulkava R, Tilvis R. White matter hyperintensities on MRI in the neurologically nondiseased elderly. Analysis of cohorts of consecutive subjects aged 55 to 85 years living at home. Stroke. 1995;26: 1171-7.
54. Schmidt R, Hayn M, Fazekas F, Kapeller P, Esterbauer H. Magnetic resonance imaging white matter hyperintensities in clinically normal elderly individuals. Correlations with plasma concentrations of naturally occurring antioxidants. Stroke. 1996;27:2043-7.
55. Hijdra A, Verbeeten B, Jr., Verhulst JA. Relation of leukoaraiosis to lesion type in stroke patients. Stroke. 1990;21:890-4.
56. van Swieten JC, Kappelle LJ, Algra A, van Latum JC, Koudstaal PJ, van Gijn J. Hypodensity of the cerebral white matter in patients with transient ischemic attack or minor stroke: influence on the rate of subsequent stroke. Dutch TIA Trial Study Group. Ann Neurol. 1992;32:177-83.
57. Jorgensen HS, Nakayama H, Raaschou HO, Olsen TS. Leukoaraiosis in stroke patients. The Copenhagen Stroke Study. Stroke. 1995;26:588-92.
58. Wiszniewska M, Devuyst G, Bogousslavsky J, Ghika J, van Melle G. What is the significance of leukoaraiosis in patients with acute ischemic stroke? Arch Neurol. 2000;57:967-73.

3 Risk factors for cerebral small vessel disease

3.1

The association between blood pressure, hypertension and cerebral white matter lesions

Abstract

Cerebral white matter lesions are frequently observed on magnetic resonance imaging (MRI) scans in elderly people and are associated with stroke and dementia. Elevated blood pressure is presumed one of the main risk factors, although data are almost exclusively derived from cross-sectional studies. We assessed in ten European cohorts the relation between concurrently and previously measured blood pressure levels, hypertension, its treatment and severe cerebral white matter lesions on MRI.

In total 1,805 non-demented subjects aged 65-75 years were sampled from on-going community-based studies that were initiated 5-20 years prior to the MRI. White matter lesions in the periventricular and subcortical region were rated separately using semi-quantitative measures. We did logistic regression analyses adjusted for potential confounders in 1,625 people with complete data.

Concurrently and formerly assessed diastolic and systolic blood pressure levels were positively associated with severe white matter lesions. Both, increase and decrease in diastolic blood pressure were associated with more severe periventricular white matter lesions. Increase in systolic blood pressure levels was associated with more severe periventricular and subcortical white matter lesions. People with poorly controlled hypertension had a higher risk of severe white matter lesions than those without hypertension, or those with controlled or untreated hypertension.

Higher blood pressure was associated with an increased risk of severe white matter lesions. Successful treatment of hypertension may reduce this risk. A potential negative effect of decreasing diastolic blood pressure level on the occurrence of severe periventricular white matter lesions should be taken into account.

INTRODUCTION

Cerebral white matter lesions are frequently observed on magnetic resonance imaging (MRI) scans in elderly people.[1-3] These lesions are associated with an increased risk of stroke, cognitive decline and dementia.[4-6] Although the exact pathogenesis of these lesions is not fully understood, they are considered to reflect ischemic small vessel disease. Elevated levels of blood pressure are thought to contribute to these lesions.[7]

Hypertension is extremely prevalent in elderly people.[8] Cross-sectional population-based MRI studies have shown a positive association between higher blood pressure and severity of white matter lesions.[3] Longitudinal data are however scarce. Midlife blood pressure reportedly is associated with white matter lesions in the elderly.[1,9-11] The Rotterdam Scan Study previously found that both an increase and a decrease in blood pressure levels are related to white matter lesions.[11] People with uncontrolled hypertension seem to have a higher prevalence of severe white matter lesions than people without hypertension or with controlled hypertension.[1,10]

We conducted a study in ten European cohorts to assess the relation between concurrent and earlier assessed blood pressure levels and cerebral white matter lesions in nondemented elderly. We also assessed the relation between change in blood pressure over time and white matter lesion severity. Finally, we assessed the relation between hypertension, its treatment, and severe white matter lesions.

METHODS

Study population

The Cardiovascular Determinants Of Dementia (CASCADE) Study is a multi-center collaborative study in Europe designed to study the etiology and natural history of brain abnormalities.[12] The study is based on ten on-going community-based cohorts that had been established 5 to 20 years prior to the CASCADE measurement.[2,13-20] Excluded for the CASCADE study were persons with a known clinical diagnosis of dementia or with Mini Mental State Examination scores <15 and those with contraindications for the MRI. In total 1,805 men and women aged 65-75 years, were randomly selected from the consecutive baseline cohorts. Since data on blood pressure-lowering medication were missing for the cohort from the United Kingdom (n=180), this study was excluded leaving 1,625 participants.

Informed consent was obtained at each center in accordance with guidelines from local Institutional Review Boards. All the individuals who took part in the exam were mobile and competent to understand the nature of their participation.

Measurement of risk factors

Data collection for CASCADE (i.e. concurrent) took place between 1996-1998 and included blood pressure measurements, assessments of cognitive function and brain MRI. Also available to CASCADE were the risk factor data collected in previous surveys of the individual cohorts except for Austria and France. The average time between the CASCADE and the preceding assessment was 5.7 (SD 2.2) years and ranged from 2.5-10 years, not taking into account the Zoetermeer Study in which it was 20 years.

Blood pressure was measured twice by a physician or trained research-nurse, on the right arm with the participant in sitting position and except for Italy with the use of a random-zero sphygmomanometer. The average of the two measurements was used. The methodology used to asses blood pressure levels followed MONICA protocol or a comparable protocol and was the same in all cohorts at all times the blood pressure was measured.[21] Hypertension was defined as a systolic blood pressure of ≥ 160mmHg, or a diastolic blood pressure of ≥ 95mmHg or the use of blood pressure lowering-medication, according to the WHO-guidelines for the treatment of hypertension at the time of the MRI assessment.[22] Diuretics, calcium antagonists, beta-blockers and ACE-inhibitors or comparable preparations were considered as blood pressure-lowering medication. Subjects were considered as successfully treated if they used blood pressure-lowering medication and their systolic and diastolic blood pressures were <160mmHg and <95mmHg, respectively. If their blood pressure still fulfilled criteria of hypertension despite blood pressure-lowering medication, they were classified as poorly controlled.

Diabetes was defined as a history of diabetes confirmed by the treating physician or as the use of oral anti-diabetics or insulin or as a fasting glucose level of 7mmol/l or greater, or a non-fasting glucose level of ≥11.1mmol/l. Smoking status was categorized as never, former and current cigarette smoking. Hypercholesterolemia was defined as a total cholesterol level of ≥6.5mmol/l. Body mass index was calculated as weight divided by height squared.

White matter lesions

All MRI scans were made with a 1.0T or 1.5T machine. The core protocol included T1-, T2-, and proton density (PD)-weighted images with 20 axial slices, 5 or 6mm thick with an interslice gap of 20 percent. The same mobile MRI machine (1.0T, Siemens, Germany) was sent to five study sites (Spain, Italy, Poland, Sweden, and UK). The German and Dutch cohorts acquired images on a 1.5T machine using the core MRI protocol. Subsequent to the start of CASCADE, two other centers with already collected scans were included (France and Austria); those scans had been obtained with comparable protocols.

White matter lesions were considered present if visible as hyperintense on PD and T2-weighted images, without prominent hypointensity on T1-weighted scans. Periventricular white matter lesions grade (range 0-9) was assessed by summing three region

specific semiquantitative grades (lesions adjacent to the frontal horns, the lateral walls, and the occipital horns of the lateral ventricle). We counted subcortical white matter lesions in three size categories based on their maximal diameter (<3mm, 3-10mm, >10mm). A total volume was approximated by assuming that these subcortical lesions were spherical with a fixed diameter (range 0-23ml).[11]

One neuro-radiologist trained three raters who scored hard copies of the images. One rater (Reading A) scored the scans from all the studies except from the Netherlands. The rater was blinded to center. The scans from the Netherlands were read by the two other raters (Reading B). There was no significant intra-reader difference in the measurements between reading A and B. Both intrarater and interrater studies (n=100 from the Dutch cohorts) showed a good to excellent agreement (κ=0.79-0.90, r=0.88-0.95).[11]

Data analysis

White matter lesion distributions were dichotomized at the upper quintile to represent severe white matter lesions (for periventricular white matter lesions grade $\geq$4 and for subcortical white matter volume $\geq$1.5ml). We calculated relative risks as estimated by the odds ratio, by means of multivariate logistic regression to quantify the association between blood pressure measurements and severe white matter lesions.

Changes in blood pressure were calculated as change in mmHg per year to adjust for differences in length of follow-up. Subsequently it was grouped in five categories (for diastolic blood pressure: <-2.5; -2.5 to -0.5; -0.5 to 0.5; 0.5 to 2.5; >2.5 mmHg/year and for systolic blood pressure: <-2.5; -2.5 to 0; 0 to 2.5; 2.5 to 5; >5 mmHg/year). The middle category was used as the reference category after data inspection. Because of the J-shaped association with change in diastolic blood pressure reported earlier in the Dutch cohorts, we repeated this analysis excluding the participants from these two cohorts to confirm this association in the other cohorts.[11] Adjustment was made for age, sex, study site and potential confounding cardiovascular risk factors. Analyses with previous blood pressure data were additionally adjusted for follow-up time.

Prior to data pooling we visually inspected the cohort specific estimates and assessed heterogeneity by formal testing of the study × blood pressure interaction. For the German cohort the effect of blood pressure on severe white matter lesions was significantly different from the other cohorts. Therefore we assessed pooled estimates with and without the German cohort.

We did logistic regression analysis with dummy variables for people with untreated hypertension, successfully treated hypertension and poorly controlled hypertension, to estimate the relative risks for severe white matter lesions compared to people with no hypertension.

Table 1. Characteristics of the study cohorts overall and stratified on study site.

	Total (n = 1625)	Austria (n = 169)	France (n = 192)	Germany (n = 194)	Italy (n = 167)	NL-RS (n = 233)	NL-ZS (n = 267)	Poland (n = 154)	Spain (n = 110)	Sweden (n = 139)
Age, y	70 (3)	68 (2)	68 (3)	71 (3)	70 (3)	70 (3)	70 (3)	70 (3)	70 (3)	69 (4)
Woman	51	46	60	51	55	48	49	49	46	53
Diabetes	10	11	7	8	11	6	8	14	19	8
Smoking (ever)	51	44	42	52	38	72	66	30	49	48
Hypercholesterolemia	36	30	42	58	33	25	26	16	30	62
Severe periventricular WML	19	10	16	10	16	30	20	23	30	15
Severe subcortical WML	21	18	20	23	23	21	14	19	30	25
Use of BP medication	34	27	34	45	26	35	31	48	20	32
Elevated blood pressure*	30	32	11	22	31	25	37	49	14	45
Hypertension†	50	47	38	57	44	46	55	71	26	61
SBP (concurrent), mmHg	146 (21)	148 (19)	133 (18)	145 (17)	149 (20)	145 (19)	150 (22)	155 (26)	136 (19)	151 (23)
SBP (previous), mmHg	137 (20)	n.a.	n.a.	139 (18)	151 (20)	134 (19)	132 (16)	143 (22)	130 (17)	137 (20)
DBP(concurrent), mmHg	82 (12)	87 (9)	77 (11)	83 (9)	79 (11)	78 (11)	82 (12)	90 (13)	73 (10)	87 (12)
DBP (previous), mmHg	80 (11)	n.a.	n.a.	82 (10)	84 (10)	74 (11)	82 (10)	81 (12)	75 (10)	88 (10)
Duration follow-up, y	5.7 (2.2)‡	n.a.	n.a.	8.0 (0.2)	3.5 (0.5)	4.8 (1.0)	20 (-)	5.0 (0.3)	9.7 (0.5)	4.0 (0.3)

Numbers are unadjusted means (standard deviation) or percentages; NL-RS = Netherlands Rotterdam Study; NL-ZS = Netherlands Zoetermeer Study; SBP = systolic blood pressure; DBP = diastolic blood pressure; n.a. = not assessed

* blood pressure ≥160/95 mmHg; † blood pressure ≥160/95 mmHg or use of blood pressure-lowering medication; ‡ exclusive NL-ZS

RESULTS

Table 1 presents characteristics of the study participants overall and stratified by study site. Blood pressure levels, the prevalence of hypertension and elevated blood pressure were highest in Poland and lowest in Spain and France.

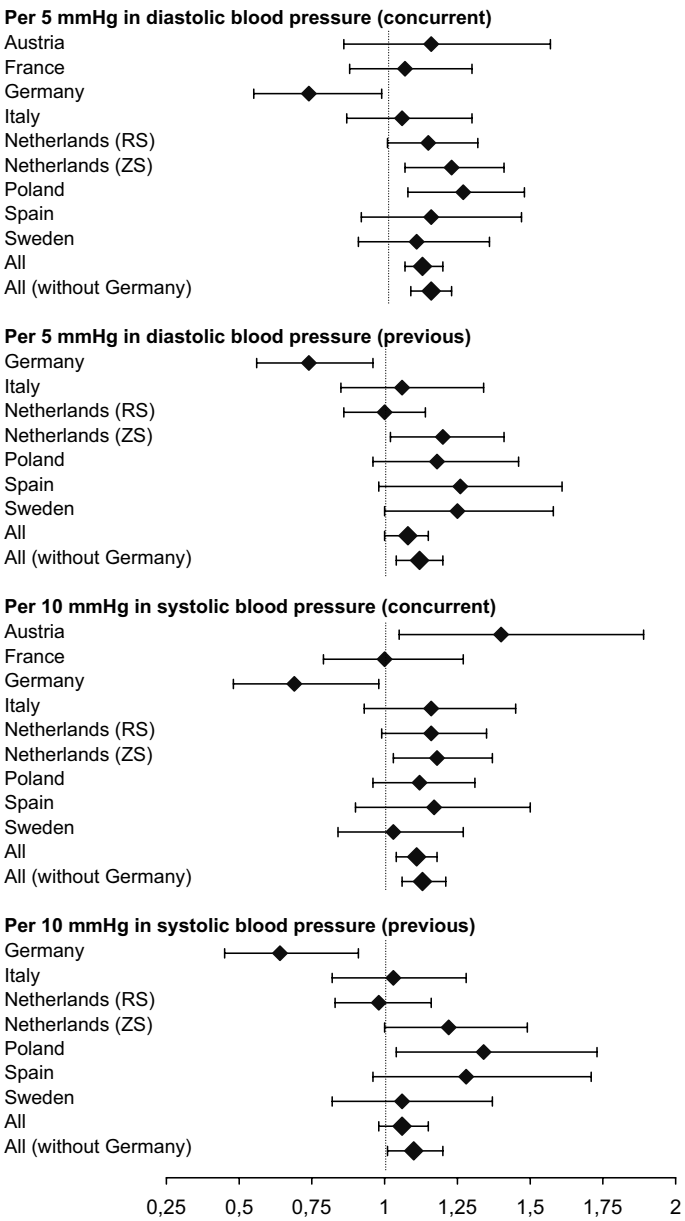

Figure 1. Odds ratios with 95% confidence intervals of concurrently and previously assessed diastolic (per 5mmHg) and systolic blood pressure (per 10 mmHg) for severe periventricular white matter lesions overall and stratified on study site (adjusted for age, sex, diabetes, smoking, hypercholesterolemia, use of blood pressure-lowering medication and study site).

Higher concurrent and previous diastolic and systolic blood pressures were equally associated with an increased risk of severe white matter lesions (figures 1 and 2). The estimates for the German cohort in relation to periventricular white mater lesions were significantly different from the others. The pooled fully adjusted relative risks (without Germany) of concurrently measured diastolic (per 5mmHg) and systolic (per 10mmHg)

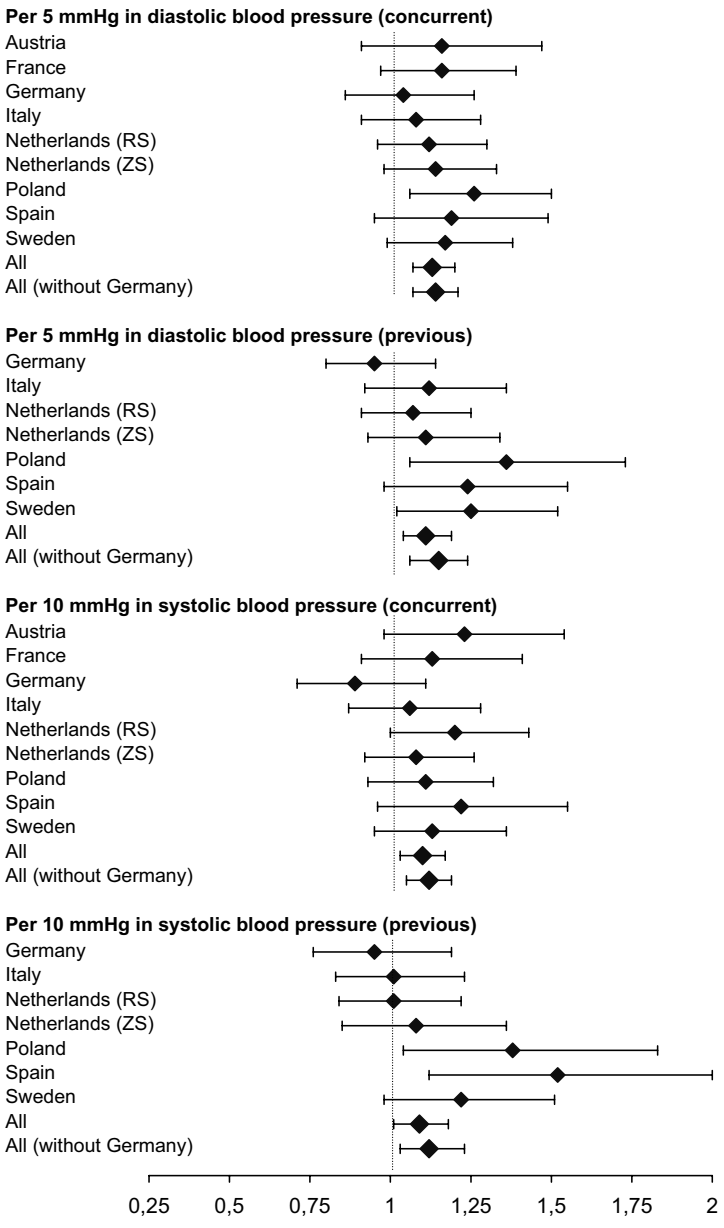

Figure 2. Odds ratios with 95% confidence intervals of concurrently and previously assessed diastolic (per 5mmHg) and systolic blood pressure (per 10mmHg) for severe subcortical white matter lesions overall and stratified on study site (adjusted for age, sex, diabetes, smoking, hypercholesterolemia, use of blood pressure-lowering medication and study site).

Table 2. Odds ratios (95%CI) of change in diastolic blood pressure per year for severe white matter lesions (exclusive Germany).

Severe white matter lesions	Change in diastolic blood pressure per year				
	<-2.5 mmHg/y (n = 105)	-2.5 to -0.5 mmHg/y (n = 255)	-0.5 to 0.5 mmHg/y (n = 268)	0.5 to 2.5 mmHg/y (n = 260)	>2.5 mmHg/y (n = 122)
Periventricular					
Model 1	2.2 (1.1;4.3)	1.6 (1.0;2.5)	1.0 (ref)	1.7 (1.1;2.8)	2.8 (1.6;5.2)
Model 2	1.9 (1.0;3.8)	1.4 (0.9;2.3)	1.0 (ref)	1.8 (1.1;2.8)	2.8 (1.5;5.1)
Model 3	4.1 (1.6;10.8)	1.8 (0.8;4.1)	1.0 (ref)	1.8 (0.8;4.0)	4.4 (1.7;11.2)
Subcortical					
Model 1	1.7 (0.9;3.2)	1.4 (0.9;2.3)	1.0 (ref)	1.6 (1.0;2.6)	1.3 (0.7;2.4)
Model 2	1.5 (0.8;2.9)	1.4 (0.8;2.2)	1.0 (ref)	1.6 (1.0;2.7)	1.3 (0.7;2.4)
Model 3	2.3 (1.0;5.3)	1.6 (0.8;3.3)	1.0 (ref)	1.5 (0.7;3.1)	1.9 (0.8;4.4)

Model 1 Adjusted for age, sex, diabetes, smoking, hypercholesterolemia and study site
Model 2 Additionally adjusted for blood pressure-lowering medication
Model 3 As model 2 and the 2 Dutch cohorts excluded

blood pressure were respectively 1.15 (95%CI 1.09;1.23) and 1.13 (95%CI 1.06;1.21) for severe periventricular white matter lesions and 1.14 (95%CI 1.07;1.21) and 1.12 (95%CI 1.05;1.19) for severe subcortical white matter lesions. Additional adjustment for length of follow-up time or body mass index did not alter the association between previously assessed blood pressure and severe white matter lesions.

Both a decrease and an increase in diastolic blood pressure, were associated with a more than doubled risk of severe periventricular white matter lesions, compared to stable blood pressure levels over time (table 2). Additional adjustment for blood pressure-lowering medication did not change these associations. The same pattern was observed in relation to severe subcortical white matter lesions, however these effects were not statistically significant. These J-shaped associations with changes in diastolic blood pressure became slightly stronger after exclusion of the two Dutch cohorts, in which this association was described before. A clear increase in systolic blood pressure was associated with a higher risk of severe periventricular and subcortical white matter lesions (table 3).

Hypertension is strongly related to severe subcortical and periventricular white matter lesions (table 4). People who were treated for high blood pressure and still had elevated levels had the highest risk of severe white matter lesions, compared to people without hypertension. They also had a significantly higher risk of severe white matter lesions than people with untreated or controlled hypertension. Exclusion of the German cohort did not alter these results.

Table 3. Odds ratios (95%CI) of change in systolic blood pressure per year for severe white matter lesions (exclusive Germany).

Severe white matter lesions	Change in systolic blood pressure per year				
	<-2.5 mmHg/y (n=125)	-2.5 to 0 mmHg/y (n=177)	0 to 2.5 mmHg/y (n=400)	2.5 to 5 mmHg/y (n=155)	>5 mmHg/y (n=154)
Periventricular					
Model 1	1.2 (0.7;2.2)	1.0 (0.6;1.6)	1.0 (ref)	1.2 (0.7;1.9)	1.8 (1.1;3.0)
Model 2	1.1 (0.6;1.9)	0.9 (0.6;1.4)	1.0 (ref)	1.1 (0.7;1.9)	1.8 (1.1;3.0)
Model 3	1.9 (0.9;4.0)	1.3 (0.7;2.7)	1.0 (ref)	1.4 (0.7;3.0)	2.4 (1.2;5.1)
Subcortical					
Model 1	1.6 (0.9;2.8)	1.4 (0.9;2.2)	1.0 (ref)	1.4 (0.9;2.4)	1.6 (1.0;2.8)
Model 2	1.5 (0.8;2.6)	1.3 (0.8;2.0)	1.0 (ref)	1.4 (0.9;2.3)	1.6 (1.0;2.7)
Model 3	1.4 (0.7;2.7)	1.6 (0.9;3.0)	1.0 (ref)	1.2 (0.6;2.4)	1.2 (0.6;2.4)

Model 1 Adjusted for age, sex, diabetes, smoking, hypercholesterolemia and study site
Model 2 Additionally adjusted for blood pressure-lowering medication
Model 3 As model 2 and the 2 Dutch cohorts excluded

Table 4. Odds ratios (95% CI) of hypertension and its treatment status for severe white matter lesions compared to normotensives who did not use blood lowering medication.

Severe white matter lesions	No hypertension	Hypertension	Hypertension treatment status		
	(n=814)	All (n=811)	Untreated (n=267)	Treated successfully (n=336)	Poorly controlled (n=208)
Periventricular	1.0 (ref)	1.6 (1.3;2.2)	1.5 (1.0;2.2)	1.4 (1.0;2.0)	2.3 (1.6;3.4)
Subcortical	1.0 (ref)	1.4 (1.1;1.8)	1.1 (0.8;1.7)	1.3 (0.9;1.8)	2.0 (1.4;2.9)

Adjusted for age, sex, diabetes, smoking, hypercholesterolemia, and study site

DISCUSSION

This study demonstrates that concurrently and formerly assessed higher systolic and diastolic blood pressure levels are associated with a higher prevalence of severe periventricular and subcortical white matter lesions. These findings were observed in all individual cohorts from different European countries except for Germany. Both people with a clear increase and clear decrease in diastolic blood pressure had more periventricular white matter lesions compared to people whose blood pressure remained stable. Increase in systolic blood pressure was associated with a higher prevalence of severe

periventricular and subcortical white matter lesions. People with uncontrolled hypertension had more often severe white matter lesions than those without hypertension or those with controlled or untreated hypertension.

The strengths of this study are its large number of participants originating from different countries reflecting the wide range of cardiovascular risk in Europe, and its longitudinal design in blood pressure measurements. Individual studies on risk factors of white matter lesions are often hard to compare due to methodological differences.[23] The standardized blood pressure and white matter lesions assessment and comparable study designs made it possible to pool the individual studies in CASCADE.

Some methodological limitations of this study need to be considered. First, participants had to be survivors of the cohort they originated from. People with high blood pressure levels or extreme changes in blood pressure levels over time, may have preferentially died and hence will be underrepresented.[24] If this non-participation was preferential in people with severe white matter lesions, which could be due to possible shared vascular risk factors and susceptibility with myocardial infarction and stroke, selection bias would have led to underestimation of the real associations.

A second potential source of selection bias was the incomplete response.[12] Non-response was most likely in people with high blood pressure and more severe white matter lesions.[10] Therefore, selection may have biased our results most likely towards an underestimation of the real associations.

Third, the association between blood pressure and white matter lesions might be confounded by other cardiovascular risk factors, study site, or length of follow-up time. However, adjustment for these factors did not significantly alter the association between blood pressure and white matter lesions, which suggests that white matter lesions are independently related to blood pressure levels and changes.

We did not observe differences between the cohorts from the different European countries with respect to the association between blood pressure and white matter lesions, except for Germany. There was no north-south gradient as seen in cardiovascular mortality.[25] We do not have a biological explanation for the deviant results in the German cohort. Selection, as mentioned above, may have preferentially effected the German study. Additional analyses for the deviant findings in the German study confirm a clear selection effect (non-participants had a significant higher blood pressure, more vascular co-morbidities and a lower self perceived general health status than participants of the German cohort).

Concurrent and previous diastolic and systolic blood pressure levels had a similar magnitudes of association to more severe white matter lesions in this sample of non-demented men and women. The main hypothesis regarding this association is that long-standing hypertension causes structural changes of the cerebral small vessels, such as thickening of the vessel walls with narrowing of the lumen, hyalinosis of the media resulting in stiffness, and tortuous elongation.[7] Together these changes lead to increased

vascular resistance and hence hypoperfusion. Furthermore, longstanding hypertension may impair cerebral autoregulation. In healthy people the mean arterial pressure remains within limits to assure perfusion during fluctuations in systemic blood pressure. These limits may shift upwards with chronic hypertension, resulting in transient falls in cerebral blood flow during periods of lower blood pressure.[26] Episodes of hypotension may then lead to hypoperfusion and ischemia of the white matter. Yet another pathogenetic mechanism may be involved. Hypertension may cause disturbances in the blood-brain barrier, which may cause lesions in the white matter by cerebral oedema, activation of astrocytes, destructive enzymes or other toxins which pass through the damaged vessel walls.[27]

This study confirms the association between decrease in diastolic blood pressure and periventricular white matter lesions, that was observed in the Rotterdam Scan Study.[11] Most vulnerable for hypoperfusion are the areas that are already marginally perfused and lack a collateral circulation. The periventricular white matter represents such an arterial border zone, which in combination with insufficient autoregulation is highly sensitive for drops in blood pressure levels.[28,29] Hypotension might be contributed to a too aggressive anti-hypertension treatment in elderly people with up-shifted autoregulation limits. People with the highest blood pressure levels in the past, who are therefore at a high risk, are most likely to drop substantially in blood pressure, due to treatment, change in life style, and aging. A drop in diastolic blood pressure may also be a consequences of arterial stiffening as part of progression of atherosclerosis, which is associated with white matter lesions.[30,31]

We found that people with poorly controlled hypertension have a higher risk of severe white matter lesions than people without hypertension. People with controlled or non-treated hypertension had an intermediate risk. This observed association is compatible with reported data on Caucasians in a comparable age range.[1] A possible explanation for our finding is that adequate control of hypertension may lead to a lesser degree of cerebral small vessel disease. The observation that people with poorly controlled hypertension have more severe white matter lesion than people without treatment may probably be explained by less severe and shorter duration of hypertension in the untreated group.

Perspectives

Our results may offer potential therapeutic possibilities in preventing and reducing the attendant cognitive decline and dementia. However, a randomized clinical trial would be needed to evaluate the effect of treatment of hypertension on the development of white matter lesions and its possible consequences.[32] The potential adverse effect of lowering the diastolic blood pressure should be born in mind and data on white matter lesion progression should be noticed.[33]

References

1. Liao D, Cooper L, Cai J, Toole JF, Bryan NR, Hutchinson RG, Tyroler HA. Presence and severity of cerebral white matter lesions and hypertension, its treatment, and its control. The ARIC Study. Atherosclerosis Risk in Communities Study. Stroke. 1996;27:2262-70.
2. de Leeuw FE, de Groot JC, Achten E, Oudkerk M, Ramos LM, Heijboer R, Hofman A, Jolles J, van Gijn J, Breteler MM. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. J Neurol Neurosurg Psychiatry. 2001;70:9-14.
3. Longstreth WT, Jr., Manolio TA, Arnold A, Burke GL, Bryan N, Jungreis CA, Enright PL, O'Leary D, Fried L. Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people. The Cardiovascular Health Study. Stroke. 1996;27:1274-82.
4. O'Brien JT, Erkinjuntti T, Reisberg B, Roman G, Sawada T, Pantoni L, Bowler JV, Ballard C, DeCarli C, Gorelick PB, Rockwood K, Burns A, Gauthier S, DeKosky ST. Vascular cognitive impairment. Lancet Neurol. 2003;2:89-98.
5. Vermeer SE, Hollander M, Van Dijk EJ, Hofman A, Koudstaal PJ, Breteler MM. Silent brain infarcts and white matter lesions increase stroke risk in the general population: the rotterdam scan study. Stroke. 2003;34:1126-9.
6. Vermeer SE, Prins ND, den Heijer T, Hofman A, Koudstaal PJ, Breteler MM. Silent brain infarcts and the risk of dementia and cognitive decline. N Engl J Med. 2003;348: 1215-22.
7. Pantoni L, Garcia JH. Pathogenesis of leukoaraiosis: a review. Stroke. 1997;28:652-9.
8. Wolf HK, Tuomilehto J, Kuulasmaa K, Domarkiene S, Cepaitis Z, Molarius A, Sans S, Dobson A, Keil U, Rywik S. Blood pressure levels in the 41 populations of the WHO MONICA Project. J Hum Hypertens. 1997;11:733-42.
9. Dufouil C, de Kersaint-Gilly A, Besancon V, Levy C, Auffray E, Brunnereau L, Alperovitch A, Tzourio C. Longitudinal study of blood pressure and white matter hyperintensities: the EVA MRI Cohort. Neurology. 2001;56:921-6.
10. de Leeuw FE, de Groot JC, Oudkerk M, Witteman JC, Hofman A, van Gijn J, Breteler MM. Hypertension and cerebral white matter lesions in a prospective cohort study. Brain. 2002;125:765-72.
11. de Leeuw FE, de Groot JC, Oudkerk M, Witteman JC, Hofman A, van Gijn J, Breteler MM. A follow-up study of blood pressure and cerebral white matter lesions. Ann Neurol. 1999;46:827-33.
12. Launer LJ, Oudkerk M, Nilsson LG, Alperovitch A, Berger K, Breteler MM, Fuhrer R, Giampaoli S, Nissinen A, Pajak A, Sans S, Schmidt R, Hofman A. CASCADE: a European collaborative study on vascular determinants of brain lesions. Study design and objectives. Neuroepidemiology. 2000;19:113-20.
13. Nilsson LG, Backman L, Erngrund K, Nyberg L, Adolfsson R, Bucht G, Karlsson S, Widing M, Winblad B. The Betula prospective cohort study: Memory, health, and aging. Aging Neuropsych Cogn. 1997;4:1-32.
14. Auperin A, Berr C, Bonithon-Kopp C, Touboul PJ, Ruelland I, Ducimetiere P, Alperovitch A. Ultrasonographic assessment of carotid wall characteristics and cognitive functions in a community sample of 59- to 71-year-olds. The EVA Study Group. Stroke. 1996;27:1290-5.
15. Giampaoli S, Poce A, Sciarra F, Lo Noce C, Dima F, Minoprio A, Santaquilani A, Caiola de Sanctis P, Volpe R, Menditto A, Menotti A, Urbinati GC. Change in cardiovascular risk factors during a 10-year community intervention program. Acta Cardiol. 1997;52:411-22.

16. Hense HW, Stieber J, Filipiak B, Keil U. Five-year changes in population blood pressure and hypertension prevalence. Results from the MONICA Augsburg surveys 1984/85 and 1989/90. Ann Epidemiol. 1993;3:410-6.
17. Marmot MG, Smith GD, Stansfeld S, Patel C, North F, Head J, White I, Brunner E, Feeney A. Health inequalities among British civil servants: the Whitehall II study. Lancet. 1991;337:1387-93.
18. Schmidt R, Lechner H, Fazekas F, Niederkorn K, Reinhart B, Grieshofer P, Horner S, Offenbacher H, Koch M, Eber B, et al. Assessment of cerebrovascular risk profiles in healthy persons: definition of research goals and the Austrian Stroke Prevention Study (ASPS). Neuroepidemiology. 1994;13:308-13.
19. Sznajd J, Pajak A, Magdon M, Misiowiec P, Malczewska-Malec M, Idzior-Walus B, Celinski A, Baczynska E. Pol-MONICA Cracow on-going study: initial findings. Acta Med Scand Suppl. 1988;728:106-12.
20. Sans S, Paluzie G, Balana L, Puig T, Balaguer-Vintro I. [Trends in prevalence, awareness, treatment and control of arterial hypertension between 1986 and 1996: the MONICA-Catalonia study] Tendencias de la prevalencia, conocimiento, tratamiento y control de la hipertension arterial entre 1986 y 1996: estudio MONICA-Cataluna. Med Clin (Barc). 2001;117:246-53.
21. Tunstall-Pedoe H, Kuulasmaa K, Amouyel P, Arveiler D, Rajakangas AM, Pajak A. Myocardial infarction and coronary deaths in the World Health Organization MONICA Project. Registration procedures, event rates, and case-fatality rates in 38 populations from 21 countries in four continents. Circulation. 1994;90:583-612.
22. 1993 guidelines for the management of mild hypertension: memorandum from a World Health Organization/International Society of Hypertension meeting. Guidelines Sub-Committee. J Hypertens. 1993; 11:905-18.
23. van Dijk EJ, Prins ND, Vermeer SE, Koudstaal PJ, Breteler MM. Frequency of white matter lesions and silent lacunar infarcts. J Neural Transm Suppl. 2002:25-39.
24. Hansson L, Zanchetti A, Carruthers SG, Dahlof B, Elmfeldt D, Julius S, Menard J, Rahn KH, Wedel H, Westerling S. Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomised trial. HOT Study Group. Lancet. 1998;351: 1755-62.
25. Sans S, Kesteloot H, Kromhout D. The burden of cardiovascular diseases mortality in Europe. Task Force of the European Society of Cardiology on Cardiovascular Mortality and Morbidity Statistics in Europe. Eur Heart J. 1997;18:1231-48.
26. Paulson OB, Strandgaard S, Edvinsson L. Cerebral autoregulation. Cerebrovasc Brain Metab Rev. 1990;2:161-92.
27. Wardlaw JM, Sandercock PA, Dennis MS, Starr J. Is breakdown of the blood-brain barrier responsible for lacunar stroke, leukoaraiosis, and dementia? Stroke. 2003;34: 806-12.
28. Matsushita K, Kuriyama Y, Nagatsuka K, Nakamura M, Sawada T, Omae T. Periventricular white matter lucency and cerebral blood flow autoregulation in hypertensive patients. Hypertension. 1994;23:565-8.
29. De Reuck J. The human periventricular arterial blood supply and the anatomy of cerebral infarctions. Eur Neurol. 1971;5: 321-34.
30. Witteman JC, Grobbee DE, Valkenburg HA, van Hemert AM, Stijnen T, Burger H, Hofman A. J-shaped relation between change in diastolic blood pressure and progression of aortic atherosclerosis. Lancet. 1994;343:504-7.
31. Bots ML, van Swieten JC, Breteler MM, de Jong PT, van Gijn J, Hofman A, Grobbee DE. Cerebral white matter lesions and atherosclerosis in the Rotterdam Study. Lancet. 1993;341:1232-7.

32. Forette F, Seux ML, Staessen JA, Thijs L, Birkenhager WH, Babarskiene MR, Babeanu S, Bossini A, Gil-Extremera B, Girerd X, Laks T, Lilov E, Moisseyev V, Tuomilehto J, Vanhanen H, Webster J, Yodfat Y, Fagard R. Prevention of dementia in randomised double-blind placebo-controlled Systolic Hypertension in Europe (Syst-Eur) trial. Lancet. 1998;352:1347-51.
33. Schmidt R, Enzinger C, Ropele S, Schmidt H, Fazekas F. Progression of cerebral white matter lesions: 6-year results of the Austrian Stroke Prevention Study. Lancet. 2003;361:2046-8.

3.2

Homocysteine, silent brain infarcts and white matter lesions

Abstract

Silent brain infarcts and white matter lesions are frequently seen on magnetic resonance imaging in healthy elderly people and both are associated with an increased risk of stroke and dementia. Plasma total homocysteine may be a potentially modifiable risk factor for stroke and dementia. We examined whether elevated total homocysteine levels are associated with silent brain infarcts and white matter lesions. The Rotterdam Scan Study is a population-based study of 1077 people aged 60 to 90 years who had cerebral magnetic resonance imaging. The cross-sectional relation of total homocysteine with silent infarcts and white matter lesions was analyzed with adjustment for cardiovascular risk factors. The mean plasma total homocysteine level was 11.5 μmol/l (standard deviation 4.1). The risk of silent brain infarcts increased with increasing total homocysteine levels (odds ratio 1.24/standard deviation increase, 95% confidence interval 1.06- 1.45). The severity of periventricular white matter lesions and extent of subcortical white matter lesions were also significantly associated with total homocysteine levels, even after excluding those with silent brain infarcts. The overall risk of having either a silent brain infarct or severe white matter lesions was strongly associated with total homocysteine levels (odds ratio 1.35/standard deviation increase, 95% confidence interval 1.16- 1.58). We concluded that total homocysteine levels are associated with silent brain infarcts and white matter lesions independent of each other and of other cardiovascular risk factors.

INTRODUCTION

Silent brain infarcts and white matter lesions are frequently seen on brain magnetic resonance imaging (MRI) in healthy elderly and either is associated with an increased risk of stroke[1] and dementia.[2,3] The presence of these lesions has been associated with several cardiovascular risk factors.[4,5] Both prospective and retrospective studies reported that an elevated total homocysteine (tHcy) concentration is a potentially modifiable risk factor for stroke.[6-9] More recently, several retrospective studies have shown that elevated levels of tHcy may also be a risk factor for dementia.[10,11] A recent study suggested that elderly patients with elevated tHcy levels had an increased risk of silent brain infarcts, but that study did not examine any associations with white matter lesions.[12] We examined whether elevated plasma tHcy, as a potentially modifiable risk factor,[13] is associated with an increased risk of silent brain infarcts and white matter lesions in elderly people and assessed the extent to which any such associations are independent of other cardiovascular risk factors and of each other.

PATIENTS AND METHODS

The Rotterdam Scan Study was designed to study the etiology and natural history of age-related brain changes in the elderly. In 1995 to 1996, we randomly selected participants aged 60 to 90 years by sex and by age in 5-year age strata from the population-based Zoetermeer[14] and Rotterdam[15] studies. A total of 1077 non-demented elderly persons participated in our study (overall response 63%).[16] Each person gave informed consent to participate in our study, which had been approved by the medical ethics committee.

Total plasma homocysteine level

We collected nonfasting blood samples into vacutainers containing sodium citrate in 1995 to 1996. Samples were put on ice immediately, centrifuged within 60 minutes, and aliquots of plasma were stored at -80^{0}C. In 1999 to 2000, plasma levels of tHcy were determined by fluorescence polarisation immunoassay on an IMx analyser (Abbott Laboratories, Chicago, IL). This method has an intralaboratory imprecision of less than 5% and showed linearity throughout the 5 to 45 μmol/l range.[17] Blood samples were not available for 39 participants because of failure to obtain a sample. Seven participants were excluded because their tHcy level fell outside this range.

Cardiovascular risk factors

We obtained information on the following variables by interview and physical examination in 1995 to 1996: systolic blood pressure, antihypertensive drugs, diabetes mellitus, pack years of smoking, and vitamin use.[18] The presence of carotid artery plaques, the

intima-media thickness of the common carotid artery, and the presence of peripheral arterial disease were assessed as noninvasive markers of atherosclerotic disease.[19]

Cerebral infarcts and white matter lesions

We obtained axial T1, T2 weighted and proton density MRI scans of the brain on 1.5 Tesla MRI scanners (MR Gyroscan, Philips, Best, the Netherlands, and MR VISION, Siemens, Erlangen, Germany) in 1995 and 1996. The slice thickness was 5 or 6 mm (scanner dependent) with a 20% interslice gap.

Infarcts were rated by a single rater and were defined as focal hyperintensities on T2 weighted images, 3 mm in size or larger. Proton density scans were used to distinguish infarcts from dilated perivascular spaces. Lesions in the white matter also had to have corresponding prominent hypointensities on T1 weighted images in order to distinguish them from cerebral white matter lesions.[18] We obtained a history of stroke and transient ischaemic attack (TIA) by self-report and by checking medical records in all 1,077 participants. A neurologist subsequently reviewed the medical history and scans and categorized the infarcts as silent or symptomatic. We defined silent brain infarcts as evidence of one or more infarcts on MRI, without a history of a (corresponding) stroke or TIA. Participants with both symptomatic and silent infarcts were categorized in the symptomatic infarct group. Twenty participants with a confirmed history of stroke had no infarcts on MRI. Three of them experienced a hemorrhagic stroke; the 17 others with ischaemic (n = 12) or unspecified (n = 5) stroke had minor symptoms.

White matter lesions were considered present if visible as hyperintense on proton density and T2 weighted images, without prominent hypointensity on T1 weighted scans. We scored periventricular and subcortical white matter lesions separately. Periventricular white matter lesions were rated semiquantitatively (grade range 0-9). A total volume of subcortical white matter lesions was approximated based on number and size of lesions (volume range 0-29.5 ml).[20]

Statistical analysis

Participants with symptomatic infarcts were excluded from all analyses. We analyzed the association between quintiles of tHcy and presence of silent brain infarcts using multiple logistic regression. Using a dichotomous approach, the reference group comprised all participants without infarcts visible on MRI. No distinction was made between participants with one or more infarcts on their scan. We evaluated the association of quintiles of tHcy and white matter lesions using analysis of covariance. The relationship of tHcy levels and white matter lesions was also analyzed continuously using multiple linear regression models. We performed separate analyses for periventricular and subcortical lesions. All analyses were adjusted for age, sex, systolic blood pressure, antihypertensive drugs, diabetes mellitus, and pack years of smoking. Additionally, we adjusted for markers of atherosclerotic disease to examine whether such associations

with tHcy were mediated by atherosclerosis.

Since the presence of silent brain infarcts and white matter lesions on MRI are highly correlated, we tested whether the association of tHcy with white matter lesions was explained by the relation between tHcy and silent brain infarcts. We therefore repeated the above analyses with white matter lesions after exclusion of participants with silent infarcts on MRI, and vice versa for the association with silent brain infarcts. Finally, we analyzed the association of tHcy with the presence of either silent infarcts or severe white matter lesions, defined as white matter lesions in the upper quintile of their distribution.

RESULTS

Selected characteristics of the study population are shown in Table 1. Twenty percent had one or more silent brain infarcts on MRI, 80% of the 1077 participants had any periventricular, and 92% any subcortical white matter lesions. The mean plasma tHcy was 11.5 μmol/l (SD 4.1). Plasma tHcy concentrations significantly increased with age (1.5 μmol/l increase/10 yrs) and were about 1.1 μmol/l higher in men than in women, 1.2

Table 1. Characteristics of the study population in 1995-1996.

	All participants n = 1077
Age (yr)	72.2 (SD 7.4)
Women	51.5%
Plasma total homocysteine (μmol/l)	11.5 (SD 4.1)
Systolic blood pressure (mm Hg)	147 (SD 22)
Use of antihypertensive drugs	34.7%
Diabetes mellitus	5.8%
Smoking: never (0 pack yr)	34.6%
> 0 and < 20 pack yr	29.6%
≥ 20 pack yr	35.8%
Use of vitamins	5.8%
Participants with infarcts on MRI: silent	20.1%
symptomatic	2.4%
both	1.5%
White matter lesions: periventricular (grade)	2.4 (SD 2.2)
subcortical (ml)	1.4 (SD 2.9)

Values are unadjusted means (standard deviation) or percentages.
SD = standard deviation.

μmol/l higher in participants who took antihypertensive drugs, and 1.4 μmol/l lower in participants who used vitamins than those who did not. Plasma tHcy levels were 0.6 μmol/l higher for every increase in plaque category. However, this association disappeared after adjustment for age, sex and other confounders (0.1 μmol/l per increase in plaque category (95% confidence interval –0.2-0.4)). Plasma tHcy was also not associated with intima-media thickness nor with peripheral artery disease (data not shown).

Silent brain infarcts were 2.5 times as common in the top quintile compared with the bottom quintile of plasma tHcy levels (Table 2). Because the crude and adjusted risk estimates and 95% confidence intervals were almost identical, only the adjusted risk estimates are presented. The results were unaltered by further adjustment for markers of atherosclerosis (data not shown). Participants were 24% more likely to have silent infarcts per standard deviation increase in tHcy (95% confidence interval 6-45%). The association of silent brain infarcts with tHcy was also significant in participants after exclusion of those with periventricular white matter lesions (odds ratio 1.79/SD increase, 95% confidence interval 1.08-2.98).

White matter lesions were also associated with plasma tHcy levels (Figure). Participants in the top quintile of tHcy had more than double the prevalence of severe white matter lesions on MRI compared with those in the bottom quintile (see Table 2). Severity of both periventricular and subcortical white matter lesions increased with higher tHcy levels (Table 3). These associations were unaltered by further adjustment for atherosclerotic disease. After exclusion of those with silent infarcts on MRI, the association of tHcy

Table 2. Association of quintiles of plasma total homocysteine (tHcy) level with silent brain infarcts, severe white matter lesions, and both on magnetic resonance imaging.

tHcy in quintiles (μmol/l)		SBI OR (95% CI)[c]	Severe WML[a] OR (95% CI)[c]	SBI and/or severe WML[b] OR (95% CI)[c]
I	5.0- 8.5	1 (ref)	1 (ref)	1 (ref)
II	8.6- 9.8	1.4 (0.8-2.5)	1.9 (1.0-3.4)	1.7 (1.0-2.9)
III	9.9-11.3	1.7 (1.0-3.1)	2.1 (1.1-3.7)	2.0 (1.2-3.3)
IV	11.4-13.7	1.6 (0.9-2.9)	2.0 (1.1-3.7)	2.3 (1.4-3.8)
V	13.8-45.0	2.5 (1.4-4.5)	2.3 (1.3-4.2)	3.0 (1.8-5.2)

[a]Presence of severe periventricular and/or severe subcortical white matter lesions, defined by the upper quintile of their distribution.
[b]Presence of silent brain infarcts and/or severe periventricular and/or severe subcortical white matter lesions.
[c]Adjusted for age, sex, systolic blood pressure, antihypertensive drugs, diabetes mellitus, and smoking.
SBI = silent brain infarcts; OR = odds ratio; CI = confidence interval; WML = white matter lesions.

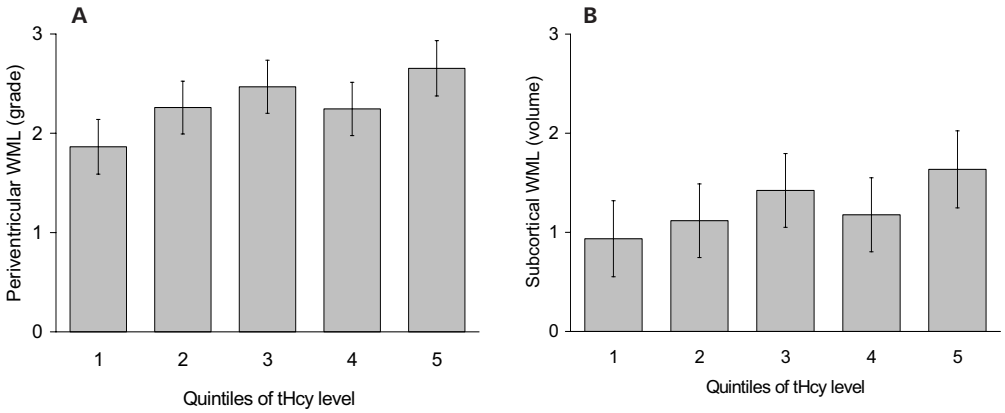

Figure. Association between quintiles of plasma total homocysteine (tHcy) level and periventricular (A) and subcortical (B) white matter lesions (mean grade or volume (ml) and 95% confidence intervals, adjusted for age, sex, systolic blood pressure, antihypertensive drugs, diabetes mellitus, and smoking).

Table 3. Association of plasma total homocysteine (tHcy) levels with periventricular and subcortical white matter lesions

	Periventricular white matter lesions (Mean increase in grade, 95% CI)[a]		Subcortical white matter lesions (Mean increase in volume [ml], 95% CI)[a]	
	All participants	Participants without infarcts	All participants	Participants without infarcts
Model 1[b]	0.19 (0.06-0.31)	0.21 (0.08-0.33)	0.24 (0.07-0.42)	0.14 (0.003-0.28)
Model 2[c]	0.18 (0.05-0.30)	0.19 (0.06-0.32)	0.25 (0.08-0.42)	0.14 (0.001-0.28)
Model 3[d]	0.17 (0.05-0.30)	0.16 (0.03-0.29)	0.25 (0.08-0.43)	0.11 (–0.03-0.25)

[a] Mean increase in grade or volume (ml) per standard deviation increase in tHcy level.
[b] Adjusted for age and sex.
[c] Additionally adjusted for systolic blood pressure, antihypertensive drugs, diabetes mellitus, and smoking.
[d] Adjustments as in Model 2, with atherosclerotic markers included in the model.
CI = confidence interval.

with periventricular white matter lesions was unaltered; the association of tHcy with subcortical white matter lesions was attenuated but remained significant.

A total of 378 (36%) of the 1,077 participants had either infarcts on MRI, severe white matter lesions or both. Plasma total tHcy levels were increased in these participants (see Table 2). The risk of having either a silent brain infarct or severe white matter lesions was strongly associated with tHcy levels (odds ratio 1.35/SD increase, 95% confidence interval 1.16- 1.58), and further adjustment for atherosclerotic markers did

not alter these associations. Exclusion of participants who used vitamins or who had a history of stroke but no infarct on MRI did not change any of the results above.

DISCUSSION

The present study demonstrated a strong and significant association between plasma tHcy levels and silent brain infarcts and white matter lesions on MRI. We showed that plasma total tHcy is an independent risk factor for the presence of silent brain infarcts, white matter lesions, and both. The relationships of these MRI lesions with tHcy levels were continuous and graded, with no obvious threshold below which lower tHcy levels were not associated with lower risks of disease.

The strengths of this study are the population-based design and the large number of elderly participants. However, because the response rate in our study was about 63%, it is possible that selection bias may have influenced the results. Those people who agreed to participate were significantly younger and had a lower prevalence of hypertension compared with nonresponders.[20] Old age and hypertension are known risk factors for the presence of both silent brain infarcts and white matter lesions. Hence, people with infarcts and severe white matter lesions on MRI may be somewhat underrepresented in our study. This bias might be expected to result in an attenuation of any association of tHcy with MRI lesions.

The plasma tHcy level was measured without knowledge of other risk factors or presence of lesions on MRI. Silent brain infarcts and white matter lesions were scored also blind to all other data. Therefore, any misclassification will be random and result in an underestimation of the strength of any risk associations. The use of a single tHcy measurement to classify persons may have underestimated the strength of any associations because of regression dilution by 10% to 15%.[21]

Elevated tHcy levels reflect nutritional deficiencies, genetic defects, or renal impairment. We do not have data on any of these determinants of tHcy concentrations and hence the present study is unable to address these issues. Further studies are required to address the extent to which the differences caused by tHcy levels reflect vitamin B12 or folate deficiency or renal impairment.

Recently, a study involving 153 participants reported a fourfold increased risk of silent brain infarcts in elderly with a tHcy greater than 15 μmol/l, but it did not present an odds ratio of silent brain infarcts after adjustment for all other confounders.[12] This study showed no attenuation of the association between tHcy and silent brain infarcts after adjustment for renal function. The strong association between tHcy and silent brain infarcts is consistent with results from previous studies that showed that tHcy is a risk factor for symptomatic infarcts.[6,7,22] There have been no previous published studies that have examined the relationship between tHcy and white matter lesions. Plasma tHcy

levels showed a significant relationship with both periventricular and subcortical white matter lesions, although the latter association was attenuated after exclusion of those without infarcts on MRI. We previously suggested periventricular and subcortical white matter lesions might have a different pathophysiology.[23] The vascular supply to the subcortical white matter is believed to be superior to the periventricular region, which as an arterial border zone may be more vulnerable to hypoperfusion.[24,25] We hypothesize that this may render the periventricular white matter more susceptible to damage caused by elevated tHcy levels. This might explain our finding of a stronger relationship of tHcy levels with periventricular than with subcortical white matter lesions.

The mechanisms through which elevated tHcy levels might cause vascular damage to the brain are unclear. Elevated tHcy levels may promote atherosclerosis by damaging the vascular wall[26] or by its direct toxic effect on nerve cells.[27] We tested whether the action of tHcy may be mediated via peripheral atherothrombosis by adjusting for the additional effects of markers of atherosclerosis. This did not modify the effect of tHcy on risk of silent brain infarcts or white matter lesions in any of the analyses. This is consistent with Fassbender's finding that tHcy was associated with small vessel disease but not with large vessel disease.[28] However, Clarke and colleagues reported in a study of patients with histologically confirmed Alzheimer's disease that both patients with and without macroscopic cerebral infarcts had higher tHcy concentrations than age-matched controls.[10] Further histopathological studies in patients with suspected dementia are needed to examine the associations of tHcy (and other risk factors) with microvascular disease. Whether plasma tHcy causes direct neurotoxicity cannot be tested in vivo, so the underlying mechanism remains unclear.

In conclusion, we found a strong relation between plasma tHcy levels with silent brain infarcts and with white matter lesions. However, this is a cross-sectional study and the results should be confirmed by prospective longitudinal studies. Whereas randomised controlled trials have shown that vitamin supplements can effectively reduce plasma tHcy levels,[29] the results of ongoing large-scale trials are needed to determine whether lowering tHcy levels reduces the risk of stroke and other cardiovascular disease. Several such trials designed to assess the effects of folic acid-based vitamin supplements on cardiovascular risk include an assessment of cognitive function. However, further trials in high-risk elderly populations are needed to assess whether such therapy may reduce the risk of dementia.

References

1. Kobayashi S, Okada K, Koide H, et al. Subcortical silent brain infarction as a risk factor for clinical stroke. Stroke 1997; 28: 1932-1939.
2. Kuller LH, Shemanski L, Manolio T, et al. Relationship between ApoE, MRI findings, and cognitive function in the Cardiovascular Health Study. Stroke 1998; 29:388-398.

3. Barber R, Scheltens P, Gholkar A, et al. White matter lesions on magnetic resonance imaging in dementia with Lewy bodies, Alzheimer's disease, vascular dementia, and normal aging. J Neurol Neurosurg Psychiatry 1999; 67:66-72.
4. Longstreth WT, Jr., Bernick C, Manolio TA, et al. Lacunar infarcts defined by magnetic resonance imaging of 3660 elderly people: the Cardiovascular Health Study. Arch Neurol 1998; 55:1217-1225.
5. Breteler MMB, van Swieten JC, Bots ML, et al. Cerebral white matter lesions, vascular risk factors, and cognitive function in a population-based study: the Rotterdam Study. Neurology 1994; 44:1246-1252.
6. Perry IJ, Refsum H, Morris RW, et al. Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. Lancet 1995; 346:1395-1398.
7. Bots ML, Launer LJ, Lindemans J, et al. Homocysteine and short-term risk of myocardial infarction and stroke in the elderly: the Rotterdam Study. Arch Intern Med 1999; 159:38-44.
8. Sacco RL, Roberts JK, Jacobs BS. Homocysteine as a risk factor for ischemic stroke: an epidemiological story in evolution. Neuroepidemiology 1998; 17:167-173.
9. Eikelboom JW, Lonn E, Genest J, Jr., et al. Homocyst(e)ine and cardiovascular disease: a critical review of the epidemiologic evidence. Ann Intern Med 1999; 131:363-375.
10. Clarke R, Smith AD, Jobst KA, et al. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. Arch Neurol 1998; 55:1449-1455.
11. Lehmann M, Gottfries CG, Regland B. Identification of cognitive impairment in the elderly: homocysteine is an early marker. Dement Geriatr Cogn Disord 1999; 10:12-20.
12. Matsui T, Arai H, Yuzuriha T, et al. Elevated plasma homocysteine levels and risk of silent brain infarction in elderly people. Stroke 2001; 32:1116-1119.
13. Refsum H, Ueland PM, Nygård O, Vollset SE. Homocysteine and cardiovascular disease. Annu Rev Med 1998; 49:31-62.
14. Hofman A, van Laar A, Klein F, Valkenburg HA. Coffee and cholesterol (letter). N Engl J Med 1983; 309:1248-1249.
15. Hofman A, Grobbee DE, de Jong PTVM, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol 1991; 7:403-422.
16. de Groot JC, de Leeuw FE, Oudkerk M, et al. Cerebral white matter lesions and cognitive function: the Rotterdam Scan Study. Ann Neurol 2000; 47:145-151.
17. Nexo E, Engbaek F, Ueland PM, et al. Evaluation of novel assays in clinical chemistry: quantification of plasma total homocysteine. Clin Chem 2000; 46:1150-1156.
18. Vermeer SE, Koudstaal PJ, Oudkerk M, et al. Prevalence and risk factors of silent brain infarcts in the population-based Rotterdam Scan Study. Stroke 2001 (in press).
19. Bots ML, van Swieten JC, Breteler MMB, et al. Cerebral white matter lesions and atherosclerosis in the Rotterdam Study. Lancet 1993; 341:1232-1237.
20. de Leeuw FE, de Groot JC, Oudkerk M, et al. A follow-up study of blood pressure and cerebral white matter lesions. Ann Neurol 1999; 46:827-833.
21. Clarke R, Woodhouse P, Ulvik A, et al. Variability and determinants of total homocysteine concentrations in plasma in an elderly population. Clin Chem 1998; 44: 102-107.
22. Boushey CJ, Beresford SAA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA 1995; 274:1049-1057.
23. de Leeuw FE, de Groot JC, Bots ML, et al.

Carotid atherosclerosis and cerebral white matter lesions in a population based magnetic resonance imaging study. J Neurol 2000; 247:291-296.
24. Pantoni L, Garcia JH. Pathogenesis of leukoaraiosis: a review. Stroke 1997; 28: 652-659.
25. van Swieten JC, van den Hout JHW, van Ketel BA, et al. Periventricular lesions in the white matter on magnetic resonance imaging in the elderly. A morphometric correlation with arteriolosclerosis and dilated perivascular spaces. Brain 1991; 114: 761-774.
26. Hankey GJ, Eikelboom JW. Homocysteine and vascular disease. Lancet 1999; 354: 407-413.
27. Lipton SA, Kim WK, Choi YB, et al. Neurotoxicity associated with dual actions of homocysteine at the N-methyl-D-aspartate receptor. Proc Natl Acad Sci U S A 1997; 94:5923-5928.
28. Fassbender K, Mielke O, Bertsch T, et al. Homocysteine in cerebral macroangiography and microangiopathy (letter). Lancet 1999; 353:1586-1587.
29. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. Homocysteine Lowering Trialists' Collaboration. BMJ 1998; 316:894-898.

3.3

Arterial oxygen saturation, COPD and cerebral small vessel disease

Abstract

Objective *— To study whether lower arterial oxygen saturation (SaO_2) and chronic obstructive pulmonary disease (COPD) are associated with cerebral white matter lesions and lacunar infarcts.*
Methods *— We measured SaO_2 twice with a pulse oximeter, assessed the presence of COPD, and performed MRI in 1077 non-demented people from a general population (age 60 to 90 years). We rated periventricular white matter lesions (on a scale of 0 to 9) and approximated a total subcortical white matter lesion volume (range 0 to 29.5 ml). All analyses were adjusted for age and sex and additionally for hypertension, diabetes, body mass index, pack years smoked, cholesterol, haemoglobin, myocardial infarction, and left ventricular hypertrophy.*
Results *— Lower SaO_2 was independent of potential confounders associated with more severe periventricular white matter lesions (score increased by 0.12 per 1% decrease in SaO_2 (95% confidence interval 0.01 to 0.23)). Participants with COPD had more severe periventricular white matter lesions than those without (adjusted mean difference in score 0.70 (95% confidence interval 0.23 to 1.16)). Lower SaO_2 and COPD were not associated with subcortical white matter lesions or lacunar infarcts.*
Conclusion *— Lower SaO_2 and COPD are associated with more severe periventricular white matter lesions.*

INTRODUCTION

Cerebral white matter lesions and lacunar brain infarcts are frequently observed on magnetic resonance imaging scans of elderly people.[1-4] Evidence is accumulating that these lesions play an important role in the development of cognitive decline and dementia.[5-7] Although the exact pathogenesis of these lesion is not fully understood, they are considered to be caused by ischaemic small vessel disease, with hypertension and increased age as the most important risk factors. Degenerative changes of cerebral small vessels result in narrowing and obstruction of the arteriolar lumen and alteration of the cerebral autoregulation, both resulting in hypoperfusion of the cerebral white matter and basal ganglia.[8]

In addition to cerebral perfusion, the arterial oxygen content determines the total amount of oxygen available in the brain. Low arterial oxygen pressure strongly aggravates brain damage caused by cerebral hypoperfusion. Pure hypoxaemic insults, however, fail to cause brain damage.[9] Whether lower arterial oxygen pressure in elderly people plays a role in the pathophysiology of white matter lesions is unknown.

The assessment of the arterial oxygen pressure is a relatively invasive procedure and therefore not applicable in a large population based study. The oxygen saturation of haemoglobin, as assessed by pulse oximetry, is a non-invasive measurement that gives an indication of the arterial oxygen pressure. We studied the association between arterial oxygen saturation (SaO_2) and white matter lesions and lacunar infarcts. Chronic obstructive pulmonary disease (COPD) is a common disease among elderly people.[10-13] Patients with COPD suffer from oxygen deprivation for prolonged periods, especially during physical exercise, exacerbation of disease and sleep.[14,15] We therefore also examined the association between COPD and white matter lesions and lacunar infarcts.

METHODS

Study sample

The Rotterdam Scan Study was designed to study the aetiology and natural history of age related brain changes in the elderly. In 1995 to 1996, we randomly selected participants aged 60 to 90 years by sex and 5 year age strata from the population based Zoetermeer[16] and Rotterdam[17] studies. These populations were almost entirely white. A total of 1077 non-demented elderly persons participated in our study (overall response 63%).[3] Each person gave informed consent to participate in our study, which had been approved by the local medical ethics committee.

Arterial oxygen saturation and COPD

All participants underwent an interview and a physical examination at the time of MRI

scanning. SaO_2 was measured twice, 5 minutes apart, with a pulse oximeter (Oxycount, Andos, Hamburg, Germany) on the right index finger. The two measurements were averaged (correlation coefficient was 0.73 and the range was 88 to 99%).

Participants were asked to bring all their prescription drugs with them to the research centre. The research physicians recorded these and checked their indication.

COPD was considered present if a person was taking inhalation medication from at least one of the following groups: sympaticomimetics, parasympaticolytics, or glucocorticosteroids.

Measurements of other covariates

Blood pressure was measured twice on the right arm with a random zero sphygmomanometer, and the average of these two measurements was used. Hypertension was defined as a systolic blood pressure of ≥140 mm Hg, a diastolic blood pressure of ≥90 mm Hg, and/or the use of blood pressure lowering medication.

We collected non-fasting blood samples from all participants. We considered diabetes mellitus to be present if a person used oral anti-diabetics or insulin and/or had a glucose level ≥11.1 mmol/l. The body mass index was calculated as weight (kg) divided by height squared (m^2). A research physician obtained information on smoking habits using a structured questionnaire. Patients were categorised as having smoked or never smoked. Furthermore, we calculated for every participant the number of pack years smoked (number of cigarettes per day × years of smoking/20). Serum total cholesterol levels were measured from non-fasting blood samples using an automated enzymatic method. The presence of left ventricular hypertrophy and myocardial infarction was assessed by MEANS interpretation of a 12 lead electrocardiogram (Acta electrocardiograph; Esaote, Florence, Italy).[18] Haemoglobin levels were assessed using a standard and validated method.[19]

Cerebral white matter lesions and lacunar infarcts

All participants underwent MRI scanning of the brain. We made axial T1, T2 and proton density weighted scans on 1.5 T MRI scanners (MR Gyroscan; Philips, Best, the Netherlands, and MR Vision; Siemens, Erlangen, Germany). The slice thickness was 5 or 6 mm (scanner dependent) with an interslice distance of 1 mm.

White matter lesions were considered present if visible as hyperintense signals on proton density and T2 weighted images, without prominent hypointensity on T1 weighted scans. When the largest diameter of the white matter lesion was directly adjacent to the ventricle, it was defined as periventricular, otherwise as subcortical. The scoring method has been described in detail previously.[20] Briefly, periventricular white matter lesions were rated semiquantitatively from 0 (no lesion) to 3 (large confluent lesion) at three regions (adjacent to the frontal horns, the lateral walls, and the occipital horns of the lateral ventricle). We added the sum of the region specific scores to acquire

a total periventricular white matter lesion score (range 0 to 9). We counted subcortical white matter lesions in three size categories based on their maximal diameter: small (<3 mm), medium (3 to 10 mm), and large (>10 mm). A total volume was approximated by assuming these subcortical lesions to be spherical with a fixed maximal diameter (volume range 0 to 29.5 ml). Both inter- and intra-reader studies (n=100) showed good to excellent agreement (for periventricular white matter lesion grade the kappa values were 0.79 and 0.90, respectively, and for subcortical white matter lesion volume the intra-class correlation coefficients were 0.88 and 0.95, respectively). We defined lacunar infarcts as focal hyperintensities on T2 weighted images 3 to 20 mm in size and located in the subcortical white matter or basal ganglia. Proton density scans were used to distinguish infarcts from dilated perivascular spaces. Lesions in the white matter also had to have corresponding prominent hypointensities on T1 weighted images for us to distinguish them from cerebral white matter lesions.

Data analysis

The relationship of SaO_2 and periventricular and subcortical white matter lesions was analysed using multiple linear regression models with SaO_2 categorised in tertiles and subsequently with SaO_2 as a continuous variable. We used linear regression analysis to calculate adjusted mean differences of periventricular white matter lesion score and subcortical white matter lesion volume between participants with and without COPD. We analysed the association between SaO_2 and COPD and the presence of lacunar infarcts by multiple logistic regression analysis; from these analyses all people with cortical infarcts were excluded (n=33). All analyses were adjusted for age and sex and additionally for hypertension, diabetes, body mass index, pack years smoked, cholesterol level, haemoglobin concentration, myocardial infarction, and left ventricular hypertrophy. We performed supplementary analysis separately for those

Table 1. Characteristics of the study population in 1995-1996

	All participants (n = 1077)
Age, years	72.2 (7.4)
Women, %	51.5
Hypertension, %	73.0
Diabetes Mellitus, %	7.0
Body mass index, kg/m^2	26.7 (3.6)
Ever smoked cigarettes, %	66.4
Smoking, pack years	19.1 (24.0)
Total cholesterol, mmol/l	5.9 (1.0)
Haemoglobin, mmol/l	8.7 (0.7)
Left ventricular hypertrophy, %	2.9
Myocardial infarction, %	10.3
Oxygen saturation, %SaO_2	96.5 (88-99)
Lacunar infarcts, %*	21.6
White matter lesion severity	
Periventricular, score 0-9	2.4 (2.2)
Subcortical, ml	1.4 (2.9)

Values are percentages or unadjusted means (SD), or for oxygen saturation the median (range).
* People with cortical infarcts were excluded (n = 33)

patients who had ever smoked and those who had never smoked. All regression analyses were followed by residual analysis to confirm assumptions of the model.

RESULTS

Table 1 gives selected characteristics of the study population. We measured SaO_2 for all participants, except for 11 subjects for whom we could not get a signal from the oximeter. These 11 people did not differ in the presented characteristics from the participants that had complete data. Seventy three participants had COPD, 31 (43%) of whom had chronic bronchitis, 9 (12%) had lung emphysema, and 33 (45%) had a combination of both or was not further specified.

SaO_2 was on average lower in men, those who were ever cigarette smokers, and higher age. Participants with COPD were on average older, more often men, and were more often ever cigarette smokers. The unadjusted mean SaO_2 was 0.84% (95% confidence interval (CI) 0.55 to 1.13%) lower in participants with COPD than in those without. Among the smokers, those with COPD smoked on average more pack years than those without COPD.

Figure 1 shows the association between SaO_2 in tertiles (means 95.3%, 96.7%, and 97.8%) and white matter lesions. People in the lowest tertile of SaO_2 had more severe periventricular white matter lesions than those in the upper tertile, but did not have more severe subcortical white matter lesions. The age and sex adjusted mean periventricular white matter lesion score increased by 0.12 per 1% decrease in SaO_2 (95% CI 0.02 to 0.21). After additional adjustment for hypertension, diabetes mellitus, body mass index,

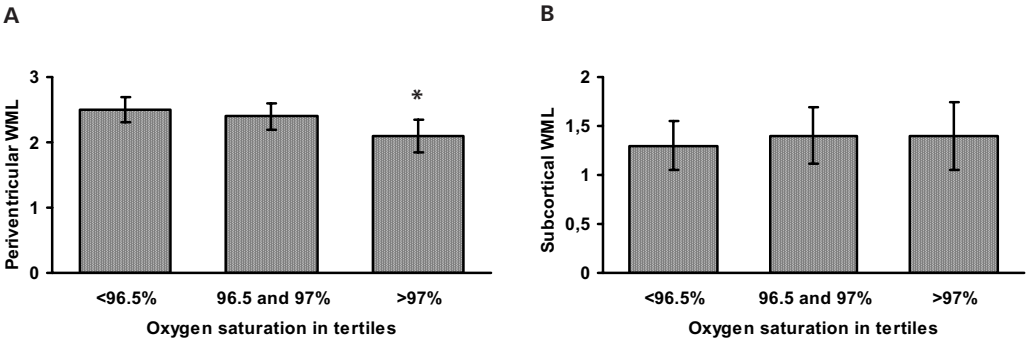

Figure 1. Association between tertiles of arterial oxygen saturation and severity of periventricular (A) and subcortical (B) white matter lesions (mean score or volume adjusted for age, sex, hypertension, diabetes mellitus, body mass index, pack years smoked, cholesterol level, haemoglobin concentration, prevalent myocardial infarction, and left ventricular hypertrophy with 95% confidence intervals). *p = 0.01 for difference between first and third tertile.

Table 2. Mean differences (95% confidence interval (95% CI)) of periventricular white matter lesion score and subcortical white matter lesion volume (ml) between participants with and without COPD

	Excess white matter lesions in participants with COPD			
	Periventricular score (95% CI)	p	Subcortical volume (95% CI)	p
Model 1*	0.70 (0.23 to 1.16)	<0.01	0.43 (-0.21 to 1.08)	0.19
Model 2†	0.69 (0.23 to 1.14)	<0.01	0.43 (-0.22 to 1.08)	0.20

*Adjusted for age and sex
†Adjusted for age, sex, hypertension, diabetes mellitus, body mass index, cholesterol level, pack years smoked

smoked pack years, cholesterol level, haemoglobin concentration, myocardial infarction, and left ventricular hypertrophy, this association remained unaltered (periventricular white matter lesions score increased by 0.12 per 1% decrease in SaO_2 (95% CI 0.01 to 0.23)). Arterial oxygen saturation was not associated with volume of subcortical white matter lesions (0.06 ml increase per 1% increase in SaO_2 (95% CI -0.07 to 0.20)) or the risk of lacunar infarcts (odds ratio 1.01 per 1% increase in SaO_2 (95% CI 0.89 to 1.15)), adjusted for age and sex. The association between SaO_2 and periventricular white matter lesion severity was not different for those who had ever and those who had never smoked (increment of 0.12 (95%CI -0.02 to 0.25) versus 0.11 (95%CI -0.08 to 0.30) per 1% decrease in SaO_2 in the fully adjusted model).

Participants with COPD had more severe periventricular white matter lesions than those without (table 2). This difference was independent of vascular risk factors, and observed in both ever and never cigarette smokers (age and sex adjusted difference for those who had ever (0.59 (95% CI 0.08 to 1.11)) and never (1.11 (95% CI 0.08 to 2.14)) smoked). Participants with and without COPD did not differ in subcortical white matter lesion volume (table 2) or the risk of lacunar infarcts (age and sex adjusted odds ratio 1.00 (95% CI 0.54 to 1.87)).

DISCUSSION

In this population based study, we found that lower SaO_2 was associated with more severe periventricular white matter lesions, but not with subcortical white matter lesions or lacunar infarcts. This association was independent of vascular risk factors, haemoglobin concentration, and measurements of cardiac function. Furthermore, we found that participants with COPD had more severe periventricular white matter lesions, but did not differ in subcortical white matter lesion volume or in prevalence of lacunar infarcts.

The strengths of this study are the population based design and the large number of elderly participants for whom MRI scans were performed. Before interpreting the results, we must address some methodological issues. The pulse oximeter accurately and precisely measures SaO_2 within the range of 70 to 100%.[21,22] All SaO_2 measurements presented in this paper were in this range. In 11 participants, the pulse oximeter failed to give a signal, probably caused by cold fingers or low peripheral perfusion. We do not think that the exclusion of this small group, which did not differ from the study population with respect to the measured covariates or outcome variables (data not shown), has markedly influenced our results.

SaO_2 is an indirect measure of arterial oxygen pressure in the cerebral arteries. The SaO_2 is linked to the arterial oxygen pressure by the oxygen-haemoglobin dissociation curve. Changes in blood CO_2 concentration, temperature and 2,3-diphosphoglycerate concentration shift the dissociation curve.[23] We did not measure these variables, but we performed the oximetry for each patient under comparable circumstances. Furthermore, pulse oximetry assesses SaO_2 in arterial blood, whereas these factors mainly affect the relation between oxygen saturation and oxygen pressure in capillary blood.[23] We therefore think that SaO_2 gives a good indication of the arterial oxygen pressure.

Defining COPD as the use of medication for this indication may have introduced misclassification. COPD cases that were not being treated will have been missed, and this may explain why the prevalence of COPD in our study is somewhat lower than in some other population based studies.[10,11,13] However, the age and sex specific prevalence of COPD in our study was comparable with a large Canadian study using health questionnaires.[12] Both SaO_2 and the presence of COPD were assessed without knowledge of other risk factors or presence of white matter lesions. The MRI scans were rated by researchers blinded to all other data. Therefore, any misclassification in the assessment of SaO_2 or COPD will be random and result in an underestimation of the strength of any association.

People with lower SaO_2 had significantly more severe periventricular white matter lesions. However, the differences were not very large and probably do not represent clinically significant disturbances on an individual level. It should be noted that we studied a large cohort of non-demented elderly people representative of the general population and not a selected group of patients. Consequently, the majority of the participants had SaO_2 values within the normal range. Within this population based range we found that a decrease in SaO_2 was associated with an increase in periventricular white matter lesion severity. This observation was supported by the consistent result with COPD.

In the Cardiovascular Health Study, an association between lower forced expiratory volume in 1 second (a measure of lung function) and white matter lesion severity was observed.[1] In that study, the association disappeared after adjusting for sex and history of smoking. Smoking is the most important determinant of COPD.[11,13] However, we also observed an association between COPD and white matter lesions in subjects who had

never smoked, indicating that this relation was not based on the potential confounding effect of smoking.

Chronic hypoperfusion is thought to play an important role in the development of white matter lesions. Experimental studies show that hypoxaemia in the absence of a reduced cerebral blood flow does not result in neuronal damage.[24-26] Hypoxaemia additional to hypoperfusion, however, strongly exacerbates ischaemic brain damage.[9] This difference in effect between pure hypoxaemia and the combination of hypoxaemia and ischaemia may be explained by the physiological increase in cerebral blood flow under conditions of reduced blood oxygen content.[27] In our study, we did not assess the cerebral blood flow of the cerebral white matter. Therefore we can only speculate on the interaction between ischaemia and hypoxaemia in the development of white matter lesions.

The periventricular white matter is an arterial border zone and therefore relatively hypoperfused, in particular when total cerebral blood flow decreases and autoregulation is impaired.[28] Areas with severe white matter lesions show hypoperfusion on perfusion weighted MRI and PET scan studies.[29-31] Furthermore vasomotor reactivity is diminished in people with severe white matter lesions.[32,33] As a consequence, the physiological compensation of hypoxaemia might be insufficient in people with cerebral small vessel disease, especially in border zone areas. Altered cerebral autoregulation may be the main cause of periventricular white matter lesions, whereas concentric narrowing of the arteriolar lumen may be the main cause of subcortical white matter lesions and lacunar infarcts. Lower SaO_2 is possibly more detrimental in combination with chronic or intermittent hypoperfusion, as in disturbed autoregulation, than in instances of acute hypoperfusion, as in arteriolar obstruction.[25]

In conclusion, our study shows that COPD and lower SaO_2 are associated with more severe periventricular white matter lesions. This finding suggests that not only cerebral hypoperfusion, but also hypoxaemia may contribute to the aetiology of periventricular white matter lesions.

References

1. Longstreth WT, Jr., Manolio TA, Arnold A, et al. Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people. The Cardiovascular Health Study. Stroke 1996;27: 1274-82.
2. Liao D, Cooper L, Cai J, et al. The prevalence and severity of white matter lesions, their relationship with age, ethnicity, gender, and cardiovascular disease risk factors: the ARIC Study. Neuroepidemiology 1997;16:149-62.
3. de Leeuw FE, de Groot JC, Achten E, et al. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. J Neurol Neurosurg Psychiatry 2001;70:9-14.
4. Vermeer SE, Koudstaal PJ, Oudkerk M, et al. Prevalence and risk factors of silent

brain infarcts in the population-based Rotterdam Scan Study. Stroke 2002;33:21-5.
5. de Groot JC, de Leeuw FE, Oudkerk M, et al. Cerebral white matter lesions and cognitive function: the Rotterdam Scan Study. Ann Neurol 2000;47:145-51.
6. Kuller LH, Shemanski L, Manolio T, et al. Relationship between ApoE, MRI findings, and cognitive function in the Cardiovascular Health Study. Stroke 1998;29:388-98.
7. van Gijn J. Leukoaraiosis and vascular dementia. Neurology 1998;51:S3-8.
8. Pantoni L, Garcia JH. Pathogenesis of leukoaraiosis: a review. Stroke 1997;28: 652-9.
9. Miyamoto O, Auer RN. Hypoxia, hyperoxia, ischemia, and brain necrosis. Neurology 2000;54:362-71.
10. Soriano JB, Maier WC, Egger P, et al. Recent trends in physician diagnosed COPD in women and men in the UK. Thorax 2000;55:789-94.
11. Lange P, Groth S, Nyboe J, et al. Chronic obstructive lung disease in Copenhagen: cross-sectional epidemiological aspects. J Intern Med 1989;226:25-32.
12. Lacasse Y, Brooks D, Goldstein RS. Trends in the epidemiology of COPD in Canada, 1980 to 1995. COPD and Rehabilitation Committee of the Canadian Thoracic Society. Chest 1999;116:306-13.
13. Pena VS, Miravitlles M, Gabriel R, et al. Geographic variations in prevalence and underdiagnosis of COPD: results of the IBERPOC multicentre epidemiological study. Chest 2000;118:981-9.
14. Chaouat A, Weitzenblum E, Kessler R, et al. Outcome of COPD patients with mild daytime hypoxaemia with or without sleep-related oxygen desaturation. Eur Respir J 2001;17:848-55.
15. Plywaczewski R, Sliwinski P, Nowinski A, et al. Incidence of nocturnal desaturation while breathing oxygen in COPD patients undergoing long-term oxygen therapy. Chest 2000;117:679-83.
16. Hofman A, van Laar A, Klein F, et al. Coffee and cholesterol (letter). N Engl J Med 1983;309:1248-1249.
17. Hofman A, Grobbee DE, de Jong PT, et al. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol 1991;7:403-22.
18. de Bruyne MC, Kors JA, Hoes AW, et al. Diagnostic interpretation of electrocardiograms in population-based research: computer program research physicians, or cardiologists? J Clin Epidemiol 1997;50: 947-52.
19. Brittin GM, Brecher G, Johnson CA. Evaluation of the Coulter Counter Model S. Am J Clin Pathol 1969;52:679-89.
20. de Leeuw FE, de Groot JC, Oudkerk M, et al. A follow-up study of blood pressure and cerebral white matter lesions. Ann Neurol 1999;46:827-33.
21. Yelderman M, New W, Jr. Evaluation of pulse oximetry. Anesthesiology 1983;59: 349-52.
22. Severinghaus JW, Kelleher JF. Recent developments in pulse oximetry. Anesthesiology 1992;76:1018-38.
23. Guyton AC, Hall JE. Transport of oxygen and carbon dioxide in the blood and body fluids. Textbook of medical physiology, 9th edition.;Philidelphia, USA, 1996.
24. Pearigen P, Gwinn R, Simon RP. The effects in vivo of hypoxia on brain injury. Brain Res 1996;725:184-91.
25. Simon RP. Hypoxia versus ischemia. Neurology 1999;52:7-8.
26. Rie M, Bernad P. Prolonged hypoxia in man without circulatory comprise fail to demonstrate cerebral pathology. Neurology 1980;30:443.
27. Cohen PJ, Alexander SC, Smith TC, et al. Effects of hypoxia and normocarbia on cerebral blood flow and metabolism in conscious man. J Appl Physiol 1967;23:183-9.
28. De Reuck J. The human periventricular arterial blood supply and the anatomy of cerebral infarctions. Eur Neurol 1971;5:321-34.

29. De Reuck J, Santens P, Strijckmans K, et al. Cobalt-55 positron emission tomography in vascular dementia: significance of white matter changes. J Neurol Sci 2001;193:1-6.
30. Meguro K, Hatazawa J, Yamaguchi T, et al. Cerebral circulation and oxygen metabolism associated with subclinical periventricular hyperintensity as shown by magnetic resonance imaging. Ann Neurol 1990;28:378-83.
31. Markus HS, Lythgoe DJ, Ostegaard L, et al. Reduced cerebral blood flow in white matter in ischaemic leukoaraiosis demonstrated using quantitative exogenous contrast based perfusion MRI. J Neurol Neurosurg Psychiatry 2000;69:48-53.
32. Bakker SL, de Leeuw FE, de Groot JC, et al. Cerebral vasomotor reactivity and cerebral white matter lesions in the elderly. Neurology 1999;52:578-83.
33. De Reuck J, Decoo D, Hasenbroekx MC, et al. Acetazolamide vasoreactivity in vascular dementia: a positron emission tomographic study. Eur Neurol 1999;41:31-6.

3.4
Amyloid ß, APOE and cerebral small vessel disease

3.4.1
Plasma amyloid ß, APOE, lacunar infarcts and white matter lesions

Abstract

Lacunar brain infarcts and cerebral white matter lesions are frequently observed on magnetic resonance imaging scans in elderly subjects. These lesions are also frequent in patient with cerebral amyloid angiopathy. We examined whether plasma amyloid ß peptide (Aß) levels are associated with lacunar infarcts and white matter lesions in the general population, and whether the apolipoprotein E (APOE) genotype modifies these associations.

We studied 1,077 participants within the population-based Rotterdam Scan Study, who were 60 to 90 years of age and free of dementia. Cross-sectional associations were analyzed by regression models with adjustments for age, sex, creatinine levels, and hypertension.

In APOE ε4 carriers, plasma Aß levels were positively associated with lacunar infarcts and white matter lesions, whereas in noncarriers no associations were observed. Per standard deviation increase in $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels the odds ratios for lacunar infarcts were 1.72 (95% confidence interval [CI] 1.22-2.43) and 1.93 (95% CI 1.31-2.85), the periventricular white matter lesion grade increased by 0.32 (95% CI 0.08-0.57) and 0.29 (95% CI 0.00-0.57), and the subcortical white matter lesion volume increased by 0.48ml (95% CI 0.04-0.91) and 0.24ml (95% CI -0.27-0.75).

Higher Aß levels are associated with more lacunar infarcts and white matter lesions in elderly subjects who carry an APOE ε4 allele.

INTRODUCTION

Lacunar brain infarcts and cerebral white matter lesions frequently are observed on magnetic resonance imaging (MRI) scans in elderly subjects.[1,2] These lesions are associated with an increased risk of stroke and dementia.[3-5] They are considered to be caused by ischemic small vessel disease, with hypertension and increased age as the most important risk factors. The exact pathogenesis is however not fully understood.[6-8]

Lacunar infarcts and white matter lesions also are observed frequently in patients with cerebral amyloid angiopathy (CAA).[9-12] CAA is characterized by deposits of amyloid ß peptide (Aß) in small and medium sized cerebral arteries.[13] This deposited Aß is a peptide consisting of either 42 ($Aß_{1-42}$) or 40 ($Aß_{1-40}$) amino acids, derived from the proteolytic processing of the amyloid precursor protein (APP).[14]

We investigated whether higher plasma Aß levels are associated with the presence of lacunar infarcts and severity of white matter lesions in nondemented elderly subjects. Because aggregation and clearance of Aß may be influenced by the apolipoprotein E (APOE) polymorphism, we also investigated whether any relationship was modified by the presence of an APOE ε4 allele.[15-17]

SUBJECTS AND METHODS

The Rotterdam Scan Study is a population-based study designed to study the etiology and natural history of age-related brain changes in the elderly. In 1995-1996, we randomly selected participants, aged 60 to 90 years, stratified by sex and 5-year age strata from the population-based Zoetermeer[18] and Rotterdam[19] studies. Subjects who were demented at baseline were excluded.[4] A total of 1,077 elderly participated (overall response 63%). Each person gave written informed consent to participate. The study was approved by the medical ethics committee of Erasmus Medical Center, Rotterdam.

Plasma amyloid ß levels

We collected nonfasting blood samples into vacutainers containing sodium citrate in 1995 to 1996. These samples were put on ice immediately and centrifuged within 60 minutes, and aliquots of plasma were stored at –80 ^{0}C. In 2001 to 2002, plasma levels of amyloid ß were determined by a double-antibody sandwich enzyme-linked immunosorbent assay method (Pfizer, Ann Arbor, MI).[20] The mean coefficients of within and between assays variation were 4.4% and 10.1% for $Aß_{1-40}$, and 4.9% and 14.8 % for $Aß_{1-42}$. The detection limits were 10 to 1,000 pg/ml for $Aß_{1-40}$ and 5 to 100 pg/ml for $Aß_{1-42}$. Seven participants were excluded, because their Aß levels fell outside these ranges. In 44 participants we failed to obtain blood samples.

Apolipoprotein E genotyping

Apolipoprotein E (APOE) genotyping was done on coded genomic DNA samples in 929 participants.[21] The distribution of APOE genotype and allele frequencies in this population were in Hardy-Weinberg equilibrium.

Other measurements

Hypertension was defined as a systolic blood pressure of 140 mm Hg or higher and/or a diastolic blood pressure of 90 mm Hg or over and/or the use of blood pressure-lowering medication. Diabetes mellitus was defined as the use of oral antidiabetics or insulin or a random or postload glucose level of 11.1 mmol/L or higher. Smoking habits were categorized as ever or never cigarette smoking. Plasma creatinine levels were assessed using an automated enzymatic procedure (Roche, Mannheim, Germany).

Lacunar brain infarcts and white matter lesions

We obtained axial T1-, T2-, and proton density-weighted scans on 1.5T MRI scanners (MR Gyroscan; Philips, Best, the Netherlands and MR VISION; Siemens, Erlangen, Germany). The slice thickness was 5 or 6 mm (scanner dependent) with a 20% interslice distance.

We defined infarcts as focal hyperintensities of at least 3 mm in size on T2-weighted images. Lesions in the white matter also had to have corresponding prominent hypointensities on T1-weighted images. We defined lacunar infarcts as focal hyperintensities 3 to 20 mm in size and located in the subcortical white matter or basal ganglia on T2-weighted images.

White matter lesions were considered present if visible as hyperintense on proton density and T2-weighted images, without prominent hypointensity on T1-weighted scans. We summed three region-specific semiquantitative grades (lesions adjacent to the frontal horns, the lateral walls, and the occipital horns of the lateral ventricle) to get a total periventricular white matter lesions grade (range 0-9). We counted subcortical white matter lesions in three size categories based on their maximal diameter (<3 mm, 3-10 mm, >10 mm). A total volume was approximated by assuming that these subcortical lesions were spherical with a fixed diameter (volume range 0-29.5 ml). Both intrarater and interrater studies (n=100) showed a good to excellent agreement (κ=0.79-0.90; r=0.88-0.95).[8]

Data analysis

Analyses were based on 1,026 participants with Aß levels within the limits of reliable assessment. We analyzed the association between possible confounders and Aß levels with linear regression analysis and the correlation between $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ with Pearson's correlation coefficient.

We quantified the associations between Aß levels, lacunar infarcts, and white mat-

ter lesions with multiple logistic and linear regression analyses. We analyzed Aß levels in quintiles of their distribution and as continuous variables to test for trend and to quantify the strength of the associations. The ranges of the quintiles of $Aß_{1-40}$ were 68-158, 159-186, 187-212, 213-245, and 246-537pg/ml and of $Aß_{1-42}$ were 5.6-14.1, 14.2-17.0, 17.1-20.0, 20.1-24.0, and 24.1-97.6pg/ml. All analyses were adjusted for age and sex and additionally for creatinine levels, hypertension, and APOE genotype. For missing data on APOE genotype, we used a missing indicator.

It has been suggested that the ratio of $Aß_{1-42}$ to $Aß_{1-40}$ may be more important than separate levels, at least for Alzheimer's disease.[22] Therefore we additionally analyzed the association between the ratio of plasma $Aß_{1-42}/Aß_{1-40}$ and lacunar infarcts and white matter lesions.

We evaluated the association of Aß levels with lacunar infarct visible on MRI by excluding all persons with nonlacunar infarcts. Given that 88% of the brain infarcts on MRI were lacunar infarcts, we focused on lacunar infarcts.

To assess a possible interaction between Aß levels and the APOE genotype, we did analyses stratified on the presence of an APOE ε4 allele. The significance of this interaction was tested through regression models with Aß levels, the presence of an APOE ε4 allele, and the interaction term as independent variables. We were not able to analyze the association in ε4 homozygotes separately because of low numbers (n=22).

RESULTS

Characteristics of the study participants are given in Table 1. Plasma $Aß_{1-40}$ and $Aß_{1-42}$ levels increased with age and creatinine levels. $Aß_{1-40}$ levels were higher in hypertensives and $Aß_{1-42}$ levels were lower in APOE ε4 carriers. Sex, smoking and diabetes were not associated with plasma Aß levels. $Aß_{1-40}$ and $Aß_{1-42}$ levels were positively correlated (Pearson's $r=0.59$, $p<0.001$).

Plasma Aß levels were positively associated with lacunar infarcts (Table 2). Stratified analysis on APOE genotype showed that this was entirely caused by an association in ε4 carriers. The interaction between Aß levels and the APOE ε4 genotype was statistically significant (p-values interaction terms $Aß_{1-40}$ × APOE ε4 and $Aß_{1-42}$ × APOE ε4 0.03 and 0.01). Additional adjustments for creatinine and hypertension hardly changed these associations. The associations for any brain infarcts were almost identical (data not shown).

A similar pattern was seen for white matter lesions. Plasma Aß levels were positively associated with both periventricular and subcortical white matter lesions in APOE ε4 carriers, but not in noncarriers (Table 3). The interaction between Aß levels and the APOE ε4 genotype was statistically significant (p-values interaction terms $Aß_{1-40}$ × APOE ε4 and $Aß_{1-42}$ × APOE ε4 in relation to periventricular white matter lesions <0.001

Table 1. Characteristics of study population

Characteristic	All participants (n = 1026)
Age, yr (SD)	72.2 (7.4)
Women, %	52
Hypertension, %	72
Ever smoked cigarettes, %	66
Diabetes, %	7
Creatinine mmol/L, mean (SD)	88.9 (18.6)
$Aß_{1-40}$ pg/ml, mean (SD)	204.8 (57.8)
$Aß_{1-42}$ pg/ml, mean (SD)	19.8 (8.1)
$Aß_{1-42}$ / $Aß_{1-40}$, mean (SD)	0.10 (0.03)
APOE ε4 carrier, %[a]	29
White matter lesions	
Periventricular, grade 0-9, mean (SD)	2.4 (2.2)
Subcortical, (ml), mean (SD)	1.4 (2.9)
Brain infarcts, %	24
Lacunar brain infarcts, %[b]	22

[a] Data available for 929 participants
[b] Percentage within people without other infarcts (n = 995)
SD = standard deviation

Table 2. Odds ratio of lacunar infarcts per standard deviation increase in amyloid ß levels with 95% confidence intervals in all participants and stratified on APOE ε4 genotype.

Amyloid ß	Odds ratio for lacunar infarcts (95%CI)[a]		
	All	APOE ε4 non-carriers	APOE ε4 carriers
1-40 (per SD)			
Model 1	1.22 (1.04-1.42)	1.14 (0.93-1.40)	1.75 (1.27-2.41)
Model 2	1.14 (0.96-1.35)	1.01 (0.81-1.16)	1.72 (1.22-2.43)
1-42 (per SD)			
Model 1	1.17 (1.02-1.36)	1.09 (0.91-1.30)	1.93 (1.31-2.85)
Model 2	1.15 (0.98-1.35)	1.02 (0.83-1.25)	1.93 (1.28-2.92)

Model 1: adjusted for age and sex
Model 2: adjusted for age, sex, creatinine, hypertension and APOE genotype (for the analyses in all participants).
[a] Subjects with nonlacunar infarcts were excluded from the analysis.
APOE = apolipoprotein E; CI = confidence interval; SD = standard deviation.

and 0.03; in relation to subcortical white matter lesions <0.001 and 0.05). Additional adjustments hardly changed these associations. The ratio of $Aß_{1-42}$ to $Aß_{1-40}$ was not associated with either lacunar infarcts or white matter lesions (data not shown).

Table 3. Increase in severity of white matter lesions per standard deviation increase in amyloid ß levels with 95% confidence intervals in all participants and stratified on APOE ε4 genotype

	Increase in white matter lesion severity (95% CI)					
	Periventricular (grade)			Subcortical (ml)		
Amyloid ß	All	APOE ε4 non-carriers	APOE ε4 carriers	All	APOE ε4 non-carriers	APOE ε4 carriers
1-40 (per SD)						
Model 1	0.10 (-0.03-0.22)	0.00 (-0.15-0.16)	0.41 (0.17-0.64)	0.15 (-0.03-0.32)	0.07 (-0.12-0.25)	0.54 (0.13-0.95)
Model 2	0.06 (-0.08-0.19)	-0.04 (-0.20-0.12)	0.32 (0.08-0.57)	0.15 (-0.04-0.33)	0.06 (-0.13-0.25)	0.48 (0.04-0.91)
1-42 (per SD)						
Model 1	0.14 (0.02-0.26)	0.09 (-0.05-0.23)	0.38 (0.11-0.66)	0.06 (-0.10-0.23)	0.03 (-0.13-0.19)	0.33 (-0.16-0.82)
Model 2	0.13 (0.00-0.25)	0.07 (-0.07-0.22)	0.29 (0.00-0.57)	0.09 (-0.08-0.27)	0.04 (-0.12-0.20)	0.24 (-0.27-0.75)

Model 1: adjusted for age and sex
Model 2: adjusted for age, sex, creatinine, hypertension and APOE genotype (for the analyses in all participants)
APOE = apolipoprotein E; CI = confidence interval; SD = standard deviation.

Figures 1 and 2 illustrate the linear increase in prevalence of lacunar infarcts and severity of white matter lesions with increasing plasma Aß levels in APOE ε4 carriers, but not in noncarriers.

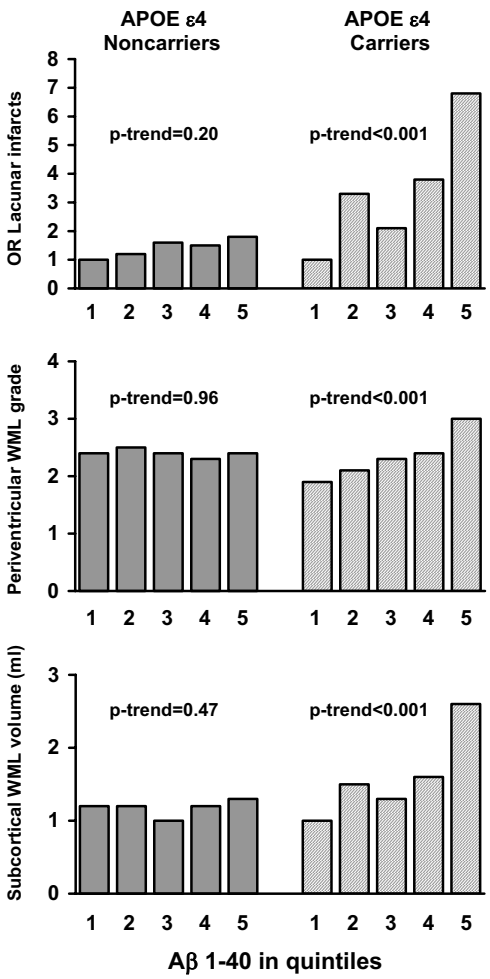

Figure 1. Association between quintiles of plasma amyloid ß 1-40 levels and lacunar infarcts (odds ratios (OR)), periventricular white matter lesions (WMLs) (grade) and subcortical white matter lesions volume (ml) stratified on the presence of an apolipoprotein E (APOE) ε4 allele, adjusted for age and sex (p-trend for continuous linear trend per stratum).

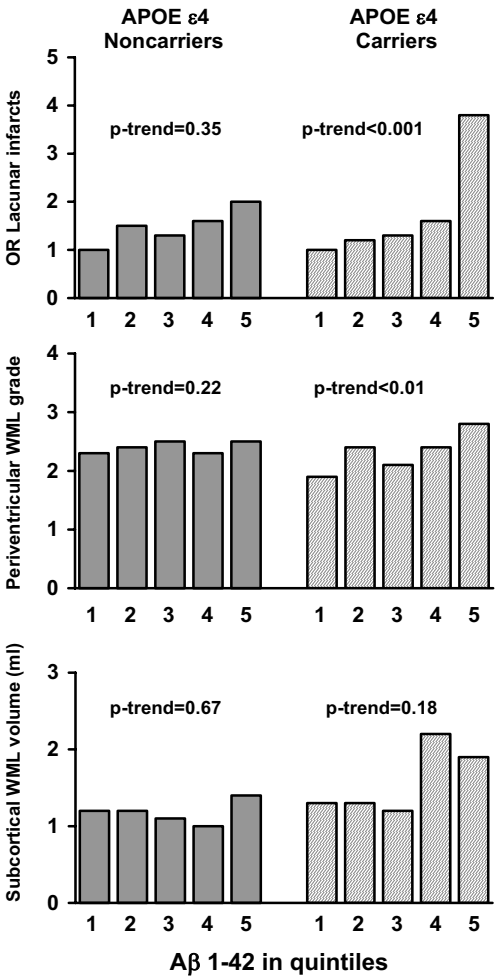

Figure 2. Association between quintiles of plasma amyloid ß 1-42 levels and lacunar infarcts (odds ratios (OR)), periventricular white matter lesions (WMLs) (grade) and subcortical white matter lesions volume (ml) stratified on the presence of an apolipoprotein E (APOE) ε4 allele, adjusted for age and sex (p-trend for continuous linear trend per stratum).

DISCUSSION

This study demonstrates that plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ levels are strongly associated with the presence of lacunar infarcts and severity of white matter lesions in nondemented elderly subjects who carry an APOE ε4 allele.

The strengths of this study are the population-based design and the large number of elderly nondemented participants in whom both brain imaging and plasma Aß assessments were performed. The plasma Aß levels were measured without knowledge of other risk factors or presence of brain lesions. MRI rating was also performed blinded to all other data. Therefore, misclassification, if any, will have been random and will have resulted in an underestimation of the strength of any risk associations. We adjusted in the analyses for all known confounders. However, because we do not exactly know what determines plasma amyloid ß levels, lacunar infarcts, and white matter lesions, we cannot exclude the possibility of confounding by yet unknown factors.

Several mechanisms may lead to increased plasma Aß levels. Plasma Aß levels are elevated in all genetic forms of early-onset Alzheimer's disease due to increased production.[23] In nondemented subjects, cerebrospinal fluid and plasma Aß levels are in a dynamic equilibrium.[24] An increased efflux from the cerebrospinal fluid to plasma may result in higher plasma levels.[25] Furthermore, chronic hypoperfusion may upregulate APP expression and thereby increase Aß production.[26] Finally, altered peripheral clearance may increase plasma Aß levels. Aß levels are positively correlated with plasma creatinine levels, indicating that with altered renal function peripheral Aß levels increase.[27]

It is not clear to what extent the above mechanisms contribute to increased plasma Aß levels in the general population. Our study consisted of only nondemented elderly subjects, and we adjusted in our analyses for creatinine levels as a measure of renal function. We consider that overproduction is probably the most important source of higher plasma Aß levels in our study.

Effects of Aß on the vessel wall are particularly described for $A\beta_{1-40}$, whereas $A\beta_{1-42}$ is mainly involved in senile plaque formation.[14,28] Because of the strong correlation between $A\beta_{1-40}$ and $A\beta_{1-42}$ plasma levels, we could not assess the individual effects of $A\beta_{1-40}$ conditional on $A\beta_{1-42}$ and vice versa.

We found an association between higher plasma Aß levels and lacunar infarcts and white matter lesions in APOE ε4 carriers, but not in noncarriers. This may be explained by different mechanisms. Firstly, fibrillary deposits of Aß in the vessel wall lead to obliteration of vessel luminae and loss of vascular smooth muscle cells necessary to cerebral autoregulation. This subsequently may result in lacunar infarcts and white matter lesions.[9,13,29,30] Overproduction of Aß may lead to deposition in the media and adventitia of meningiocortical arteries and arterioles.[31] The apolipoprotein ε4 isoform supports ß-sheet transformation of Aß, which has a stronger tendency to aggregate and deposit.[14-17] Furthermore, APOE ε4 may facilitate the transport of Aß from the cerebrospinal fluid

into smooth muscle cells of the vessel wall.[32]

Secondly, soluble Aß may directly effect cerebral vasoreactivity by enhancing endothelium dependent vasoconstriction.[29,30] This effect might be stronger in APOE ε4 carriers.[33] Altered autoregulation will result in hypoperfusion of the brain, specifically in regions with limited collateral blood flow, and consequently may lead to white matter lesions and lacunar infarcts.[9] APOE ε4 carriers may have a more generally increased susceptibility to ischemic cerebral damage.[34]

A third possible explanation is that higher Aß levels and lacunar infarcts and white matter lesions both result from cerebral hypoperfusion.[26] Hypoperfusion might induce higher Aß levels which, in turn, via vascular depositions could reinforce hypoperfusion. Because adjusting for vascular risk factors did not effect the association, we consider hypoperfusion due to vascular risk factors a less likely explanation.

Our observations in nondemented elderly subjects are in line with findings from pathological and imaging studies in patients with Alzheimer's disease and CAA-related intracerebral hemorrhage and otherwise selected patient groups that found an association between CAA and brain infarcts and white matter lesions.[9-12,35] The observation that plasma Aß level in combination with the APOE ε4 allele is strongly associated with cerebral small vessel disease-related lesions in asymptomatic elderly is novel. Therefore, confirmation of our results in other data sets and prospectively is needed.

References

1. Liao D, Cooper L, Cai J et al. The prevalence and severity of white matter lesions, their relationship with age, ethnicity, gender, and cardiovascular disease risk factors: the ARIC Study. Neuroepidemiology. 1997;16:149-162
2. Longstreth WT, Jr., Dulberg C, Manolio TA et al. Incidence, manifestations, and predictors of brain infarcts defined by serial cranial magnetic resonance imaging in the elderly: the Cardiovascular Health Study. Stroke. 2002;33:2376-2382
3. Vermeer SE, Hollander M, Van Dijk EJ et al. Silent brain infarcts and white matter lesions increase stroke risk in the general population: the rotterdam scan study. Stroke. 2003;34:1126-1129
4. Vermeer SE, Prins ND, den Heijer T et al. Silent brain infarcts and the risk of dementia and cognitive decline. N Engl J Med. 2003;348:1215-1222
5. Brun A, Englund E. A white matter disorder in dementia of the Alzheimer type: a pathoanatomical study. Ann Neurol. 1986;19:253-262
6. Pantoni L, Garcia JH. Pathogenesis of leukoaraiosis: a review. Stroke. 1997;28: 652-659
7. Fisher CM. Lacunes: small, deep cerebral infarcts. Neurology. 1965;15:774-784
8. de Leeuw FE, de Groot JC, Oudkerk M et al. A follow-up study of blood pressure and cerebral white matter lesions. Ann Neurol. 1999;46:827-833
9. Gray F, Dubas F, Roullet E, Escourolle R. Leukoencephalopathy in diffuse hemorrhagic cerebral amyloid angiopathy. Ann Neurol. 1985;18:54-59

10. Cadavid D, Mena H, Koeller K, Frommelt RA. Cerebral beta amyloid angiopathy is a risk factor for cerebral ischemic infarction. A case control study in human brain biopsies. J Neuropathol Exp Neurol. 2000;59: 768-773
11. Olichney JM, Hansen LA, Hofstetter CR et al. Cerebral infarction in Alzheimer's disease is associated with severe amyloid angiopathy and hypertension. Arch Neurol. 1995;52:702-708
12. Bornebroek M, Haan J, Maat-Schieman ML et al. Hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D): I--A review of clinical, radiologic and genetic aspects. Brain Pathol. 1996;6:111-114
13. Vinters HV. Cerebral amyloid angiopathy. A critical review. Stroke. 1987;18:311-324
14. Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. Physiol Rev. 2001;81: 741-766
15. Holtzman DM, Fagan AM, Mackey B et al. Apolipoprotein E facilitates neuritic and cerebrovascular plaque formation in an Alzheimer's disease model. Ann Neurol. 2000;47:739-747
16. Bales KR, Verina T, Dodel RC et al. Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. Nat Genet. 1997;17:263-264
17. Greenberg SM, Rebeck GW, Vonsattel JP et al. Apolipoprotein E epsilon 4 and cerebral hemorrhage associated with amyloid angiopathy. Ann Neurol. 1995;38:254-259
18. Hofman A, van Laar A, Klein F, Valkenburg H. Coffee and cholesterol (letter). N Engl J Med. 1983;309:1248-1249
19. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-422
20. Mehta PD, Pirttila T, Patrick BA et al. Amyloid beta protein 1-40 and 1-42 levels in matched cerebrospinal fluid and plasma from patients with Alzheimer disease. Neurosci Lett. 2001;304:102-106
21. Wenham PR, Price WH, Blandell G. Apolipoprotein E genotyping by one-stage PCR. Lancet. 1991;337:1158-1159
22. Kanai M, Matsubara E, Isoe K et al. Longitudinal study of cerebrospinal fluid levels of tau, A beta1-40, and A beta1-42(43) in Alzheimer's disease: a study in Japan. Ann Neurol. 1998;44:17-26
23. Scheuner D, Eckman C, Jensen M et al. Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nat Med. 1996;2:864-870
24. DeMattos RB, Bales KR, Parsadanian M et al. Plaque-associated disruption of CSF and plasma amyloid-beta (Abeta) equilibrium in a mouse model of Alzheimer's disease. J Neurochem. 2002;81:229-236
25. DeMattos RB, Bales KR, Cummins DJ et al. Brain to plasma amyloid-beta efflux: a measure of brain amyloid burden in a mouse model of Alzheimer's disease. Science. 2002;295:2264-2267
26. Bennett SA, Pappas BA, Stevens WD et al. Cleavage of amyloid precursor protein elicited by chronic cerebral hypoperfusion. Neurobiol Aging. 2000;21:207-214
27. Arvanitakis Z, Lucas JA, Younkin LH et al. Serum creatinine levels correlate with plasma amyloid Beta protein. Alzheimer Dis Assoc Disord. 2002;16:187-190
28. Verbeek MM, Eikelenboom P, de Waal RM. Differences between the pathogenesis of senile plaques and congophilic angiopathy in Alzheimer disease. J Neuropathol Exp Neurol. 1997;56:751-761
29. Thomas T, Thomas G, McLendon C et al. beta-Amyloid-mediated vasoactivity and vascular endothelial damage. Nature. 1996;380:168-171
30. Niwa K, Kazama K, Younkin L et al. Cerebrovascular autoregulation is profoundly impaired in mice overexpressing amyloid precursor protein. Am J Physiol Heart Circ Physiol. 2002;283:H315-323

31. Calhoun ME, Burgermeister P, Phinney AL et al. Neuronal overexpression of mutant amyloid precursor protein results in prominent deposition of cerebrovascular amyloid. Proc Natl Acad Sci U S A. 1999;96:14088-14093
32. Urmoneit B, Prikulis I, Wihl G et al. Cerebrovascular smooth muscle cells internalize Alzheimer amyloid beta protein via a lipoprotein pathway: implications for cerebral amyloid angiopathy. Lab Invest. 1997;77:157-166
33. Paris D, Town T, Parker TA et al. Isoform-specific vasoconstriction induced by apolipoprotein E and modulation of this effect by Alzheimer's beta-amyloid peptide. Neurosci Lett. 1998;256:73-76
34. Horsburgh K, McCarron MO, White F, Nicoll JA. The role of apolipoprotein E in Alzheimer's disease, acute brain injury and cerebrovascular disease: evidence of common mechanisms and utility of animal models. Neurobiol Aging. 2000;21:245-255
35. Greenberg SM, Vonsattel JP, Stakes JW et al. The clinical spectrum of cerebral amyloid angiopathy: presentations without lobar hemorrhage. Neurology. 1993;43: 2073-2079

3.4.2
Plasma amyloid ß, APOE and impaired cerebral vasomotor reactivity

Abstract

Background and purpose *— Amyloid ß (Aβ) may disturb cerebral autoregulation in elderly people through deposition in the walls of small cerebral blood vessels, or through its vasoactive properties. We assessed whether previous or concurrent plasma $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels were associated with impaired cerebral vasomotor reactivity. Furthermore, we assessed whether the APOE gene modified this association.*

Methods *— Plasma $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels were assessed in a random sample of the longitudinal population based Rotterdam Study at time of its baseline and second follow-up survey. At the second follow-up survey, cerebral vasomotor reactivity to hypercapnia was measured with transcranial Doppler ultrasonography (TCD) in 442 people, aged 60 to 90 years. The association between Aβ levels and impaired vasomotor reactivity was analyzed by age and sex adjusted logistic regression analysis.*

Results *— Plasma $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels assessed on average 6.5-year before TCD were linearly associated with impaired vasomotor reactivity (odds ratio 1.48 (95%CI 1.19; 1.84) per standard deviation increase in $A\beta_{1\text{-}40}$, and 1.36 (95%CI 1.09; 1.70) per standard deviation increase in $A\beta_{1\text{-}42}$). Such an association was not present for Aβ assessed concurrently with the TCD measurement. Decrease in plasma $A\beta_{1\text{-}40}$ in the 6.5-year period preceding TCD measurements was associated with a higher prevalence of impaired vasomotor reactivity. These associations were not significantly different for APOE ε4 carriers and non-carriers.*

Conclusions *— High plasma $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels measured on average 6.5 years earlier, and decline in $A\beta_{1\text{-}40}$ levels over time, are associated with impaired vasomotor reactivity.*

INTRODUCTION

Small cerebral blood vessels have the intrinsic property to constantly adjust the cerebral blood flow to changes in systemic blood pressure.[1] Impairment in this cerebral autoregulation can result in cerebral hypoperfusion and may consequently lead to ischemic white matter lesions and lacunar brain infarcts.[2-5] Insufficient vasomotor response to vasodilatory stimuli is observed in major carotid artery stenosis and in cerebral small vessel disease.[4,6] Hypertension and increased age are associated with cerebral autoregulation disturbances. The exact mechanism by which cerebral autoregulation is impaired is however unclear.

Animal studies have shown that amyloid beta peptide (Aß) may play a role in the disturbance of the cerebral autoregulation.[7] Aß is a peptide consisting of 40 ($Aß_{1-40}$) or 42 ($Aß_{1-42}$) amino acids.[8] Aggregates of Aß in medium and small sized cerebral arteries, as seen in cerebral amyloid angiopathy (CAA), are cytotoxic to the smooth muscle cells of the vessel walls, which play an important role in the cerebral autoregulation.[9] Next to this cytotoxic effect, Aß also has endothelium dependent vasoactive properties, enhancing endothelin-1 induced vasoconstriction.[10,11]

Until now no study has assessed the relationship between plasma Aß levels and cerebral vasomotor reactivity in humans. The autoregulatory capacity of arterioles can be assessed by measurement of the vasomotor response to a dilatory stimulus, such as carbon dioxide, with transcranial Doppler ultrasonography (TCD).[6]

The aim of our study was to investigate the relationship between plasma Aß levels and impaired vasomotor reactivity assessed by TCD in a general elderly population. Since aggregation and clearance of Aß could be influenced by the apolipoprotein E (APOE) polymorphism, we also investigated whether this relationship depended on the presence of an APOE ε4 allele.[12-14]

PARTICIPANTS AND METHODS

Participants

The study was conducted as part of the Rotterdam Study, a prospective population-based cohort study aimed at investigating determinants of chronic and disabling diseases in the elderly.[15] The study started in 1990 when all inhabitants aged 55 years or over of Ommoord, a district of Rotterdam, were invited to participate. Of 10,275 eligible subjects 7,983 (78%) agreed to participate. A first follow-up survey was done in 1993-1994, and a second follow-up survey in 1997-1999.

From the baseline cohort, we drew a random sample of 1,756 people in which we assessed plasma Aß levels in baseline blood as well as in blood of the second follow up survey if available. Of the 1,756 people, 293 had died and 374 refused the second follow-

up examination, which resulted in 1,089 people for whom we had repeated plasma Aß assessment available.

As part of the second follow-up survey, we performed transcranial Doppler examinations in a random set of 2,735 participants. In 1,755 participants we succeeded to measure the cerebral vasomotor reactivity. Failure to complete the measurement was mainly due to bilateral window failure (n=687). The frequency of bilateral window failure is higher in women and increases with age.[3]

A total of 466 people were part of both the random sample in which plasma Aß levels were assessed and the random sample in which cerebral vasomotor reactivity measurements were obtained. In 23 participants an $Aß_{1-40}$ or $Aß_{1-42}$ assessment was missing at the first or third survey, and in 2 participants the levels were outside the reliable measurement range, leaving 441 participants available for the present analyses.

Each person gave written informed consent to participate in our study, which had been approved by the medical ethics committee of the Erasmus Medical Center Rotterdam.

Plasma amyloid ß levels

We collected non-fasting blood samples from participants into vacutainers containing sodium citrate in 1990-1993 and in 1997-1999. These whole blood samples were put on ice immediately, and centrifuged within 60 minutes, and aliquots of plasma were stored at –80 ^{0}C. In 2001-2002, plasma levels of amyloid ß were determined by a double-antibody sandwich ELISA method (Pfizer, USA).[16] The mean coefficients of within and between assays variation were 4.4% and 4.9 % for $Aß_{1-40}$, and 10.1% and 14.8 % for $Aß_{1-42}$. The detection limits were 10-1,000 pg/ml for $Aß_{1-40}$ and 5-100 pg/ml for $Aß_{1-42}$. We calculated the change in Aß level per year, by dividing the difference in Aß levels between 1990-1993 and 1997-1999 by the time between both assessments.

APOE genotyping

Apolipoprotein E (APOE) genotyping was done on coded genomic DNA samples. The APOE gene was amplified by means of the primers and amplification conditions described by Wenham et al.[17] The distribution of the APOE genotype and the allele frequencies in this population were in Hardy-Weinberg equilibrium.

Measurements of other covariates

Hypertension was defined as a systolic blood pressure of 140 mm Hg or over, a diastolic blood pressure of 90 mm Hg or over, and/or the use of blood pressure lowering medication. We considered diabetes mellitus to be present if a person used oral anti-diabetics or insulin and/or had a non-fasting glucose level over 11.1 mmol/l. Smoking habits were assessed by a structured questionnaire and categorized as ever or never cigarette smoking.

Participants underwent ultrasonography of both carotid arteries with a 7.5 MHz linear array transducer and a Duplex scanner (ATL Ultra-Mark IV, Advanced Technology Laboratories, USA). The intima-media thickness was calculated as the mean thickness of four locations: the near and far wall of both right and left common carotid artery.[18]

Vasomotor reactivity

Transcranial Doppler ultrasonography monitoring was performed (Multi-Dop X-4, DWL, Germany) as earlier described.[3] Cerebrovascular CO_2 reactivity measurements were performed as follows: the cerebral blood flow velocity (cm/s) was continuously measured in the middle cerebral artery on both sides, if possible. First, participants breathed room air through an anesthetic mask until a steady expiratory end-tidal CO_2 was obtained. Subsequently, participants inhaled a mixture of 5% carbon dioxide and 95% oxygen for 2 minutes. Cerebrovascular 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 period (%/kPa). End-tidal CO_2 pressure (kPa) was recorded continuously with a CO_2 analyser (Multines, Datascope, The Netherlands). End-expiratory CO_2 was assumed to reflect arterial CO_2. TCD-8 DWL special software VMR-CO_2 was used. Changes in blood pressure due to hypercapnia may introduce misclassification in the vasomotor reactivity assessment.[19,20] We monitored the blood pressure dynamically during the vasomotor reactivity assessment in a random sub-sample of 134 participants, to assess whether blood pressure changes during hypercapnia influenced the associations under study. Right and left hemodynamic indices were highly correlated. We used their mean for all the analyses. In case of one-sided window-failure, the contralateral cerebral hemodynamic parameter was used in the analysis. We defined impaired vasomotor reactivity as the lowest tertile of its distribution, which corresponded with measures lower than 30%/kPa. The upper two tertiles were considered normal. This cut-off is consistent with that used by others.[6]

Data analysis

We assessed the association between age, sex, APOE genotype and Aß levels with linear regression analysis. We calculated Pearson's correlation coefficient to quantify the correlation between $Aß_{1\text{-}40}$ and $Aß_{1\text{-}42}$. We performed multiple logistic regression analysis to assess the relation between Aß levels and impaired vasomotor reactivity. We analyzed Aß levels in quintiles of its distribution and as a continuous variable (per standard deviation). The cut-off values for the quintiles of $Aß_{1\text{-}40}$ at time of TCD were 155, 185, 207, and 243 pg/ml and for the measurements on average 6.5-years earlier 152, 173, 191, and 217 pg/ml. For $Aß_{1\text{-}42}$ the cut-off values at time of TCD were 12.0, 15.0, 18.4, and 23.2 pg/ml and for the measurement on average 6.5-year earlier 13.7, 16.0, 18.2, and 21.0 pg/ml.

We assessed the relation between change in Aß levels per year (in quintiles) and im-

paired vasomotor reactivity with multiple logistic regression analysis. The cut-off values of the quintiles of change in $Aß_{1-40}$ were –4.3, 0.0, 3.7, and 8.7 pg/ml/year and for the change in $Aß_{1-42}$ –1.1, –0.4, 0.2, and 0.9 pg/ml/year. We used the middle quintile, which contained the people who did not change over time, as the reference group.

It has been suggested that the ratio of $Aß_{1-42}$ to $Aß_{1-40}$ may be more important than separate levels, at least for AD.[22] Therefore we additionally analyzed the association between the ratio of plasma $Aß_{1-42}/Aß_{1-40}$ and lacunar infarcts and white matter lesions.

To assess a possible interaction between Aß levels and the APOE genotype, we analyzed the association between Aß levels and impaired vasomotor reactivity stratified on the presence of an APOE ε4 allele. To test whether this interaction was statistically significant, we did regression analysis with Aß levels, the presence of an APOE ε4 allele and the product of both variables (interaction term) as independent variables in one model.

All analyses were adjusted for age and sex. In additional analyses we adjusted for hypertension, diabetes mellitus, smoking, and carotid intima-media thickness. We subsequently excluded people with prevalent stroke (n=11) or dementia (n=4) at time of TCD. In the group of 134 people with complete monitoring of blood pressure dynamics during the vasomotor reactivity assessment, we made additional adjustment for systolic and diastolic blood pressure changes during the assessment.

Table 1. Characteristics of participants on average 6.5-years before and at time of TCD

	All participants (n = 441)	
	6.5-years before TCD	At time of TCD
Age, y	63.7 (5.8)	70.2 (5.8)
Women, %	48	48
Hypertension, %	46	65
Diabetes, %	3	4
Ever Smoked, %	71	72
Dementia, %	0	1
Stroke, %	1	3
$Aß_{1-40}$, pg/ml	188.4 (51.6)	207.9 (70.1)
$Aß_{1-42}$, pg/ml	18.1 (6.6)	18.3 (8.3)
APOE ε4 carrier, %	30	30
IMT CCA*, mm	-	0.87 (0.15)

Values are percentages or unadjusted means (standard deviation)
* Mean intima media thickness of the common carotid arteries

RESULTS

Selected characteristics of the 441 study participants at time of TCD, and on average 6.5-years (SD 0.4) earlier are given in table 1. In the 6.5-years between the two assessments, plasma Aß levels on average increased ($Aß_{1-40}$ 3.0 pg/ml/year (SD 11.4) and $Aß_{1-42}$ 0.04 pg/ml/year (SD 1.39)). No differences were observed in mean plasma Aß levels between men and women and between APOE ε4 carriers and non-carriers. Plasma $Aß_{1-40}$ and

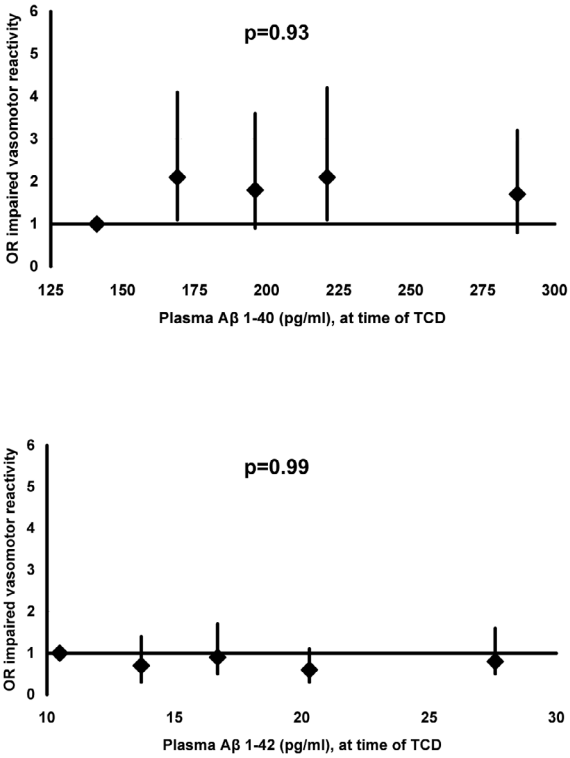

Figure 1. The relation between plasma $Aß_{1-40}$ and $Aß_{1-42}$ levels in quintiles, assessed at time of TCD, and impaired vasomotor reactivity (expressed as odds ratios with 95% confidence intervals, adjusted for age and sex). Odds ratios are plotted at the median $Aß_{1-40}$ and $Aß_{1-42}$ levels of the different quintiles (p-value for continuous linear trend).

Table 2. Relation between plasma Aß levels (per standard deviation) assessed 6.5-years before and at time of TCD and impaired vasomotor reactivity (odds ratio's with 95% confidence intervals, adjusted for age and sex)

	Odds ratios of impaired vasomotor reactivity (95% CI)	
	Aß 6.5-years before TCD	Aß at time of TCD
$Aß_{1-40}$ (per standard deviation)	1.48 (1.19; 1.84)	1.01 (0.87; 1.17)
$Aß_{1-42}$ (per standard deviation)	1.36 (1.09; 1.70)	1.00 (0.85; 1.18)

$Aß_{1-42}$ levels were positively correlated (at time of TCD Pearson's r=0.61 p<0.001, and 6.5-years earlier r=0.68, p<0.001).

Plasma $Aß_{1-40}$ and $Aß_{1-42}$ levels at time of TCD were not related with impaired vasomotor reactivity (figure 1, table 2). Higher $Aß_{1-40}$ and $Aß_{1-42}$ levels 6.5-years before TCD were linearly associated with a higher prevalence of impaired vasomotor reactivity (figure 2, table 2). Figure 3 shows the association between categories of change in Aß levels and impaired vasomotor reactivity. People who had clearly dropped in their $Aß_{1-40}$ levels (first quintile, range -45.1; -4.3 pg/ml/year) had twice as frequently impaired vasomotor reactivity (odds ratio 1.9 (95% CI 1.0; 3.6)) compared to people with an average change in $Aß_{1-40}$ levels (middle quintile, range 0 - 3.7 pg/ml/year). People who increased in $Aß_{1-40}$ levels (upper two quintiles) did not differ in impaired vasomotor reactivity prevalence from people with an average change (middle quintile). Changes in $Aß_{1-42}$ levels were not associated with impaired vasomotor reactivity.

The associations between Aß levels assessed 6.5-years before TCD and impaired vasomotor reactivity were similar for APOE ε4 carriers and non-carriers: odds ratios for $Aß_{1-40}$ and $Aß_{1-42}$ (per SD increase) were 1.49 (95% CI 0.99; 12.25) and 1.40 (95% CI 0.90; 2.18) in ε4 carriers and 1.47 (95% CI 1.13; 1.91) and 1.34 (95% CI 1.04;1.73) in non-carriers. In APOE ε4 carriers, people who dropped in their Aß levels over time tended to

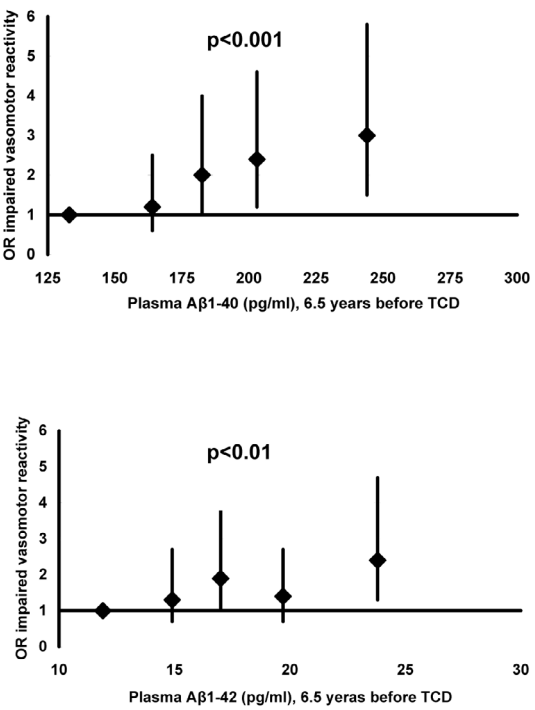

Figure 2. The relation between plasma $Aß_{1-40}$ and $Aß_{1-42}$ levels in quintiles, assessed 6.5-years before, and impaired vasomotor reactivity (expressed as odds ratios with 95% confidence intervals, adjusted for age and sex). Odds ratios are plotted at the median $Aß_{1-40}$ and $Aß_{1-42}$ levels of the different quintiles(p-value for continuous linear trend).

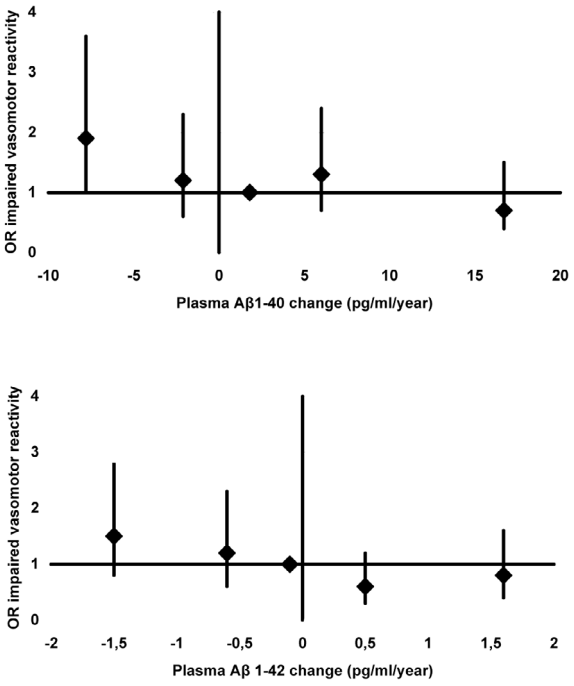

Figure 3. The relation between quintiles of change in plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ per year and impaired vasomotor reactivity (expressed as odds ratios with 95% confidence intervals, adjusted for age and sex). Odds ratios are plotted at the median of $A\beta_{1-40}$ and $A\beta_{1-42}$ change in level per year of the different quintiles, with the third quintile as the reference category.

have a higher prevalence of impaired vasomotor reactivity than non-carriers (odds ratio third versus first quintile of $A\beta_{1-40}$ in carriers 3.16 (95% CI 0.89; 11.19) and in non-carriers 1.46 (95% CI 0.68; 3.13), and odds ratio third versus first quintile of $A\beta_{1-42}$ in carriers 1.82 (95% CI 0.63; 2.78), and in non-carriers 1.32 (95% CI 0.63; 2.78)). These interactions were however not statistically significant (p-values 0.16 and 0.87).

Neither additional adjustment for hypertension, smoking, diabetes, and carotid intima-media thickness nor exclusion of people with prevalent stroke or dementia at time of TCD changed the associations (data not shown). The ratio of $A\beta_{1-42}/A\beta_{1-40}$ was not associated with either lacunar infarcts or white matter lesions (data not shown).

The average increases in systolic and diastolic blood pressure during the vasomotor reactivity assessment were 10.6 mmHg (SD 11.5) and 5.3 mmHg (SD 6.5). Additional adjustment for changes in systolic and diastolic blood pressure during the TCD procedure did not change the odds ratios for $A\beta_{1-40}$ and $A\beta_{1-42}$ in relation to impaired vasomotor reactivity.

DISCUSSION

We found that in elderly people impaired cerebral vasomotor reactivity is related to

higher plasma $Aß_{1-40}$ and $Aß_{1-42}$ levels on average 6.5-years earlier but not to concurrent Aß-levels. Furthermore, a decrease in plasma $Aß_{1-40}$ levels over time is associated with a higher prevalence of impaired vasomotor reactivity.

The strengths of this study are the population-based design and the large number of elderly participants in whom TCD and plasma Aß assessments at two points in time were performed. The plasma Aß levels were measured without knowledge of other risk factors or presence of impaired vasomotor reactivity. Therefore, misclassification, if any, will have been random and may have resulted in an underestimation of the strength of any risk associations.

TCD measurements were not obtained in all participants at the second follow-up survey of the Rotterdam Study. The selection of people for TCD measurements was random so this will not have biased the results. However, TCD measurements could not be successfully completed in 38% of those invited. People with unsuccessful completion of the TCD protocol were on average older and more often women. In older age and in postmenopausal women the thickness of the temporal bone increases, resulting in a higher frequency of window failure.[21] Furthermore, hyperostosis, which is predominantly found in women, is also thought to preclude TCD measurements. We do not think that this selection has significantly biased our results, because Aß levels of participants were not different from those of non-participants. Moreover, the association between Aß levels and impaired vasomotor reactivity was not modified by age or sex (data not shown).

Several factors may be responsible for the variance and change in plasma Aß levels in elderly people. Genetic factors may be responsible for a higher Aß production, given the elevated plasma Aß levels in all genetic forms of early-onset AD.[22,23] Higher plasma Aß levels may also reflect a change in the dynamic equilibrium of CSF and plasma Aß.[24,25] Clearance of Aß from the CSF to plasma can be disturbed by either obstruction of drainage pathways with amyloid deposits, or by a diminished soluble fraction of CSF Aß due to progressive aggregation and deposition of Aß.[16,25-27] The two latter mechanisms will result in lower peripheral Aß levels with progression of intracerebral Aß deposition. This could possibly explain the difference in associations of Aß levels assessed 6.5-years before and at time of the TCD with impaired vasomotor reactivity.

Impaired vasomotor reactivity can result from either cerebral small vessel disease or hemodynamic changes with maximally dilated arterioles. The latter is the case in high grade carotid stenosis or occlusion, in which cerebral perfusion can become directly dependent of the systemic arterial blood pressure.[2,3,20] It is unlikely that large vessel disease has substantially contributed to our findings, given that high grade stenosis or occlusion were not present in our study sample. Furthermore Aß levels were not associated with carotid intima media thickness, which is a measure of large vessel pathology, and consequently adjustment for carotid intima media thickness did not change the associations. On the other hand, we recently found that plasma Aß levels are associated with white matter lesions and lacunar infarcts, which support the selective relationship

between plasma Aß and cerebral small vessel disease.[28]

Higher plasma Aß levels could be related to impaired vasomotor reactivity through two different mechanisms. Firstly, higher levels of Aß could lead to depositions in the media and adventitia of meningocortical arteries and arterioles. These deposits lead to obliteration of vessel lumina and loss of vascular smooth muscle cells necessary to autoregulation.[7,9,10,29] Secondly, Aß may, apart from its cytotoxicity to smooth muscle cells, enhance the extent and duration of endothelial dependent vasoconstriction.[7,10,11] This could alter the ability of arterioles to dilate in response to blood pressure changes or hypercapnia.We observed an association between impaired vasomotor reactivity and Aß measured 6.5-years earlier, but not with concurrently measured Aß levels. This difference in associations could be explained by differences in direct and long term effects of Aß.

High Aß levels assessed several years before the TCD assessment might reflect a tendency for vascular Aß deposition in the future, and may explain an association between impaired vasomotor reactivity 6.5-years later (long term effect). Aß levels assessed at time of the TCD assessment may be related to the observed vasomotor reactivity through a direct vasoactive effect (short term effect). Our observations are more compatible with Aß levels representing vascular deposits years later. However, they do not exclude a direct vasoactive effect.

In conclusion impaired cerebral vasomotor reactivity is associated with higher plasma $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels assessed 6.5-years earlier and with decline in $A\beta_{1\text{-}40}$ levels over a 6.5-year time period, but not with concurrently assessed Aß levels. Plasma Aß might be involved in the impairment of cerebral autoregulation in the elderly via deposition in the small cerebral arteries.

References

1. Paulson OB, Strandgaard S, Edvinsson L. Cerebral autoregulation. Cerebrovasc Brain Metab Rev. 1990;2:161-92.
2. Yamauchi H, Fukuyama H, Nagahama Y, Shiozaki T, Nishizawa S, Konishi J, Shio H, Kimura J. Brain arteriolosclerosis and hemodynamic disturbance may induce leukoaraiosis. Neurology. 1999;53:1833-8.
3. Bakker SL, de Leeuw FE, de Groot JC, Hofman A, Koudstaal PJ, Breteler MM. Cerebral vasomotor reactivity and cerebral white matter lesions in the elderly. Neurology. 1999;52:578-83.
4. Terborg C, Gora F, Weiller C, Rother J. Reduced vasomotor reactivity in cerebral microangiopathy : a study with near-infrared spectroscopy and transcranial Doppler sonography. Stroke. 2000;31:924-9.
5. Pfefferkorn T, von Stuckrad-Barre S, Herzog J, Gasser T, Hamann GF, Dichgans M. Reduced cerebrovascular CO(2) reactivity in CADASIL: A transcranial Doppler sonography study. Stroke. 2001;32:17-21.
6. Ringelstein EB, Sievers C, Ecker S, Schneider PA, Otis SM. Noninvasive assessment of CO2-induced cerebral vasomotor response in normal individuals and patients with internal carotid artery occlusions.

Stroke. 1988;19:963-9.
7. Niwa K, Kazama K, Younkin L, Younkin SG, Carlson GA, Iadecola C. Cerebrovascular autoregulation is profoundly impaired in mice overexpressing amyloid precursor protein. Am J Physiol Heart Circ Physiol. 2002;283:H315-23.
8. Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. Physiol Rev. 2001;81: 741-66.
9. Vinters HV. Cerebral amyloid angiopathy. A critical review. Stroke. 1987;18:311-24.
10. Thomas T, Thomas G, McLendon C, Sutton T, Mullan M. beta-Amyloid-mediated vasoactivity and vascular endothelial damage. Nature. 1996;380:168-71.
11. Crawford F, Suo Z, Fang C, Mullan M. Characteristics of the in vitro vasoactivity of beta-amyloid peptides. Exp Neurol. 1998;150:159-68.
12. Holtzman DM, Fagan AM, Mackey B, Tenkova T, Sartorius L, Paul SM, Bales K, Ashe KH, Irizarry MC, Hyman BT. Apolipoprotein E facilitates neuritic and cerebrovascular plaque formation in an Alzheimer's disease model. Ann Neurol. 2000;47:739-47.
13. Bales KR, Verina T, Dodel RC, Du Y, Altstiel L, Bender M, Hyslop P, Johnstone EM, Little SP, Cummins DJ, Piccardo P, Ghetti B, Paul SM. Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. Nat Genet. 1997;17:263-4.
14. Greenberg SM, Rebeck GW, Vonsattel JP, Gomez-Isla T, Hyman BT. Apolipoprotein E epsilon 4 and cerebral hemorrhage associated with amyloid angiopathy. Ann Neurol. 1995;38:254-9.
15. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-22.
16. Mehta PD, Pirttila T, Patrick BA, Barshatzky M, Mehta SP. Amyloid beta protein 1-40 and 1-42 levels in matched cerebrospinal fluid and plasma from patients with Alzheimer disease. Neurosci Lett. 2001;304:102-6.
17. Wenham PR, Price WH, Blandell G. Apolipoprotein E genotyping by one-stage PCR. Lancet. 1991;337:1158-9.
18. Bots ML, van Swieten JC, Breteler MM, de Jong PT, van Gijn J, Hofman A, Grobbee DE. Cerebral white matter lesions and atherosclerosis in the Rotterdam Study. Lancet. 1993;341:1232-7.
19. Hetzel A, Braune S, Guschlbauer B, Dohms K. CO2 reactivity testing without blood pressure monitoring? Stroke. 1999; 30:398-401.
20. Dumville J, Panerai RB, Lennard NS, Naylor AR, Evans DH. Can cerebrovascular reactivity be assessed without measuring blood pressure in patients with carotid artery disease? Stroke. 1998;29:968-74.
21. Bass A, Krupski WC, Schneider PA, Otis SM, Dilley RB, Bernstein EF. Intraoperative transcranial Doppler: limitations of the method. J Vasc Surg. 1989;10:549-53.
22. Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kukull W, Larson E, Levy-Lahad E, Viitanen M, Peskind E, Poorkaj P, Schellenberg G, Tanzi R, Wasco W, Lannfelt L, Selkoe D, Younkin S. Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nat Med. 1996;2:864-70.
23. Tokuda T, Fukushima T, Ikeda S, Sekijima Y, Shoji S, Yanagisawa N, Tamaoka A. Plasma levels of amyloid beta proteins Abeta1-40 and Abeta1-42(43) are elevated in Down's syndrome. Ann Neurol. 1997;41: 271-3.
24. DeMattos RB, Bales KR, Cummins DJ, Paul SM, Holtzman DM. Brain to plasma amyloid-beta efflux: a measure of brain amyloid burden in a mouse model of Alzheimer's disease. Science. 2002;295:2264-7.
25. DeMattos RB, Bales KR, Parsadanian M,

O'Dell MA, Foss EM, Paul SM, Holtzman DM. Plaque-associated disruption of CSF and plasma amyloid-beta (Abeta) equilibrium in a mouse model of Alzheimer's disease. J Neurochem. 2002;81:229-36.

26. Weller RO, Massey A, Newman TA, Hutchings M, Kuo YM, Roher AE. Cerebral amyloid angiopathy: amyloid beta accumulates in putative interstitial fluid drainage pathways in Alzheimer's disease. Am J Pathol. 1998;153:725-33.
27. Jensen M, Schroder J, Blomberg M, Engvall B, Pantel J, Ida N, Basun H, Wahlund LO, Werle E, Jauss M, Beyreuther K, Lannfelt L, Hartmann T. Cerebrospinal fluid A beta42 is increased early in sporadic Alzheimer's disease and declines with disease progression. Ann Neurol. 1999;45:504-11.
28. van Dijk EJ, Prins ND, Vermeer SE, Hofman A, van Duijn CM, Koudstaal PJ, Breteler MMB. Plasma amyloid ß, APOE, lacunar brain infarcts, and white matter lesions. Ann Neurol;in press.
29. Gray F, Dubas F, Roullet E, Escourolle R. Leukoencephalopathy in diffuse hemorrhagic cerebral amyloid angiopathy. Ann Neurol. 1985;18:54-9.

4 Progression of cerebral small vessel disease

4.1
Progression of cerebral white matter lesions

Abstract

Background and purpose *— Cerebral white matter lesions in elderly people are mostly caused by cerebral small vessel disease and have been associated with an increased risk of stroke, dementia and depression. We studied the rate of white matter lesion progression over time, and its relation with cardiovascular risk factors.*

Methods *— Six hundred sixty-eight people, aged 60 to 90 years, underwent repeated MRI scanning within a 3-year follow-up, as part of the prospective population-based Rotterdam Scan Study. We rated change in periventricular and subcortical white matter lesion severity with a semiquantitative scale, and graded progression as no, minor or marked. We assessed risk factors for white matter lesion progression by means of logistic and multinomial logistic regression analysis.*

Results *— Twenty-seven percent of participants showed any and 9% showed marked progression of white matter lesions in the periventricular region. Thirty-two percent showed any and 10% showed marked progression in the subcortical region. White matter lesion severity and presence of brain infarcts at baseline, higher age, high blood pressure, and current smoking were positively associated with progression of white matter lesions. Women had more marked progression of subcortical white matter lesions compared to men.*

Conclusion *— Approximately one-third of elderly people have progression of white matter lesions in 3-years. Presence and severity of white matter lesion and brain infarcts at baseline, higher age, female sex, elevated blood pressure and current cigarette smoking are associated with white matter lesion progression.*

INTRODUCTION

Cerebral white matter lesions are frequently seen on magnetic resonance imaging (MRI) scans in elderly people.[1,2] These lesions mostly reflect demyelination and axon loss due to chronic ischemia associated with cerebral small vessel disease.[3,4] People with more severe white matter lesions have an increased risk of stroke, dementia, and depression.[5-7]

Cross-sectional studies have reported a higher prevalence and increased severity of white matter lesions with older age. Hypertension is considered the main risk factor, but other cardiovascular risk factors may be related to these lesions as well.[4,8-13] In contrast, data on change of white mater lesions in community dwelling people are scarce.[14] The Austrian Stroke Prevention Study reported progression of white matter lesions within three years in 18% of the 273 participants. Diastolic blood pressure and severe white matter lesions at baseline were the only predictors of lesion progression.[14,15]

We investigated in a large population-based sample of elderly people, the rate, location and configuration of change of cerebral white matter lesions. Furthermore, we studied the association of age, sex, severity and presence of white matter lesions and brain infarcts at baseline, and cardiovascular risk factors with progression of cerebral white matter lesions.

METHODS

Subjects

The Rotterdam Scan Study is a prospective population-based cohort study designed to study the causes and consequences of age related brain changes in elderly people.[9] We randomly selected people 60 to 90 years of age, stratified on age and sex, from two ongoing population based studies, the Rotterdam Study and the Zoetermeer study.[16,17] A total of 1,077 elderly people without dementia participated (response rate 63%). The medical ethics committee of the Erasmus Medical Center approved the study, and each participant gave written informed consent. The baseline examination in 1995 to 1996 comprised a structured interview, physical examination, blood sampling, and neuropsychological tests, as well as a cerebral MRI scan.

In 1999 to 2000, we re-invited 951 people who were eligible for a second MRI examination from the cohort of 1,077 people who had a MRI examination at baseline. In total 668 participated (response rate 70%). One-hundred-twenty-six participants were ineligible to undergo a second MRI for the following reasons: 82 died, 19 were institutionalized, 19 had MRI contraindications, 3 moved abroad, and 3 could not be reached. The reasons for refusal to participate in the second examination were as follows: claustrophobia developed at the baseline MRI (n=98), too much hassle (n=90), no interest (n=77), and other reasons (n=18).

MRI scanning

At baseline, we made axial T1-, T2-, and proton-density- (PD) weighted cerebral MR scans on a 1.5-Tesla scanner (for participants from Zoetermeer, MR Gyroscan, Philips; for participants from Rotterdam MR VISION, Siemens, with comparable pulse sequences).[18] In 1999 to 2000 all second MRI scans were made with the MR VISION scanner and the same sequences.

Lesion rating

We considered white matter lesions to be periventricular if they were directly adjacent to the ventricle; otherwise we considered them subcortical. We scored periventricular white matter lesions semiquantitatively in order to obtain a total periventricular score (range 0-9).[18] For subcortical white matter lesion, we approximated a total volume (range 0-29.5ml).[18] We defined infarcts as focal hyperintensities on T2-weighted images, 3mm in size or larger. Lesions in the white matter also had to have corresponding prominent hypointensities on T1-weighted images, in order to distinguish them from cerebral white matter lesions. We defined lacunar infarcts as infarcts sized 3 to 20mm and located in the subcortical white matter or basal ganglia.

To assess change of white matter lesions, we transferred digital images to a Linux PC, and used viewing software developed in Matlab (MathWorks). Two raters independently analyzed progression of white matter lesions severity on PD- and T2-weighted images by direct scan comparison. We used a new white matter lesions change scale, since commonly used rating scales designed for cross-sectional assessments of white matter lesions are not well suited for measuring change.[19] Baseline and follow-up series were downloaded side by side, with the baseline and follow-up scan randomly appearing on the right or left side of the screen. We assessed the difference in white matter lesion severity between the right and left images. In the periventricular region, we assessed difference in size of lesions in the frontal caps, lateral bands, and occipital caps, in the left and right hemisphere (negative difference -1 point, no difference 0 points, positive difference +1 point, resulting in a score of -6 to +6). In the subcortical region, we assessed difference in number, size, or confluence of lesions in the frontal, parietal, temporal, and occipital lobes of the left and right hemisphere (negative difference -1 point, no difference 0 points, positive difference +1 point, resulting in a score of -8 to +8). If periventricular lesion change extended beyond 10mm outside the border of the ventricle, we considered the change to have occurred in both the periventricular and subcortical region. Raters were blinded to all clinical information, including scan date, for participants derived from the Rotterdam Study. This was not possible for participants derived from the Zoetermeer Study due to the change in MRI between baseline and follow-up. If raters disagreed one point or less on the scale, we used the mean of the ratings, otherwise we held a consensus meeting. The change rating showed good interobserver agreement (intraclass correlation coefficient periventricular region 0.79; subcortical region

0.75), and good to very good intraobserver agreement (intraclass correlation coefficient periventricular region 0.70-0.89; subcortical region 0.78-0.93). We defined progression as an increase of 1 point or more between baseline and follow-up. Progression was categorized into minor progression (score 1-2.5) and marked progression (score 3 or higher). Digital images were not available in 52 participants due to technical problems. In these participants, we assessed white matter lesion change on hardcopy.

We evaluated the effect of non-blinding of the scan date.[19] The difference between non-blinded and blinded scores was 0.075 (95%CI -0.71;+0.86) points for periventricular white matter lesions, and 0.025 (95%CI -0.84;+0.89) points for subcortical white matter lesions. Therefore, non-blinding in the Zoetermeer Study part of our study did not introduce bias.

Cardiovascular risk factors

Blood pressure was measured twice on the right arm with a random-zero sphygmomanometer. The two measurements were averaged. Hypertension was defined according to WHO-ISH guidelines at time of blood pressure measurement, as systolic blood pressure of ≥160mmHg, diastolic blood pressure of ≥95mmHg, or the use of blood pressure lowering medication. Smoking habits were classified as never, former and current cigarette smoking. We considered diabetes mellitus to be present if the random glucose level was ≥11.1mmol/l or if a person was taking oral antidiabetics or insulin. To determine plasma total homocysteine levels, we used a fluorescence polarisation immunoassy on an IMx analyser (Abbott Laboratories).[8] Partial arterial oxygen saturation was measured twice, with a pulse oximeter (Andos, Oxycount). The two measurements were averaged (range 86-99%).[12] Participants underwent ultrasonography of both carotid arteries with a 7.5-MHz linear-array transducer and a Duplex scanner (ATL UltraMark IV, Advanced Technology Laboratories). We calculated the mean common carotid intima-media thickness as the mean of the near and far walls of both the right and left common carotid arteries.[13] Apolipoprotein E (APOE) genotyping was done on coded genomic DNA samples.[20] The distribution of the APOE genotype in this population was in Hardy-Weinberg equilibrium.

Data analysis

We used age and sex adjusted analysis of covariance to assess whether baseline risk factors differed between people with and without a second MRI assessment. We calculated Spearman's rho for correlation between periventricular and subcortical white matter lesion progression. We used logistic and multinomial logistic regression analyses to study the association of risk factors with any, minor and marked white matter lesion progression. People with a negative difference in white matter lesion severity over time were added to the group of people with no progression. All analyses were adjusted for age and sex, and additionally for cardiovascular risk factors. We consider baseline white matter

lesion severity as an intermediate factor in the relation between risk factors and white matter lesion progression. Therefore, we did not adjust for baseline lesions. To assess whether the effect of blood pressure on lesion progression was different in people with already severe white matter lesions at baseline, we did separate analyses in people with and without severe white matter lesions at baseline. We defined severe periventricular and subcortical white matter lesions as the upper quintile of their baseline distribution.[9]

RESULTS

People who underwent a second MRI were younger and less often used blood pressure lowering medication than people who refused a second examination (table 1). They were also younger, healthier and more often female than people who were ineligible for a second MRI (table 1). The mean follow-up between the two MRI assessments was 3.3 (SD 0.2) years.

Table 1. Baseline characteristics of people who had a 2nd MRI assessment and for those who refused or were ineligible.

	People with 2nd MRI assessment n = 668	People who refused 2nd MRI assessment n = 283	People ineligible for 2nd MRI assessment n = 126
Age, year	71 (7)	74 (7)*	77 (8)*
Women	52	57	41*
Systolic blood pressure, mmHg	147 (21)	149 (22)	146 (23)
Diastolic blood pressure, mmHg	79 (12)	79 (12)	75 (12)*
Use of antihypertensive medication	30	42*	43
Hypertension	49	58	58
Current smoking	16	13	21*
Former smoking	51	50	52
Diabetes	5	8	13*
Total homocysteine, μmol/l	11.0 (3.6)	11.7 (4.2)	13.9 (5.4)*
Oxygen saturation, $\%SpO_2$	96.4 (1.3)	96.3 (1.2)	96.3 (1.5)
Intima media thickness, mm	0.86 (0.15)	0.87 (0.13)	0.93 (0.17)
Periventricular white matter lesions (range 0-9)	2.2 (2.1)	2.5 (2.1)	3.5 (2.5)*
Subcortical white matter lesions, ml	1.2 (2.5)	1.5 (3.1)	2.1 (4.1)
Brain infarcts	22	25	34
Lacunar infarcts	20	23	28
APOE ε4 allele	30	30	22

Values are unadjusted means (SD) or percentages
*Age and sex adjusted mean or percentage is significantly different ($p < 0.05$) from people with 2nd MRI assessment

Table 2. Relationship between periventricular and subcortical white matter lesion (WML) progression

		Periventricular WML progression			
		no	minor	marked	totals
Subcortical WML progression	no	407 (61)	38 (6)	6 (1)	451 (68)
	minor	72 (11)	59 (9)	19 (3)	150 (23)
	marked	9 (1)	23 (3)	35 (5)	67 (10)
	totals	488 (73)	120 (18)	60 (9)	668 (100)

Numbers are absolute numbers of people and percentages of overall total

Twenty-seven percent of the people had any progression of periventricular white matter lesions and 32% had any progression of subcortical white matter lesions, whereas 39% had any progression of white matter lesions in at least one of these regions (table 2). Spearman's rho for the correlation between progression in the periventricular and subcortical region was 0.60 ($p<0.001$)). Two people had minor regression of white matter lesions in the periventricular and 13 in the subcortical region. Within the group with periventricular white matter progression, 17% had progression of the frontal caps, 56% of the bands, and 73% of the occipital caps. Within the group with subcortical white matter lesion progression, 71% had progression in the frontal, 63% in the parietal, 12% in the occipital, and 7% in the temporal lobe. The hemispheres were equally affected. Marked subcortical white matter progression predominantly consisted of growth and confluence of lesions, whereas minor progression mostly consisted of new small lesions (figure 1).

White matter lesion severity and the presence of brain infarcts, of which 90% were lacunar, on baseline MRI were strongly related to progression of white matter lesions (table 3). None of the people without lesions at baseline, and three people with only small punctate lesions (<3 mm) at baseline, had marked subcortical white matter lesion pro-

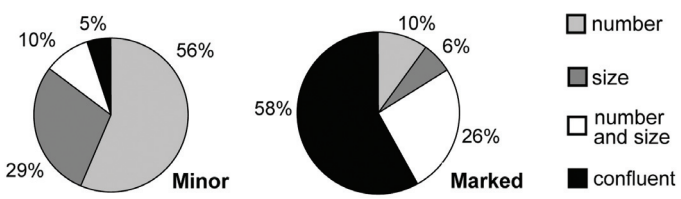

Figure 1. Configuration of minor and marked subcortical white matter lesion progression.

Table 3. Relationship between baseline brain lesions and progression of white matter lesions.

Baseline brain lesions	Odds ratio periventricular WML progression (95%CI)			Odds ratio subcortical WML progression (95%CI)		
	Any	Minor	Marked	Any	Minor	Marked
Periventricular WML (range 0-9)	1.90 (1.69; 2.14)	1.77 (1.56; 2.00)	2.35 (1.97; 2.80)	1.64 (1.48; 1.81)	1.45 (1.30; 1.62)	2.33 (1.97; 2.76)
Subcortical WML (per ml)	1.77 (1.54; 2.04)	1.67 (1.41; 1.89)	2.03 (1.73; 2.39)	1.74 (1.51; 2.01)	1.63 (1.41; 1.89)	2.03 (1.73; 2.39)
Infarcts	3.20 (2.12; 4.84)	2.38 (1.48; 3.82)	5.84 (3.21; 10.60)	3.11 (2.06; 4.70)	2.13 (1.35; 3.36)	5.62 (3.18; 9.91)
Lacunar infarcts	3.05 (1.99; 4.69)	2.13 (1.29; 3.52)	5.95 (3.24; 10.94)	2.94 (1.98; 4.37)	2.17 (1.35; 3.50)	6.22 (3.49; 11.09)

Age and sex adjusted odds ratio per grade increase in periventricular and per millilitre increase in subcortical white matter lesions and for presence of infarcts with people without progression as the reference (95% confidence intervals)

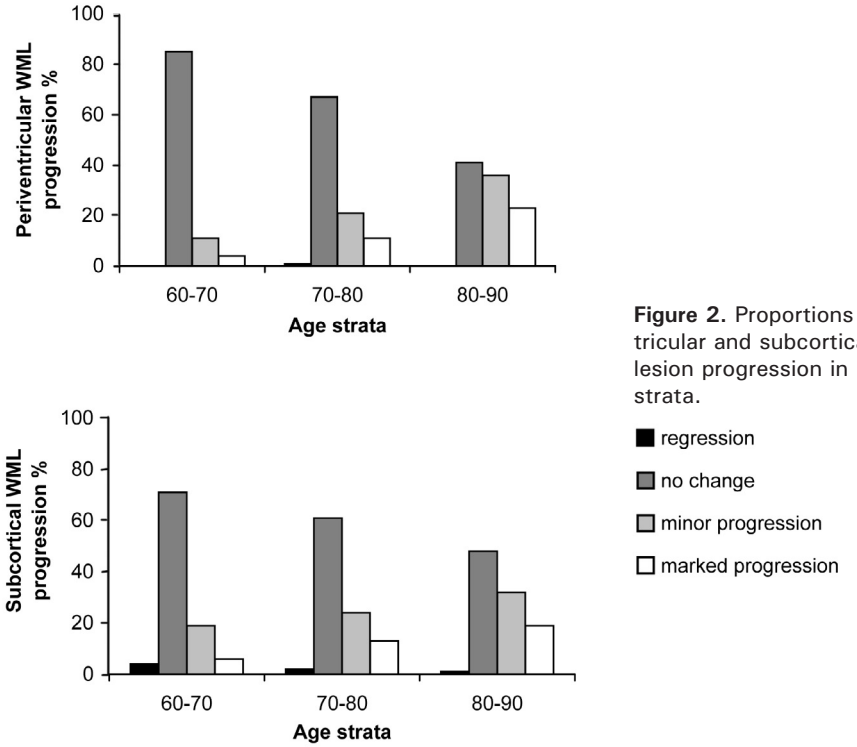

Figure 2. Proportions of periventricular and subcortical white matter lesion progression in 10 years age strata.

gression. The extent of progression of both periventricular and subcortical white matter lesions was strongly related to age (figure 2). Women had a higher risk of subcortical white matter lesions progression than men, and this difference reached significance for marked subcortical white matter lesions (table 4). Systolic and diastolic blood pressure were positively associated with white matter lesion progression (table 4), which remained after adjustment for use of blood pressure lowering medication. Current cigarette smoking was positively associated with marked white matter lesion progression (table 4). We did not observe a relation between carotid intima-media thickness, homocysteine levels, oxygen saturation and white matter lesion progression. The association of blood pressure and smoking with progression of white matter lesions hardly changed after additional adjustment for other cardiovascular risk factors (data not shown).

In subgroup analyses, the associations between blood pressure and white matter lesion progression was present in the people without severe white matter lesions at baseline (per standard deviation increase in respectively diastolic and systolic blood pressure, the age and sex adjusted odd ratios were 1.36 (95%CI 1.08;1.71) and 1.25 (95%CI 0.99;1.59) for any periventricular white matter lesion progression and 1.15 (95%CI 0.94;1.42) and 1.12 (95%CI 0.90;1.39) for any subcortical white matter lesion

Table 4. Relationship between baseline determinants and progression of white matter lesions (WML)

	Odds ratios periventricular WML progression (95%CI)			Odds ratios subcortical WML progression (95%CI)		
	Any	Minor	Marked	Any	Minor	Marked
Age (per year)	1.12 (1.09; 1.15)	1.11 (1.07; 1.14)	1.15 (1.11; 1.20)	1.07 (1.04; 1.09)	1.06 (1.03; 1.08)	1.10 (1.06; 1.14)
Sex (women)	1.21 (0.84; 1.74)	1.10 (0.72; 1.66)	1.58 (0.89; 2.79)	1.09 (0.78; 1.52)	0.89 (0.61; 1.29)	1.80 (1.05; 3.10)
SBP (per SD)	1.23 (1.02;1.48)	1.24 (1.00; 1.53)	1.21 (0.90; 1.61)	1.18 (0.99; 1.40)	1.13 (0.93; 1.37)	1.28 (0.98; 1.67)
DBP (per SD)	1.32 (1.10; 1.59)	1.29 (1.05; 1.59)	1.38 (1.04; 1.84)	1.21 (1.03; 1.44)	1.14 (0.95; 1.39)	1.38 (1.06; 1.80)
Hypertension	1.41 (0.97; 2.04)	1.48 (0.97; 2.26)	1.26 (0.71; 2.25)	1.22 (0.87; 1.73)	1.37 (0.93; 2.01)	1.01 (0.60; 1.75)
Current smoking	1.87 (1.04; 3.34)	1.51 (0.77; 2.95)	2.96 (1.25; 7.04)	1.45 (0.86; 2.45)	1.08 (0.59; 1.98)	2.82 (1.25; 6.36)
Former smoking	1.06 (0.66; 1.70)	0.99 (0.58; 1.70)	1.21 (0.58; 2.51)	0.96 (0.62; 1.46)	0.77 (0.47; 1.24)	1.58 (0.81; 3.12)
Diabetes	0.79 (0.35; 1.78)	0.53 (0.18; 1.56)	1.38 (0.48; 3.95)	0.61 (0.28; 1.33)	0.39 (0.13; 1.14)	1.14 (0.41; 3.17)
Homocysteine (per SD)	1.08 (0.90; 1.30)	1.13 (0.92; 1.39)	0.98 (0.73; 1.31)	0.95 (0.80; 1.14)	0.97 (0.79; 1.18)	0.92 (0.70; 1.22)
Oxygen saturation (per SD)	0.87 (0.72; 1.06)	0.85 (0.69; 1.05)	0.92 (0.69; 1.24)	1.05 (0.88; 1.25)	1.05 (0.86; 1.28)	1.05 (0.80; 1.39)
Carotid IMT (per SD)	1.00 (0.82; 1.23)	0.98 (0.78; 1.24)	1.05 (0.78; 1.42)	1.12 (0.33; 3.82)	1.03 (0.84; 1.27)	0.97 (0.72; 1.32)
Carotid plaques (range 0-6)	1.09 (0.97; 1.24)	1.15 (1.00; 1.32)	0.98 (0.81; 1.19)	1.11 (0.99; 1.25)	1.16 (1.03; 1.32)	0.98 (0.81; 1.18)
APOE ε4 carrier	0.91 (0.59; 1.40)	0.84 (0.51; 1.38)	1.07 (0.55; 2.07)	1.07 (0.73; 1.58)	1.11 (0.72; 1.70)	1.00 (0.53; 1.87)

Age and sex adjusted odds ratios with people without progression as the reference (95% confidence intervals). SBP = systolic blood pressure; DBP = diastolic blood pressure; IMT = intima-media thickness; APOE = apolipoprotein E

progression). In people with severe white matter lesions at baseline the effect of blood pressure was not present (per standard deviation increase in respectively diastolic and systolic blood pressure, the age and sex adjusted odd ratios were 0.89 (95%CI 0.60;1.32) and 0.98 (95%CI 0.67;1.44) for any periventricular white matter lesion progression and 0.91 (95%CI 0.62;1.35) and 1.01 (95%CI 0.70;1.45) for any subcortical white matter lesion progression).

DISCUSSION

We found that approximately 30% of people between 60 and 90 years of age had any progression and 10% had marked progression of periventricular or subcortical white matter lesions within 3-years. Older age, female sex, higher blood pressure, current cigarette smoking and high white matter lesion severity and the presence of brain infarcts at baseline were associated with progression of white matter lesions. The associations were independent of other cardiovascular risk factors.

The strengths of the present study are its large number of participating elderly people from the general population, its prospective design, the good interobserver agreement between raters, and the assessment of a large number of risk factors. Still, some methodological issues should be considered. First, there is a possibility of selection bias. People who participated were younger and healthier compared to those who were ineligible or refused a second MRI scan. Therefore, the progression of white matter lesions in our study may be an underestimation of the progression in the population at large. The same selection may also have attenuated the estimates for the associations between risk factors and progression of white matter lesions. Second, we changed MRI scanner in half of our cohort, which made it not possible to blind for study date in that part. However, we evaluated the effect of non-blinding and found that it did not cause overestimation of progression in our study.[19] Furthermore, the rate of progression and the strength of the associations were not different for the blinded and non-blinded group. Third, we used a semiquantitative measurement tool, which gives less precise estimates of total lesion progression than a volumetric measurement. The used scale showed however a good correlation with volumetric measurements.[19] Furthermore, misclassification in lesion assessment, if any, would have resulted in an underestimation of the relation with risk factors due to regression towards the mean.

It is difficult to compare the distribution of white matter lesions between studies, due to differences in study population, imaging techniques, lesion rating, lesion categorization and risk factor distributions, and the same holds for comparing progression of white matter lesions.[2] In the Austrian Stroke Prevention Study, 18% of the participants had any, and 8% had marked progression within 3 years of follow-up.[14,15] The lower proportion of progression of white matter lesions in that study compared to our findings could

be explained by the exclusion of people with cerebrovascular disease, the 10 years lower mean age of the participants and the lower response rate.

In cross-sectional studies, older age and higher blood pressure, in particular diastolic blood pressure, were strongly associated with white matter lesion severity.[1,18] Furthermore, women tended to have more severe white matter lesions.[1] We observed that these risk factors are also associated with progression of white matter lesions. The Austrian Stroke Prevention study reported the same associations with age and diastolic blood pressure.[14,15] In addition, we found that current cigarette smoking was associated with marked white matter lesion progression.

Our data confirm that white matter lesion severity and presence of brain infarcts at baseline are strongly associated with lesion progression.[14,15,21] The association between brain infarcts and progression of white matter lesions can be explained by a shared exposure to risk factors and susceptibility for these factors, resulting in a common pathological substrate.[21,22] Baseline white matter lesions and lacunar infarcts reflect small vessel disease and chronic hypoperfusion. Persistent hypoperfusion could, without additional vessel damage, result in white matter lesion progression. The progression of white matter lesions from punctate to large confluent lesions is probably a continuous process, which has an exponential rather than a linear relation with time.

We found that in people with severe white matter lesions at baseline, higher blood pressure was not a risk factor for white matter lesion progression any more. This is in agreement with earlier finding that blood pressure is not related to incident silent brain infarcts in people with prevalent silent brain infarcts.[22] The vast majority of these infarcts were lacunar infarcts.[22] In people with present small vessel disease, high blood pressure may lead to progression of small vessel damage on one hand and be necessary to maintain perfusion in a state of impairment autoregulation on the other. Apart from these potential opposite roles of blood pressure, another explanation of the negative results in people with vascular lesions at baseline, could be that cardiovascular risk factors do not further discriminate within a group of people already at high risk.

We previously reported on the cross-sectional association between carotid atherosclerosis[11], homocysteine levels[8], oxygen saturation[12], and white matter lesions. We did not observe an association between these factors and progression of white matter lesions. This could be explained by selective follow-up as noted earlier. Alternative explanations for these distinct findings, are the smaller samples size, and the possibility that baseline white matter lesion distribution is a more robust measure of small vessel disease since it reflects accumulation of lesions over a longer period of time.

In this study we considered progression of white matter lesions as an increase in number, size or confluence of lesions. This “lesion volume”-based paradigm may not cover the complete pathophysiology, since progression could, apart from increase in volume, also be increase in tissue damage within existing lesions. A prospective study in patients with CADASIL showed progression in tissue damage with diffusion tensor

imaging.[23] Future studies on the progression of white matter lesions should therefore also take change in integrity of the white matter into account.

References

1. de Leeuw FE, de Groot JC, Achten E, Oudkerk M, Ramos LM, Heijboer R, Hofman A, Jolles J, van Gijn J, Breteler MM. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. J Neurol Neurosurg Psychiatry. 2001;70:9-14.
2. van Dijk EJ, Prins ND, Vermeer SE, Koudstaal PJ, Breteler MM. Frequency of white matter lesions and silent lacunar infarcts. J Neural Transm Suppl. 2002:25-39.
3. van Swieten JC, van den Hout JH, van Ketel BA, Hijdra A, Wokke JH, van Gijn J. Periventricular lesions in the white matter on magnetic resonance imaging in the elderly. A morphometric correlation with arteriolosclerosis and dilated perivascular spaces. Brain. 1991;114 (Pt 2):761-74.
4. Pantoni L, Garcia JH. Pathogenesis of leukoaraiosis: a review. Stroke. 1997;28:652-9.
5. O'Brien JT, Erkinjuntti T, Reisberg B, Roman G, Sawada T, Pantoni L, Bowler JV, Ballard C, DeCarli C, Gorelick PB, Rockwood K, Burns A, Gauthier S, DeKosky ST. Vascular cognitive impairment. Lancet Neurol. 2003;2:89-98.
6. Vermeer SE, Hollander M, Van Dijk EJ, Hofman A, Koudstaal PJ, Breteler MM. Silent brain infarcts and white matter lesions increase stroke risk in the general population: the rotterdam scan study. Stroke. 2003;34:1126-9.
7. Prins ND, van Dijk EJ, Den Heijer T, Vermeer SE, Koudstaal PJ, Oudkerk M, Hofman A, Breteler MMB. Cerebral white matter lesions and the risk of dementia. The Rotterdam Scan Study. Arch Neurol (in press).
8. Vermeer SE, van Dijk EJ, Koudstaal PJ, Oudkerk M, Hofman A, Clarke R, Breteler MM. Homocysteine, silent brain infarcts, and white matter lesions: The Rotterdam Scan Study. Ann Neurol. 2002;51:285-9.
9. de Leeuw FE, de Groot JC, Oudkerk M, Witteman JC, Hofman A, van Gijn J, Breteler MM. Hypertension and cerebral white matter lesions in a prospective cohort study. Brain. 2002;125:765-72.
10. de Leeuw FE, de Groot JC, Oudkerk M, Kors JA, Hofman A, van Gijn J, Breteler MM. Atrial fibrillation and the risk of cerebral white matter lesions. Neurology. 2000;54:1795-801.
11. de Leeuw FE, de Groot JC, Bots ML, Witteman JC, Oudkerk M, Hofman A, van Gijn J, Breteler MM. Carotid atherosclerosis and cerebral white matter lesions in a population based magnetic resonance imaging study. J Neurol. 2000;247:291-6.
12. van Dijk EJ, Vermeer SE, De Groot JC, van de Minkelis J, Prins ND, Oudkerk M, Hofman A, Koudstaal PJ, Breteler MMB. Arterial oxygen saturation, COPD and cerebral small vessel disease. JNNP (in press).
13. Bots ML, van Swieten JC, Breteler MM, de Jong PT, van Gijn J, Hofman A, Grobbee DE. Cerebral white matter lesions and atherosclerosis in the Rotterdam Study. Lancet. 1993;341:1232-7.
14. Schmidt R, Fazekas F, Kapeller P, Schmidt H, Hartung HP. MRI white matter hyperintensities: three-year follow-up of the Austrian Stroke Prevention Study. Neurology. 1999;53:132-9.
15. Schmidt R, Enzinger C, Ropele S, Schmidt H, Fazekas F. Progression of cerebral

white matter lesions: 6-year results of the Austrian Stroke Prevention Study. Lancet. 2003;361:2046-8.
16. Hofman A, van Laar A, Klein F, Valkenburg H. Coffee and cholesterol (letter). N Engl J Med. 1983;309:1248-1249.
17. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-22.
18. de Leeuw FE, de Groot JC, Oudkerk M, Witteman JC, Hofman A, van Gijn J, Breteler MM. A follow-up study of blood pressure and cerebral white matter lesions. Ann Neurol. 1999;46:827-33.
19. Prins ND, van Straaten ECW, van Dijk EJ, Simoni M, Koudstaal PJ, Scheltens P, Breteler MMB, Barkhof F. Measuring progression of cerebral white matter lesions on MRI; A comparison of visual rating scales in relation to volumetric change. Neurology (in press).
20. Wenham PR, Price WH, Blandell G. Apolipoprotein E genotyping by one-stage PCR. Lancet. 1991;337:1158-9.
21. van Zagten M, Boiten J, Kessels F, Lodder J. Significant progression of white matter lesions and small deep (lacunar) infarcts in patients with stroke. Arch Neurol. 1996;53:650-5.
22. Vermeer SE, Den Heijer T, Koudstaal PJ, Oudkerk M, Hofman A, Breteler MM. Incidence and risk factors of silent brain infarcts in the population-based Rotterdam Scan Study. Stroke. 2003;34:392-6.
23. Molko N, Pappata S, Mangin JF, Poupon F, LeBihan D, Bousser MG, Chabriat H. Monitoring disease progression in CADASIL with diffusion magnetic resonance imaging: a study with whole brain histogram analysis. Stroke. 2002;33:2902-8.

4.2
C-reactive protein and cerebral small vessel disease

Abstract

Inflammatory processes are involved in the development and consequences of atherosclerosis. Whether these processes are also involved in cerebral small vessel disease is unknown. Cerebral white matter lesions and lacunar infarcts are caused by small vessel disease and commonly observed on MRI scans in elderly people. These lesions are associated with an increased risk of stroke and dementia. We assessed whether higher C-reactive protein (CRP) levels were related to the severity and progression of white matter lesion, and the prevalence and incidence of lacunar infarcts.

We based our study on 1,033 participants of the population-based Rotterdam Scan Study who had complete data on CRP levels and underwent brain MRI scanning. Subjects were 60-90 years of age and free of dementia at baseline. Six hundred thirty-six subjects had a second MRI scan on average 3.3 years later. We used multivariate regression models to assess the associations between CRP levels and markers of small vessel disease. Higher CRP levels were associated with presence and progression of white matter lesions, particularly with marked lesion progression (Odds ratios for highest versus lowest quartile of CRP 3.1 (95%CI 1.3;7.2) and 2.5 (95%CI 1.1;5.6) for marked periventricular and subcortical white matter lesion progression). These associations were independent of cardiovascular risk factors and carotid atherosclerosis. Persons with higher CRP levels tended to have more prevalent and incident lacunar infarcts.

Our study suggests that inflammation is involved in the pathogenesis of cerebral small vessel disease, and in particular in the development of white matter lesions.

INTRODUCTION

Inflammatory processes are part of the pathogenesis of atherosclerosis and a risk factor for myocardial infarction, stroke and peripheral arterial disease.[1-4] Furthermore, these processes probably also play a role in the response to ischemic events resulting from atherosclerosis.[5] Whether inflammatory processes, apart from their involvement in large vessel disease, are also part of the development and consequences of cerebral small vessel disease is yet unknown.

Lacunar brain infarcts and cerebral white matter lesions are caused by small vessel disease.[6-9] These lesions are commonly observed on magnetic resonance imaging (MRI) scans of elderly people and are associated with an increased risk of stroke, dementia and depression.[10-15] Although the pathophysiology of cerebral small vessel disease is not exactly clear increased age and hypertension are considered the main risk factors. Narrowing of the vascular lumen and failure of cerebral autoregulation result in ischemic damage of the cerebral white and subcortical gray matter.[8,16]

The acute phase reactant C-reactive protein (CRP) has proven to be a sensitive systemic marker of inflammation.[17,18] The objective of the present study was to assess whether higher levels of this inflammation marker are related to the presence and progression of cerebral small vessel disease in elderly people.

METHODS

Subjects

The Rotterdam Scan Study is a prospective, population based cohort study. We randomly selected participants aged 60-90 years in strata of age (5 years) and sex from two large ongoing population-based studies.[19,20] The characteristics of the 1,077 non-demented participants have been described previously.[16] Baseline examination in 1995-1996 comprised a structured interview, neuropsychological tests, physical examination, blood sampling, and a MRI scan of the brain. In 1999-2000, 668 (70%) of the 951 participants who were alive and eligible underwent a second MRI and were re-examined at the research center similar to the baseline examination.[21] Each subject gave written informed consent to participate in our study, which had been approved by the local medical ethics committee.

MRI scanning

In 1995-1996, we made axial T1-, T2-, and proton density (PD)-weighted cerebral MR scans on a 1.5-Tesla scanner (MR VISION, Siemens, MR Gyroscan, Philips).[22] In 1999-2000, participants underwent a second MRI with the use of the MR VISION scanner and the same sequences.

White matter lesions

We considered white matter lesions to be in the periventricular region if they were directly adjacent to the ventricle; otherwise we considered them subcortical. Baseline white matter lesions severity was scored on hardcopy with a visual rating scale.[22] We scored periventricular white matter lesions semiquantitatively in three regions (lesions adjacent to the frontal horns, the lateral walls, and the occipital horns of the lateral ventricle) resulting in a total score ranging from 0-9. For subcortical WML we approximated a total volume based on number and size of lesions (range 0-29.5ml).

Two raters independently assessed progression of white matter lesions severity on digital T2-weighted and PD-weighted images by direct scan comparison.[21,23] Raters were blinded to all clinical information. We scored differences in white mater lesion severity in the 3 periventricular regions of both hemispheres (periventricular score range -6 to +6) and in the subcortical white matter of the 4 lobes of both hemispheres (subcortical score range -8 to +8).[23] The change rating showed good interobserver agreement (intraclass correlation coefficient 0.75-0.79), and good to very good intraobserver agreement (intraclass correlation coefficient 0.70-0.93). If raters disagreed one point or less on the scale, we used the mean of the ratings, otherwise we held a consensus meeting. Progression was defined as an increase of one point or more between baseline and follow-up. We categorized progression into no progression (score <1), minor progression (score 1-2.5), and marked progression (score 3 or higher). Hyperintensities on PD- and T2-weighted images around an incident infarct were not considered as progression of white matter lesions.

Cerebral infarcts

The presence of brain infarcts was rated similarly at the baseline and second MRI.[15] We defined brain infarcts as areas of focal hyperintensity on T2-weighted images sized ≥3mm. Areas of hyperintensity in the white matter also had to have corresponding prominent hypointensity on T1-weighted images, in order to distinguish them from white matter lesions. We defined lacunar infarcts as infarcts sized 3-20mm and located in the subcortical white matter or basal ganglia. Non-lacunar infarcts were excluded in the analyses of lacunar infarcts.

High-sensitivity C-reactive protein (CRP)

We collected non-fasting blood samples into vacutainers in 1995-1996. Samples were put on ice immediately, centrifuged within 60 minutes, and aliquots of serum were stored at -80˚C. In 2003, serum levels of C-reactive protein were determined by Rate Near Infrared Particle Immunoassay method (Immage® high sensitive CRP, Beckman Coulter, USA). This method has an intra-laboratory imprecision of less than 5%. CRP level distribution was highly skewed. In the study population 5.2% (n=54 of whom 31 had repeated MRI assessments) had levels of CRP >10mg/L. Outliers (values three standard deviations above the population mean of log-transformed CRP, n=14 of whom 10 had

repeated MRI assessments) were excluded, since they may indicate the presence of an active inflammatory disease.[24] We assessed CRP levels in 1,047 people of the baseline cohort of whom 636 people had a second MRI scan. In the other 30 people we failed to obtain blood samples.

Cardiovascular risk factors[21]

Hypertension was defined according to WHO-ISH guidelines at time of blood pressure measurement, as systolic blood pressure of ≥160mmHg, diastolic blood pressure of ≥95mmHg, or the use of blood pressure lowering medication. Smoking habits were classified as never, former and current cigarette smoking. We considered diabetes mellitus to be present if the random glucose level was ≥11.1mmol/l or if a person was taking oral anti-diabetics or insulin. Non-fasting serum total cholesterol and high-density lipoprotein were determined. Body mass index was calculated as weight divided by height squared. Participants underwent ultrasonography of both carotid arteries to obtain an atherosclerotic plaque score (range 0-6) and an intima-media thickness measurement.[25]

Data analysis

We categorized CRP levels in quartiles of the baseline distribution and used linear regression analyses to assess its relation with periventricular and subcortical white matter lesions at baseline and multinomial logistic regression analyses to assess its relation with white matter lesion progression. We used logistic regression analyses to study the relation between quartiles of CRP levels and prevalent and incident lacunar infarcts. All analyses were adjusted for age and sex and cardiovascular risk factors, and subsequently for measurements of carotid atherosclerosis. We did additional analyses in which we excluded people who used non-steroidal anti-inflammatory drugs (NSAIDs) or statins and people with a prevalent myocardial infarction or stroke, to check whether these factors influenced the associations under study.

RESULTS

Selected baseline characteristics of all participants and of those with repeated MRI assessments are shown in table 1. The mean follow-up period between the first and second MRI was 3.3 years (standard deviation 0.2 years). During this period, 172 participants (27%) showed white matter lesion progression in the periventricular region, 59 (9%) of whom showed marked progression. Two-hundred-five participants (32%) showed progression in the subcortical region, 62 (10%) of whom showed marked progression. Ninety participants (14%) had a new cerebral infarct on the follow-up MRI, of which 76 (12%) were lacunar.

Table 1. Baseline characteristics of all participants and of the participants with repeated MRI assessments

	All participants (n = 1,033)	Participants with repeated MRI assessment (n = 636)
Age, years	72 (7)	71 (7)
Women, %	51	51
Hypertension, %	53	47
Diabetes Mellitus, %	7	6
Body mass index, kg/m^2	26.7 (3.6)	26.8 (3.7)
Current smoking, %	16	16
Former smoking, %	51	51
Cholesterol/HDL ratio	4.9 (1.5)	4.9 (1.4)
CRP, mg/l	2.0 (1.0;3.6)	1.8 (1.0;3.5)
Use of NSAIDs, %	6	6
Use of statins, %	7	7
Periventricular WML (grade)	2.4 (2.2)	2.1 (1.5)
Subcortical WML (ml)	1.4 (2.8)	1.1 (2.4)
Infarcts, %	24	22
Lacunar infarcts, %*	22	20
Carotid plaques, (range 0-6)	1.7 (1.6)	1.5 (1.5)
Intima-media thickness, mm	0.87 (0.15)	0.86 (0.15)
Prevalent myocardial infarction, %	8	6
Prevalent stroke, %	6	4

Values are percentages, unadjusted means (standard deviation), and for CRP levels medians (interquartile range) *non-lacunar infarcts excluded

People with high CRP levels (upper quartile) had more severe periventricular and subcortical white matter lesions at baseline than people with low CRP levels (lowest quartile), adjusted for age, sex and cardiovascular risk factors (table 2). This association in all 1,033 subjects was similar for the 636 subjects with repeated MRI assessments. Additional adjustment for carotid plaques and intima-media thickness hardly changed these associations (table 2). Persons with higher CRP levels tended to have more lacunar infarcts at baseline, but this was non-significant.

People with high CRP levels (upper quartile) also had more progression of periventricular and subcortical white matter lesions than people with low CRP levels (lowest quartile), adjusted for age, sex and cardiovascular risk factors (table 3). Additional adjustment for carotid plaques and intima-media thickness hardly changed these associations (table 3). The association between CRP levels and white matter lesion progression was stronger for marked than for any white matter lesion progression. People with higher CRP levels tended to have more new lacunar infarcts than people with lower CRP levels, however the association was non-significant (table 3). Exclusion of people with

Table 2. Association between levels of C-reactive protein in quartiles and white matter lesion (WML) severity and prevalent infarcts (95% confidence intervals) in 1,033 subjects at baseline.

Quartiles	Mean baseline white matter lesions severity (95%CI)		Odds ratio prevalent infarcts (95%CI)	
	Periventricular (grade)	Subcortical (ml)	All infarcts	Lacunar infarcts
Model 1				
1 (0.20-1.01mg/l)	2.3 (2.1;2.5)	1.2 (0.8;1.5)	1 (ref)	1 (ref)
2 (1.02-1.82mg/l)	2.2 (2.0;2.5)	1.2 (0.8;1.5)	1.2 (0.8;1.9)	1.3 (0.8;2.0)
3 (1.83-3.49mg/l)	2.3 (2.1;2.6)	1.4 (1.0;1.7)	1.3 (0.9;2.1)	1.4 (0.9;2.2)
4 (3.50-22.50mg/l)	2.7 (2.4;2.9)†	1.7 (1.4;2.0)†	1.3 (0.9;2.1)	1.3 (0.8;2.1)
p-trend	<0.05	<0.05	0.18	0.25
Model 2				
1 (0.20-1.01mg/l)	2.3 (2.1;2.6)	1.2 (0.9;1.5)	1 (ref)	1 (ref)
2 (1.02-1.82mg/l)	2.2 (2.0;2.5)	1.2 (0.8;1.5)	1.1 (0.7;1.8)	1.2 (0.7;1.9)
3 (1.83-3.49mg/l)	2.4 (2.1;2.6)	1.4 (1.0;1.7)	1.3 (0.9;2.0)	1.4 (0.9;2.2)
4 (3.50-22.50mg/l)	2.6 (2.4;2.9)‡	1.7 (1.4;2.0)†	1.3 (0.9;2.1)	1.3 (0.8;2.1)
p-trend	0.06	<0.05	0.14	0.19

Model 1 adjusted for age, sex, diabetes, smoking, BMI, hypertension and cholesterol/HDL ratio
Model 2 adjusted for age, sex, and carotid plaques and IMT
*p<0.01 †p<0.05 ‡p<0.10 for comparison with first quartile, p-trend over quartiles

prevalent myocardial infarction or stroke or of people who used statins or NSAIDs did not change these associations.

DISCUSSION

We found in a general population of non-demented elderly that higher CRP levels were associated with presence and progression of periventricular and subcortical white matter lesions, and in particular with marked lesion progression. These associations were independent of cardiovascular risk factors or carotid atherosclerosis. People with higher CRP levels tended to have more prevalent and incident lacunar infarcts than people with lower CRP levels, however these associations were non-significant.

Some methodological issues need to be discussed. First, non-participation both at baseline and at follow-up was associated with older age.[16,21] People with higher CRP levels and more severe white matter lesions and lacunar infarcts may have selectively not participated. The cross-sectional association between CRP levels and white matter lesions was however not different for all participants compared to the group of participants who had repeated MRI assessments. Hence, although the potential selection of

Table 3. Association between levels of C-reactive protein in quartiles and progression of white matter lesions (WML) and incident infarcts (95% confidence intervals) in 636 subjects with repeated MRI scanning.

Quartiles	Periventricular WML progression			Subcortical WML progression			Incident infarcts	
	Any OR (95%CI)	Minor OR (95%CI)	Marked OR (95%CI)	Any OR (95%CI)	Minor OR (95%CI)	Marked OR (95%CI)	All OR (95%CI)	Lacunar OR (95%CI)
Model1								
1 (0.20-1.01mg/l)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
2 (1.02-1.82mg/l)	1.4 (0.8;2.4)	1.7 (0.9;3.2)	0.7 (0.2;1.8)	1.2 (0.7;1.9)	1.2 (0.7;2.0)	1.3 (0.6;3.0)	1.0 (0.5;2.0)	0.9 (0.4;2.0)
3 (1.83-3.49mg/l)	1.3 (0.7;2.3)	1.2 (0.6;2.4)	1.6 (0.7;3.8)	0.9 (0.5;1.5)	0.9 (0.5;1.7)	0.9 (0.4;2.1)	1.5 (0.8;3.0)	1.8 (0.9;3.7)
4 (3.50-22.50mg/l)	2.2 (1.3;4.0)*	1.9 (1.0;3.7)‡	3.1 (1.3;7.2)*	1.8 (1.1;3.1)†	1.7 (0.9;3.0)‡	2.5 (1.1;5.6)†	1.5 (0.7;2.9)	1.4 (0.7;3.0)
p-trend	<0.01	0.16	<0.01	0.06	0.15	0.06	0.15	0.18
Model2								
1 (0.20-1.01mg/l)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
2 (1.02-1.82mg/l)	1.3 (0.8;2.3)	1.7 (0.9;3.2)‡	0.6 (0.2;1.6)	1.1 (0.7;1.7)	1.0 (0.6;1.8)	1.2 (0.5;2.7)	0.9 (0.5;1.9)	0.9 (0.4;2.0)
3 (1.83-3.49mg/l)	1.3 (0.7;2.3)	1.2 (0.6;2.3)	1.5 (0.6;3.4)	0.8 (0.5;1.3)	0.8 (0.4;1.4)	0.8 (0.3;1.9	1.5 (0.7;2.8)	1.7 (0.8;3.4)
4 (3.50-22.50mg/l)	2.2 (1.3;3.8)*	2.0 (1.0;3.8)†	2.7 (1.2;6.0)†	1.5 (0.9;2.5)‡	1.2 (0.8;2.3)	2.1 (1.0;4.5)†	1.5 (0.8;2.9)	1.4 (0.7;2.9)
p-trend	<0.01	0.12	<0.01	0.20	0.48	0.12	0.13	0.16

Model 1 adjusted for age, sex, diabetes, smoking, BMI, hypertension and cholesterol/HDL ratio
Model 2 adjusted for age, sex, and carotid plaques and IMT *p<0.01 †p<0.05 ‡p<0.10 for comparison with first quartile, p-trend over quartiles

participants may have somewhat limited the range of baseline and outcome measurements we do not think that it has effected the validity of the associations. Second, although two experienced raters independently assessed all MRI scans with a good agreement, there still is a possibility of misclassification of brain lesions. Also, CRP levels may have been measured with some error, since we only assessed them once.[26] However, a study in which CRP was measured regularly over a 6-month period concluded that CRP levels are tightly regulated with few short-term fluctuations.[17] Since CRP levels, covariates and brain lesions were assessed independently and blindly from each other, misclassification, if any, will have been random and resulted in an underestimation of the associations.

Elevated CRP levels are not disease specific but are sensitive markers produced in response to tissue injury, infectious agents, immunological stimuli and inflammation. Cytokines such as interleukin-1, interleukin-6 and tumor necrosis factor-α are highly correlated with CRP levels and their function.[18,27] Elevated CRP levels may be related to cerebral white matter lesions and lacunar infarcts via different mechanisms.

First, CRP levels may be a marker for arteriolosclerosis or small vessel disease, as it is for atherosclerosis. Arteriolosclerosis may result in white matter lesions and lacunar infarcts through vessel occlusion, disturbed cerebral autoregulation or increases vascular permeability.[8,28] Although no inflammatory cells are observed in the vessel wall in case of arteriolosclerosis, inflammatory endothelial activation may play a role in both small and large vessel disease.[6,7,29,30] Increased levels of inflammatory endothelial inflammation markers (e.g. ICAM and VCAM) have been reported in people with white matter lesions and lacunar infarcts.[30]

Second, inflammation may be a response to ischemic tissue damage.[31-33] Microglial activation is shown in chronic cerebral hypoperfusion and may contribute to even further tissue damage.[34,35] In a state of chronic low-grade inflammation, oligodendrocytes and neurons may be more susceptible to hypoperfusion and hence accelerate lesion progression.[36,37] Third, elevated CRP levels could reflect large vessel atherosclerosis.[1-5,18] Carotid atherosclerosis is related to cerebral white matter lesions, probably by reducing the cerebral blood flow and by production of inflammatory mediators and free-radicals that effect the micro-vascular endothelium.[4,25,38] Furthermore, carotid atherosclerosis also reflects longstanding exposure to cardiovascular risk factors, which it shares with small vessel disease. Adjusting for carotid atherosclerosis and for cardiovascular risk factors hardly changed the association, indicating that other mechanism are more likely. Fourth, inflammatory processes are strongly related to and probably part of the pathogenesis of Alzheimer's disease,[27,39,40] in which white matter lesions and lacunar infarcts are frequently observed.[41-44] Cerebral amyloid angiopathy is a part of Alzheimer's disease pathology and may result in white matter lesions and infarcts.[27,40,45] In our study, although none of the participants were demented at baseline, pre-clinical stages of Alzheimer's disease could nonetheless have been responsible for the association. At

present and based on our observations we can not conclude which mechanism underlies the relations we found between CRP levels and small vessel disease. More than one mechanism and probably a combination of mechanisms could underlie the association between inflammation and white matter lesions.[5]

The observation that inflammation may be involved in the pathophysiology of cerebral small vessel disease, is in line with observations in stroke and dementia, although confirmation by other studies is needed. Furthermore, more insight in the exact mechanisms underlying the association and their relative contribution are necessary. Since statins, NSAIDs, and aspirin could possibly reduce inflammatory activity, they are potential candidates to attenuate progression of lesions related to small vessel disease and their consequences.[46]

References

1. Rost NS, Wolf PA, Kase CS, Kelly-Hayes M, Silbershatz H, Massaro JM, D'Agostino RB, Franzblau C, Wilson PW. Plasma concentration of C-reactive protein and risk of ischemic stroke and transient ischemic attack: the Framingham study. Stroke. 2001;32:2575-9.
2. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med. 1997;336:973-9.
3. Ridker PM, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. Jama. 2001;285:2481-5.
4. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med. 1999;340:115-26.
5. Tracy RP. Inflammation in cardiovascular disease: cart, horse, or both? Circulation. 1998;97:2000-2.
6. Fazekas F, Kleinert R, Offenbacher H, Schmidt R, Kleinert G, Payer F, Radner H, Lechner H. Pathologic correlates of incidental MRI white matter signal hyperintensities. Neurology. 1993;43:1683-9.
7. van Swieten JC, van den Hout JH, van Ketel BA, Hijdra A, Wokke JH, van Gijn J. Periventricular lesions in the white matter on magnetic resonance imaging in the elderly. A morphometric correlation with arteriolosclerosis and dilated perivascular spaces. Brain. 1991;114 (Pt 2):761-74.
8. Pantoni L, Garcia JH. Pathogenesis of leukoaraiosis: a review. Stroke. 1997;28: 652-9.
9. Fisher CM. Lacunes: small, deep cerebral infarcts. Neurology. 1965;15:774-784.
10. O'Brien JT, Erkinjuntti T, Reisberg B, Roman G, Sawada T, Pantoni L, Bowler JV, Ballard C, DeCarli C, Gorelick PB, Rockwood K, Burns A, Gauthier S, DeKosky ST. Vascular cognitive impairment. Lancet Neurol. 2003;2:89-98.
11. Vermeer SE, Hollander M, Van Dijk EJ, Hofman A, Koudstaal PJ, Breteler MM. Silent brain infarcts and white matter lesions increase stroke risk in the general population: the rotterdam scan study. Stroke. 2003;34:1126-9.
12. Prins ND, van Dijk EJ, Den Heijer T, Vermeer SE, Koudstaal PJ, Oudkerk M, Hofman A, Breteler MMB. Cerebral white

matter lesions and the risk of dementia. The Rotterdam Scan Study. Arch Neurol. (in press).
13. Liao D, Cooper L, Cai J, Toole J, Bryan N, Burke G, Shahar E, Nieto J, Mosley T, Heiss G. The prevalence and severity of white matter lesions, their relationship with age, ethnicity, gender, and cardiovascular disease risk factors: the ARIC Study. Neuroepidemiology. 1997;16:149-62.
14. de Leeuw FE, de Groot JC, Achten E, Oudkerk M, Ramos LM, Heijboer R, Hofman A, Jolles J, van Gijn J, Breteler MM. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. J Neurol Neurosurg Psychiatry. 2001;70:9-14.
15. Vermeer SE, Den Heijer T, Koudstaal PJ, Oudkerk M, Hofman A, Breteler MM. Incidence and risk factors of silent brain infarcts in the population-based Rotterdam Scan Study. Stroke. 2003;34:392-6.
16. de Leeuw FE, de Groot JC, Oudkerk M, Witteman JC, Hofman A, van Gijn J, Breteler MM. A follow-up study of blood pressure and cerebral white matter lesions. Ann Neurol. 1999;46:827-33.
17. Macy EM, Hayes TE, Tracy RP. Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. Clin Chem. 1997;43:52-8.
18. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med. 2000;342:836-43.
19. Hofman A, van Laar A, Klein F, Valkenburg H. Coffee and cholesterol (letter). N Engl J Med. 1983;309:1248-1249.
20. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-22.
21. van Dijk EJ, Prins ND, Vrooman HA, Hofman A, Koudstaal PJ, Breteler MM. Progression and risk factors of cerebral white matter lesions in the population-based Rotterdam Scan Study. submitted.
22. de Groot JC, de Leeuw FE, Oudkerk M, van Gijn J, Hofman A, Jolles J, Breteler MM. Cerebral white matter lesions and cognitive function: the Rotterdam Scan Study. Ann Neurol. 2000;47:145-51.
23. Prins ND, van Straaten ECW, van Dijk EJ, Simoni M, Koudstaal PJ, Scheltens P, Breteler MMB, Barkhof F. Measuring progression of cerebral white matter lesions on MRI; A comparison of visual rating scales in relation to volumetric change. Neurology (in press).
24. van der Meer IM, Iglesias del Sol A, Hak AE, Bots ML, Hofman A, Witteman JC. Risk factors for progression of atherosclerosis measured at multiple sites in the arterial tree: the Rotterdam Study. Stroke. 2003;34:2374-9.
25. Bots ML, van Swieten JC, Breteler MM, de Jong PT, van Gijn J, Hofman A, Grobbee DE. Cerebral white matter lesions and atherosclerosis in the Rotterdam Study. Lancet. 1993;341:1232-7.
26. Kushner I, Sehgal AR. Is high-sensitivity C-reactive protein an effective screening test for cardiovascular risk? Arch Intern Med. 2002;162:867-9.
27. McGeer PL, McGeer EG. Inflammation, autotoxicity and Alzheimer disease. Neurobiol Aging. 2001;22:799-809.
28. Wardlaw JM, Sandercock PA, Dennis MS, Starr J. Is breakdown of the blood-brain barrier responsible for lacunar stroke, leukoaraiosis, and dementia? Stroke. 2003;34: 806-12.
29. Fassbender K, Bertsch T, Mielke O, Muhlhauser F, Hennerici M. Adhesion molecules in cerebrovascular diseases. Evidence for an inflammatory endothelial activation in cerebral large- and small-vessel disease. Stroke. 1999;30:1647-50.
30. Hassan A, Hunt BJ, O'Sullivan M, Parmar K, Bamford JM, Briley D, Brown MM, Tho-

mas DJ, Markus HS. Markers of endothelial dysfunction in lacunar infarction and ischaemic leukoaraiosis. Brain. 2003;126: 424-32.
31. Akiguchi I, Tomimoto H, Suenaga T, Wakita H, Budka H. Alterations in glia and axons in the brains of Binswanger's disease patients. Stroke. 1997;28:1423-9.
32. Wakita H, Tomimoto H, Akiguchi I, Kimura J. Protective effect of cyclosporin A on white matter changes in the rat brain after chronic cerebral hypoperfusion. Stroke. 1995;26:1415-22.
33. Mevorach D. Opsonization of apoptotic cells. Implications for uptake and autoimmunity. Ann N Y Acad Sci. 2000;926:226-35.
34. Wakita H, Tomimoto H, Akiguchi I, Matsuo A, Lin JX, Ihara M, McGeer PL. Axonal damage and demyelination in the white matter after chronic cerebral hypoperfusion in the rat. Brain Res. 2002;924: 63-70.
35. Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci. 1999;22: 391-7.
36. del Zoppo G, Ginis I, Hallenbeck JM, Iadecola C, Wang X, Feuerstein GZ. Inflammation and stroke: putative role for cytokines, adhesion molecules and iNOS in brain response to ischemia. Brain Pathol. 2000;10: 95-112.
37. Iadecola C, Alexander M. Cerebral ischemia and inflammation. Curr Opin Neurol. 2001;14:89-94.
38. de Leeuw FE, de Groot JC, Bots ML, Witteman JC, Oudkerk M, Hofman A, van Gijn J, Breteler MM. Carotid atherosclerosis and cerebral white matter lesions in a population based magnetic resonance imaging study. J Neurol. 2000;247:291-6.
39. Schmidt R, Schmidt H, Curb JD, Masaki K, White LR, Launer LJ. Early inflammation and dementia: a 25-year follow-up of the Honolulu-Asia Aging Study. Ann Neurol. 2002;52:168-74.
40. Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, Cooper NR, Eikelenboom P, Emmerling M, Fiebich BL, Finch CE, Frautschy S, Griffin WS, Hampel H, Hull M, Landreth G, Lue L, Mrak R, Mackenzie IR, McGeer PL, O'Banion MK, Pachter J, Pasinetti G, Plata-Salaman C, Rogers J, Rydel R, Shen Y, Streit W, Strohmeyer R, Tooyoma I, Van Muiswinkel FL, Veerhuis R, Walker D, Webster S, Wegrzyniak B, Wenk G, Wyss-Coray T. Inflammation and Alzheimer's disease. Neurobiol Aging. 2000;21:383-421.
41. Snowdon DA, Greiner LH, Mortimer JA, Riley KP, Greiner PA, Markesbery WR. Brain infarction and the clinical expression of Alzheimer disease. The Nun Study. Jama. 1997;277:813-7.
42. Brun A, Englund E. A white matter disorder in dementia of the Alzheimer type: a pathoanatomical study. Ann Neurol. 1986;19:253-62.
43. Kalaria RN. Small vessel disease and Alzheimer's dementia: pathological considerations. Cerebrovasc Dis. 2002;13 Suppl 2:48-52.
44. Barber R, Scheltens P, Gholkar A, Ballard C, McKeith I, Ince P, Perry R, O'Brien J. White matter lesions on magnetic resonance imaging in dementia with Lewy bodies, Alzheimer's disease, vascular dementia, and normal aging. J Neurol Neurosurg Psychiatry. 1999;67:66-72.
45. Gray F, Dubas F, Roullet E, Escourolle R. Leukoencephalopathy in diffuse hemorrhagic cerebral amyloid angiopathy. Ann Neurol. 1985;18:54-9.
46. Ridker PM, Rifai N, Clearfield M, Downs JR, Weis SE, Miles JS, Gotto AM, Jr. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. N Engl J Med. 2001;344:1959-65.

5 Silent brain infarcts, white matter lesions and the risk of stroke

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Abstract

Background and Purpose *– Silent brain infarcts and white matter lesions are associated with an increased risk of subsequent stroke in minor stroke patients. In healthy elderly people, silent brain infarcts and white matter lesions are common, but little is known about their relevance. We examined the risk of stroke associated with these lesions in the general population.*

Methods *– The Rotterdam Scan Study is a population-based prospective cohort study among 1077 elderly people. The presence of silent brain infarcts and white matter lesions was scored on cerebral MRI scans obtained from 1995 to 1996. Participants were followed for stroke for on average 4.2 years. We estimated the risk of stroke in relation to presence of brain lesions with Cox proportional hazards regression analysis.*

Results *– Fifty-seven participants (6%) experienced a stroke during follow-up. Participants with silent brain infarcts had a 5 times higher stroke incidence than those without. The presence of silent brain infarcts increased the risk of stroke >3-fold, independently of other stroke risk factors (adjusted hazard ratio 3.9, 95% CI 2.3 to 6.8). People in the upper tertile of the white matter lesion distribution had an increased stroke risk compared with those in the lowest tertile (adjusted hazard ratio for periventricular lesions 4.7, 95% CI 2.0 to 11.2 and for subcortical lesions 3.6, 95% CI 1.4 to 9.2). Silent brain infarcts and severe white matter lesions increased the stroke risk independently of each other.*

Conclusion *– Elderly people with silent brain infarcts and white matter lesions are at a strongly increased risk of stroke, which could not be explained by the major stroke risk factors.*

INTRODUCTION

Prior brain infarctions and cerebral white matter lesions are frequently seen on MRI scans in patients admitted with a first stroke. In patients with a minor stroke, both silent brain infarcts and white matter lesions increased the risk of recurrent stroke.[1,2] With the increased use of imaging techniques, these lesions are more often seen in non-stroke patients as well. Silent brain infarcts and white matter lesions are thought to have a vascular origin and are frequently seen in neurologically asymptomatic elderly people.[3-9] Little is known about the relevance of these lesions in the general population. Recently, a population-based study reported a 2-fold-increased risk of stroke in elderly people with silent brain infarcts.[10] We examined whether the presence of silent brain infarcts and white matter lesions increased the rate and risk of stroke in the general population. Furthermore, we quantified this relation and investigated if this was independent of the established stroke risk factors and of each other.

METHODS

Participants

The Rotterdam Scan Study was designed to study causes and consequences of brain changes in the elderly. In 1995 to 1996, we randomly selected participants aged 60 to 90 years in strata of age (5 years) and sex from two large ongoing population-based studies.[11,12] A total of 1,077 nondemented elderly people participated in our study (overall response 63%). The study design has been described in detail.[8] The medical ethics committee of the Erasmus Medical Centre approved the study and each participant gave informed consent.

Cerebral infarcts and white matter lesions

All participants underwent MRI of the brain in 1995 to 1996. We made axial T1-, T2-weighted, and proton-density scans on 1.5 Tesla MRI scanners (MR Gyroscan, Philips, and MR VISION, Siemens). The slice thickness was 5 or 6 mm with an interslice gap of 20%.

Infarcts were rated by a single rater and were defined as focal hyperintensities on T2-weighted images, 3 mm in size or larger. Proton-density scans were used to distinguish infarcts from dilated perivascular spaces. Lesions in the white matter also had to have corresponding prominent hypointensities on T1-weighted images, in order to distinguish them from cerebral white matter lesions. Intrarater study (n=110) for detecting infarcts showed good agreement (κ=0.80).[9] We obtained a history of stroke and transient ischemic attack (TIA) by self-report, and by checking medical records in all 1,077 participants. An experienced neurologist subsequently reviewed the medical history and scans

and categorized the infarcts as silent or symptomatic. We defined silent brain infarcts as evidence of 1 or more infarcts on MRI, without a history of a (corresponding) stroke or TIA. Participants with both symptomatic and silent infarcts were categorized in the symptomatic infarct group. Twenty participants with a confirmed history of stroke had no infarcts on MRI. Three of them experienced a hemorrhagic stroke; the 17 others with ischemic (n=12) or unspecified (n=5) stroke had minor symptoms. Participants with symptomatic infarcts on MRI (n=42, 16 of whose symptoms of a TIA corresponded to the infarct)[9] and participants with a previous stroke without infarcts on MRI (n=20) were excluded from all analyses.

White matter lesions were considered present if visible as hyperintense on proton-density and T2-weighted images, without prominent hypointensity on T1-weighted scans. Two raters scored periventricular and subcortical white matter lesions separately. Periventricular white matter lesions were rated semiquantitatively (grade range 0 to 9). A total volume of subcortical white matter lesions was approximated based on number and size of lesions (volume range 0 to 29.5 mL). Both intrareader and interreader studies (n=100) showed a good to excellent agreement (κ=0.79 to 0.90, r=0.88 to 0.95).[8]

Cardiovascular risk factors

We obtained the cardiovascular risk factors by interview and physical examination from 1995 to 1996. Blood pressure was measured twice on the right arm with a random-zero sphygmomanometer. We used the average of these 2 measurements. Participants were asked to bring all prescribed drugs to the research center, where a physician checked the use. Hypertension was defined as a systolic blood pressure of 140 mmHg or over, a diastolic blood pressure of 90 mmHg or over, and/or the use of blood pressure-lowering medication. We considered diabetes mellitus to be present if the random glucose level was 11.1 mmol/L or higher, or if a person used antidiabetic medication. Serum total cholesterol was determined using an automated enzymatic procedure (Hitachi analyzer, Roche Diagnostics). Plasma total homocysteine levels were determined by fluorescence polarization immunoassay on an IMx analyzer (Abbott Laboratories).[13] The presence of atrial fibrillation was assessed by MEANS interpretation of a 12-lead ECG (ACTA ECG, ESAOTE).[14] In 4 participants, ECG was missing. The intima-media thickness was measured by longitudinal 2-dimensional ultrasound of the carotid artery. We calculated the mean common carotid artery intima-media thickness as the mean of 4 locations: the near and far wall of both the right and left common carotid artery.[15] Nineteen participants lacked the measurement of the common carotid intima-media thickness. A physician assessed participants' smoking habits using a structured questionnaire and classified smoking status as current or not.

Follow-up for incident stroke

In 1999 to 2000, we reinterviewed 787 of the participants that were alive about symp-

toms of stroke and TIA using a structured questionnaire (response rate 81%). In addition, we continuously monitored the medical records of all 1,077 participants at the general practitioner's office to obtain information on the occurrence of stroke since the last visit until January 1st, 2001. For all reported strokes, we recorded information about signs and symptoms, date of onset, duration, and hospital stay. If participants had been hospitalized for a stroke, we retrieved discharge letters and radiology reports from the hospital where they had been treated. By reviewing all available information, an experienced neurologist assessed the exact day of onset and classified the stroke. Stroke was defined as an episode of relevant focal deficits with acute onset, documented by neurological examination, and lasting for >24 hours. On the basis of radiological findings strokes were further subdivided into hemorrhagic or ischemic stroke subtypes. Follow-up was complete.

Data analysis

We used the Kaplan–Meier method to estimate the rates of stroke. The follow-up time was calculated from the date the MRI scan was made until the date of stroke, death, or end of follow-up, whichever came first. We did Cox proportional hazards regression analysis to determine whether the presence of brain lesions on MRI was predictive of subsequent stroke, by estimation of its hazard ratio (HR) and 95% CI. Adjustments were made for age and sex, and for the established stroke risk factors hypertension, diabetes mellitus, atrial fibrillation, common carotid intima-media thickness, smoking, and history of TIA.[16] Separate models were used for presence of silent brain infarcts, periventricular, and subcortical white matter lesions. For silent brain infarcts, no distinction was made between participants with 1 or more infarcts on MRI. We did a subanalysis to examine whether the risk of stroke was different between participants with >1 silent infarct and those with only 1 infarct on MRI, by comparing them both to participants without infarcts. The association with periventricular and subcortical white matter lesions was analyzed in tertiles of their distribution, and continuously if the relation was linear. Furthermore, we investigated whether silent brain infarcts and white matter lesions predicted future stroke independently of each other, by including them in one model. In addition, we repeated the above analyses after exclusion of participants with previous TIA without infarcts on MRI (n=33).

RESULTS

The baseline characteristics of the study population are shown in Table 1. Fifty-seven participants (6%) experienced at least 1 stroke during 4,260 person-years (mean follow-up 4.2 years). Six of these strokes were hemorrhagic, 42 ischemic, and in 9 the stroke subtype was unspecified. Thirty-one of these 57 participants (54%) had 1 or more silent

Table 1. Baseline characteristics of all participants who were free of stroke and symptomatic infarcts on MRI at baseline.

	All participants n = 1015
Age, yr	72 ± 7
Women	526 (52%)
Hypertension	727 (72%)
Diabetes mellitus	66 (7%)
Atrial fibrillation	28 (3%)
Mean intima-media thickness, mm	0.87 ± 0.15
Current smoking	163 (16%)
History of TIA	33 (3%)
Presence of silent brain infarcts	217 (21%)
Periventricular white matter lesions, grade	2.3 ± 2.2
Subcortical white matter lesions, mL	1.3 ± 2.8

Values are unadjusted mean ± SD or no. of participants (percentages).

brain infarcts present on MRI. They had more severe periventricular and subcortical white matter lesions.

The 4-year mortality was 7% (95% CI 6% to 9%). The overall stroke rate was 11 per 1,000 person years (95% CI 8 to 15). Thirty-one of the 217 participants (14.3%) with silent brain infarcts developed a stroke during an average follow-up of 4.2 years. The absolute risk of developing stroke within 4 years was 11.7% for participants with silent brain infarcts and 2.3% for those without. This absolute risk was 5.0 times higher (95% CI 2.7 to 9.2) for participants with 1 or more silent brain infarcts on MRI compared with the ones without, both for participants younger and older than 75 years of age (Figure). There were no statistically significant differences in stroke risk between men and women (absolute risk of developing stroke within 4 years: 4.9% for men and 3.7% for women).

The presence of silent brain infarcts more than tripled the risk of stroke after adjustment for the established stroke risk factors (Table 2). Participants with >1 silent infarct (n=76) had a higher stroke risk than those with only 1 infarct on MRI (n=141), although these risk estimates were not significantly different (age- and sex-adjusted HR 4.9, 95% CI 2.5 to 9.4, and 2.8, 95% CI 1.5 to 5.3, respectively). Both participants in the upper tertile of the distribution of periventricular white matter lesions (n=291) and subcortical white matter lesions (n=336) had an increased stroke risk, independent of other stroke risk factors (Table 2). Additional adjustment for blood pressure levels did not change the results (HR for silent brain infarcts 3.5, 95% CI 2.0 to 6.0; for the upper

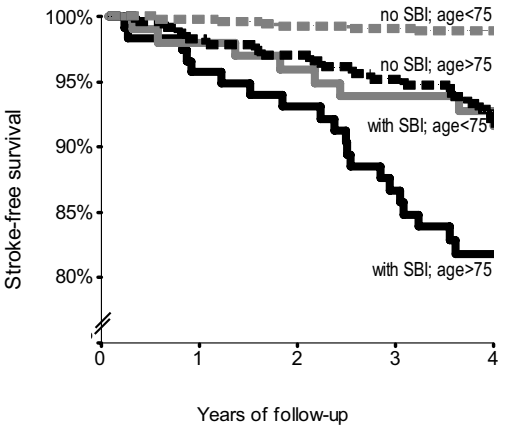

Figure. Kaplan – Meier curves of the stroke-free survival, stratified for the presence of silent brain infarcts (SBI) on MRI and age.

Table 2. Relationship between the presence of silent brain infarcts, tertiles of periventricular, and subcortical white matter lesions (WML) on MRI and the risk of stroke.

	Risk of stroke (Hazard ratio [95% CI])		
	Adjusted for age and sex	Adjusted for stroke risk factors*	Adjusted for MRI lesions†
Silent brain infarcts			
Absent	1 (reference)	1 (reference)	1 (reference)
Present	3.6 (2.1-6.1)	3.9 (2.3-6.8)	3.3 (1.8-5.9)
Periventricular WML			
1st tertile (grade 0-1.0)	1 (reference)	1 (reference)	1 (reference)
2nd tertile (grade 1.5-3.0)	2.5 (1.0-6.3)	2.5 (1.0-6.2)	2.0 (0.8-5.3)
3rd tertile (grade 3.5-9.0)	4.7 (2.0-11.2)	4.7 (2.0-11.2)	2.8 (1.0-7.6)
Subcortical WML			
1st tertile (0-0.05 ml)	1 (reference)	1 (reference)	1 (reference)
2nd tertile (0.05-0.6 ml)	2.2 (0.8-5.7)	2.3 (0.9-6.1)	1.4 (0.5-4.0)
3rd tertile (0.6-29.5 ml)	3.7 (1.5-9.1)	3.6 (1.4-9.2)	1.4 (0.5-4.0)

* Age, sex, hypertension, diabetes mellitus, atrial fibrillation, mean intima-media thickness, smoking, and history of TIA.
† Adjusted for stroke risk factors and additionally for the presence of silent brain infarcts and tertiles of periventricular and subcortical white matter lesions.

tertile of periventricular white matter lesions 4.5, 95% CI 1.9 to 10.7, and for the upper tertile of subcortical white matter lesions 3.5, 95% CI 1.4 to 8.6), nor did adjustment for the duration of hypertension or diabetes mellitus (data shown). There was no interaction between hypertension and silent brain infarcts for the risk of stroke. When silent brain infarcts, tertiles of periventricular, and subcortical white matter lesions were all included in the same model, the associations with stroke risk remained for silent brain

infarcts, but diminished for periventricular and especially subcortical white matter lesions. For subcortical white matter lesions, the risk of stroke did not increase linearly. The largest risk difference was between no and very small volumes of subcortical white matter lesions (0.05 mL). With larger volumes the stroke risk only marginally increased further. The relationship between periventricular white matter lesions and the risk of stroke was linear, and remained after adjustment for stroke risk factors (adjusted HR per grade increase of periventricular lesions 1.36, 95% CI 1.20 to 1.54), and after additional adjustment for silent brain infarcts and subcortical white matter lesions (adjusted HR 1.27, 95% CI 1.10 to 1.47). Results of above analyses were similar after exclusion of participants with previous TIA (data not shown). Additional adjustment for the less established stroke risk factors homocysteine and cholesterol levels did not change any of the associations (data not shown).

DISCUSSION

We found that elderly people with silent brain infarcts have a >3-fold-increased risk of stroke, compared with those without infarcts on MRI in the general population. The presence of more severe white matter lesions also increased stroke risk. This was independent of other established stroke risk factors and of each other.

The strengths of this study are the large number of participating elderly people and its population-based design. Furthermore, we had no losses to follow-up, and therefore no selection bias. A potential methodological limitation of our study is misclassification. Despite good agreement, we may have systematically over- or underrated infarcts or white matter lesions on MRI. We do not have pathological verification of the lesions seen on MRI. Furthermore, infarcts may have been erroneously classified as silent or symptomatic. Both the readers who identified white matter lesions and infarcts and the neurologist who classified infarcts into silent or symptomatic were blinded to all other data. Misclassification may also have occurred in the identification of strokes during follow-up. People probably underreport symptoms of TIA and minor stroke, which will have resulted in an underestimation of the true number of events. But because we obtained information about these events both by self-report and from medical records, without knowledge of baseline MRI findings, it is unlikely that this has introduced a major bias in our study. If anything, this nondifferential misclassification will have resulted in an attenuation of the relation.

We report a >3-fold-increased risk of stroke in elderly people with silent brain infarcts on MRI in the general population. This is in line with the finding of the Cardiovascular Health Study, the only other population-based study that examined this relationship.[10] This study also observed that people defined as having white matter lesions by cluster analysis had a higher risk of stroke.[17] We extended this finding and found that more

severe white matter lesions, both periventricular and subcortical located, also increased the risk of stroke. A Japanese study of healthy volunteers found an increased stroke risk when silent brain infarcts and white matter lesions were present on MRI, but this study was based on adults who wished to receive health screening at their own expense and it obtained information about 19 incident strokes by self-report only.[18] Unfortunately, numbers were too small in our study to do separate analyses for stroke subtypes.

Both silent brain infarcts, of which the majority are lacunar infarcts,[6,9] and white matter lesions reflect mainly small-vessel disease. We showed however that the increased stroke risk with the presence of silent infarcts and white matter lesions remained after adjustment for cardiovascular risk factors. These risk factors did not explain the effect of silent brain infarcts and white matter lesions on stroke risk. There may be residual confounding by the way we adjusted for these stroke risk factors, because we could not account totally for duration and severity of exposure. However, we do not think it will dispel the strong risk increase of stroke by the presence of silent brain infarcts and white matter lesions. This suggests that silent brain infarcts and white matter lesions are not just intermediates in the relation of vascular risk factors and the risk of stroke, but that these lesions might be markers for other, yet unknown, factors that lead to symptomatic stroke.

In conclusion, we found that elderly people with silent brain infarcts and white matter lesions from the general population are at a high risk of stroke. The major stroke risk factors seemed to account for only part of this increased stroke risk. Because the clinical relevance of these lesions was long unknown, no special treatment regimen has been developed for these people. The stroke risk for people with silent brain infarcts is comparable with the risk of TIA patients, of whom approximately 20% develop stroke within 4 years. Further research will have to show if treatment of these people, comparable to the treatment regimen for people with TIA, effectively prevents stroke.

References

1. Silent brain infarction in nonrheumatic atrial fibrillation. EAFT Study Group. European Atrial Fibrillation Trial. Neurology. 1996;46:159-165.
2. van Swieten JC, Kappelle LJ, Algra A, van Latum JC, Koudstaal PJ, van Gijn J. Hypodensity of the cerebral white matter in patients with transient ischemic attack or minor stroke: influence on the rate of subsequent stroke. Dutch TIA Trial Study Group. Ann Neurol. 1992;32:177-183.
3. Longstreth WT, Jr., Manolio TA, Arnold A, Burke GL, Bryan N, Jungreis CA, Enright PL, O'Leary D, Fried L. Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people. The Cardiovascular Health Study. Stroke. 1996;27:1274-1282.
4. Liao D, Cooper L, Cai J, Toole JF, Bryan NR, Hutchinson RG, Tyroler HA. Presence and severity of cerebral white matter lesions and hypertension, its treatment, and its control. The ARIC Study. Atherosclerosis Risk in Communities Study. Stroke.

1996;27:2262-2270.
5. Pantoni L, Garcia JH. Pathogenesis of leukoaraiosis: a review. Stroke. 1997;28: 652-659.
6. Longstreth WT, Jr., Bernick C, Manolio TA, Bryan N, Jungreis CA, Price TR. Lacunar infarcts defined by magnetic resonance imaging of 3660 elderly people: the Cardiovascular Health Study. Arch Neurol. 1998; 55:1217-1225.
7. Howard G, Wagenknecht LE, Cai J, Cooper L, Kraut MA, Toole JF. Cigarette smoking and other risk factors for silent cerebral infarction in the general population. Stroke. 1998;29:913-917.
8. de Leeuw FE, de Groot JC, Achten E, Oudkerk M, Ramos LMP, Heijboer R, Hofman A, Jolles J, van Gijn J, Breteler MMB. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. J Neurol Neurosurg Psychiatry. 2001;70:9-14.
9. Vermeer SE, Koudstaal PJ, Oudkerk M, Hofman A, Breteler MMB. Prevalence and risk factors of silent brain infarcts in the population-based Rotterdam Scan Study. Stroke. 2002;33:21-25.
10. Bernick C, Kuller L, Dulberg C, Longstreth WT, Jr., Manolio TA, Beauchamp N, Price TR. Silent MRI infarcts and the risk of future stroke. The cardiovascular health study. Neurology. 2001;57:1222-1229.
11. Hofman A, van Laar A, Klein F, Valkenburg HA. Coffee and cholesterol (letter). N Engl J Med. 1983;309:1248-1249.
12. Hofman A, Grobbee DE, de Jong PTVM, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-422.
13. Vermeer SE, van Dijk EJ, Koudstaal PJ, Oudkerk M, Hofman A, Clarke R, Breteler MMB. Homocysteine, silent brain infarcts, and white matter lesions: the Rotterdam Scan Study. Ann Neurol. 2002;51:285-289.
14. de Bruyne MC, Kors JA, Hoes AW, Kruijssen DACM, Deckers JW, Grosfeld M, van Herpen G, Grobbee DE, van Bemmel JH. Diagnostic interpretation of electrocardiograms in population-based research: computer program research physicians, or cardiologists? J Clin Epidemiol. 1997;50: 947-952.
15. Bots ML, van Swieten JC, Breteler MMB, de Jong PTVM, van Gijn J, Hofman A, Grobbee DE. Cerebral white matter lesions and atherosclerosis in the Rotterdam Study. Lancet. 1993;341:1232-1237.
16. Goldstein LB, Adams R, Becker K, Furberg CD, Gorelick PB, Hademenos G, Hill M, Howard G, Howard VJ, Jacobs B, Levine SR, Mosca L, Sacco RL, Sherman DG, Wolf PA, del Zoppo GJ. Primary prevention of ischemic stroke: A statement for healthcare professionals from the Stroke Council of the American Heart Association. Stroke. 2001;32:280-299.
17. Longstreth WT, Jr., Diehr P, Beauchamp NJ, Manolio TA. Patterns on cranial magnetic resonance imaging in elderly people and vascular disease outcomes (letter). Arch Neurol. 2001;58:2074.
18. Kobayashi S, Okada K, Koide H, Bokura H, Yamaguchi S. Subcortical silent brain infarction as a risk factor for clinical stroke. Stroke. 1997;28:1932-1939.

6 General discussion

The objective of this thesis was to gain more insight in the etiology of cerebral small vessel disease in elderly people. I have described the frequency, severity and progression of cerebral small vessel disease, its potential causes, and its association with stroke. In this chapter, I will summarize the main findings and discuss some of the general methodological issues. I will review the results in the broader perspective of the presumed pathophysiology of cerebral small vessel disease and discuss their clinical relevance. Finally, I will provide suggestions for further research.

MAIN FINDINGS

Frequency, distribution and progression of cerebral small vessel disease

White matter lesions and lacunar infarcts are frequently observed on CT and MRI scans of healthy elderly people, as well as in patients with dementia and stroke (chapter 2). The frequency of these lesions varies widely among studies due to differences in methodology and risk factor profiles. In the Rotterdam Scan Study, almost all participants had periventricular or subcortical white matter lesions and about one-fifth had lacunar infarcts on MRI. These numbers are in accordance with those from the Cardiovascular Health Study, a methodologically comparable study in the USA.[1,2] Given the high prevalence of white matter lesions, it may be more informative to describe its severity distribution, i.e. the frequencies of more severe white matter lesions (figure 1, chapter 2). We found that the presence and severity of white matter lesions and lacunar infarcts strongly increased with age. Silent lacunar infarcts were more common in women.[3] Women also tended to have more severe white matter lesions than men,[4] in line with other studies.[1,2,5]

Periventricular and subcortical white matter lesions showed progression in one-third of the participants within 3-years. Ten percent of all participants had marked white matter lesion progression (chapter 4.1). The Austrian Stroke Prevention Study reports any white matter lesion progression in 18% and marked progression in 8% of their participants within 3 years.[6] These participants were healthier and younger compared to ours. Marked subcortical white matter progression predominantly consisted of growth and confluence of lesions, whereas minor progression mostly consisted of new small lesions. The extent of progression of both periventricular and subcortical white matter lesions was, similar to the presence of lesions at baseline, strongly related to age. Women had a higher risk of marked subcortical white matter lesion progression than men. In our study, 14% of the people had one or more new infarcts on the second MRI, of which the vast majority were lacunar infarcts.[7] Incidence of lacunar infarcts strongly increased with age, but was not different between men and women. In the Cardiovascular Health Study, 18% of the people without a prevalent infarct had new infarcts on MRI after 5 years follow-up.[8] Over 90% of these infarcts were lacunar infarcts.

Risk factors

Blood pressure and hypertension

Higher systolic and diastolic blood pressure levels were associated with more severe periventricular and subcortical white matter lesions (chapter 3.1). The same relationship was found for concurrently and previously (on average 6 years earlier) assessed blood pressure levels. Increase in blood pressure over time was strongly related with periventricular white matter lesions in particular. We did not observe large differences in these associations among nine European cohorts. Higher blood pressure was also related to the progression of white matter lesions (chapter 4.1). People who were successfully treated for hypertension had a lower risk of severe white matter lesion compared to those with uncontrolled hypertension (chapter 3.1). These data confirm the eminent role of high blood pressure and are in line with the observation that longstanding hypertension is the main risk factor in the development of white matter lesions.[9]

A clear decrease in diastolic blood pressure over time approximately doubled the risk of severe periventricular white matter lesions (chapter 3.1). The observation among nine pooled European cohorts that both an increase and a decrease in diastolic blood pressure is related to severe white matter lesions, confirms earlier findings in the Rotterdam Scan Study.[10] We found that in people with severe white matter lesions at baseline, higher blood pressure was no longer a risk factor for white matter lesion progression (chapter 4.1). This is in agreement with an earlier finding that blood pressure is not related to incident silent brain infarcts in people with prevalent silent brain infarcts.[7]

Homocysteine

Plasma homocysteine levels are associated with silent brain infarcts and white matter lesions (chapter 3.2). The relationship between these MRI lesions and homocysteine levels was continuous. People within the top quintile of homocysteine had 3 times the risk of either silent brain infarcts or severe white matter lesions compared to those in the lowest quintile. Other studies have confirmed these observations.[11-13]

Arterial oxygen saturation and chronic obstructive pulmonary disease

We found that people with COPD or lower arterial oxygen saturation had more severe periventricular white matter lesions, but not subcortical white matter lesions or lacunar infarcts (chapter 3.3). However, we did not find an association with the progression of periventricular white matter lesions.

Plasma amyloid β

Plasma $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels are strongly associated with white matter lesions and lacunar brain infarcts in people who carry an APOE ε4 allele, while in non-carriers there were no or only weak associations (chapter 3.4.1).

Additionally, we found that in the elderly participants of the Rotterdam Study impaired cerebral vasomotor reactivity, as measured with transcranial Doppler ultrasonography, is related to higher plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ levels measured on average 6.5 years earlier, but not to concurrent Aß levels (chapter 3.4.2). Furthermore, a decrease in plasma $A\beta_{1-40}$ levels over time is associated with a higher prevalence of impaired vasomotor reactivity.

Cigarette smoking

Current cigarette smoking was associated with an almost 3 times increased risk of marked white matter lesion progression compared to never smoking (chapter 4.1). People who formally smoked cigarettes did not have a higher risk of white matter lesion progression compared to people who never smoked. As in other studies, smoking was not associated with lacunar infarcts in our population.[2,14]

Inflammation

Higher CRP levels were associated with baseline severity and progression of white matter lesions, and in particular with marked lesion progression (chapter 4.2). These associations were independent of cardiovascular risk factors and carotid atherosclerosis. Higher CRP levels showed a non-significant trend toward more lacunar infarcts at baseline and new lacunar infarcts during follow-up.

Diabetes

We did not observe an association between diabetes and white matter lesions (chapter 4.1). People with diabetes, who did not have an infarct at baseline had a 3 times increased risk of an incident silent brain infarct. Other population-based studies did not observe an association between diabetes and cerebral small vessel disease either.[2,6,8,15-17]

Small vessel disease and the risk of lesion progression and stroke

Elderly people with silent brain infarcts or severe white matter lesions had a more than 3-fold increased risk of stroke, which could not be explained by established stroke risk factors (chapter 5). This is in line with observations from the Cardiovascular Health Study and the Atherosclerosis Risk In Community study.[18,19]

People with lacunar infarcts had about 6 times the risk of having marked progression of periventricular or subcortical white matter lesions within 3 years, compared to those without infarcts (chapter 4.1). The strongest predictor of progression of white matter lesions was the severity of white matter lesions at baseline. Progression of white matter lesions was strongly related to new infarcts on MRI and incident stroke.[20] The Cardiovascular Health Study reported that in people without infarcts on MRI, white matter lesions severity was the strongest predictor of incident lacunar infarcts.[8]

METHODOLOGICAL CONSIDERATIONS

We aimed to study the etiology of cerebral small vessel disease. The success of identifying causes depends on the precision of measurements, the validity of the results and the possibility of inference from these results. In the previous chapters, I have discussed the methodological issues of our studies separately. Here I would like to discuss the main issues in a more general perspective. I will do so on the basis of principles described in detail by Rothman and Greenland.[21]

Precision of measurements

Precision of measurements involves minimizing random error. Random error may result either in imprecise effect estimates (broad confidence intervals) or in underestimated effects (regression dilution bias). The latter occurs in particular when one investigates associations with determinants that have a large within-person variability such as blood pressure levels. We reduced random error first by having a large overall sample size. Next, we sampled participants in strata of age and sex to obtain equal precision over the entire age and sex distribution. Third, we optimized the accuracy of individual measurements by repeating the majority of the measurements of both the determinants and the outcome and subsequently using the average values. Finally, we used tools with a high reproducibility and also designed a new rating method to more precisely measure change in white matter lesions seen on MRI.[22]

Despite these efforts to optimize the measurements, precision could have been further improved. Double assessment of blood sample level could prevent underestimation of the risk association, for instance for homocysteine up to 10 to 15%.[23] Automated white matter lesions detection with volumetric measurement could reduce measurement error in the outcome assessment. Extensive periventricular and subcortical white matter lesions on MRI are sometimes hard to distinguish just as lacunar infarcts, microbleeds and widened Virchow-Robin spaces.[24,25] Pathological confirmation of brain lesions on MRI could in theory further improve precision.

Validity of results

Validity of results involves restricting systematic error or bias. Three general types of bias can be identified: selection bias, information bias and confounding.

Selection bias

Selection bias results from the selective (non-)participation of people who have a different association between a determinant and an outcome compared to those who participate in the study. A population-based sample greatly reduces the potential for selection bias. Unlike most clinical-based samples, inclusion of persons in our study was random and therefore not distorted by determinants related to disease. However,

the response at baseline and at follow-up was not complete. People that did not participate at baseline were older and had a higher frequency of hypertension in the past than participants. Since age, hypertension, white matter lesions and lacunar infarcts predict stroke, depression, and dementia independently of each other, older people with hypertension and severe brain lesions may have refused to participate more often than people with a higher age and hypertension without severe brain lesions.[18-20,26,27] This would have resulted in an underestimation of the effects of age and hypertension on cerebral small vessel disease. Selection at follow-up may have also occurred and therefore risk estimates in relation to progression of lesions could have been underestimated even further. As it is not possible to correct for selection bias in the analyses, the only remedy is a maximal response rate. Our response rate at baseline was equal to that of the Cardiovascular Health Study, and higher than in most other studies (chapter 2). The relatively demanding MRI procedure and the high mortality in elderly people, inherent to this kind of research, make some selection almost unavoidable.

Information bias

Information bias results from measurement error of the determinant that depends on the outcome or of the outcome that depends on the determinant. In our studies, the assessments of the risk factors, white matter lesions, lacunar infarcts and incident strokes were performed independently and blindly from each other. Therefore, it is very unlikely that misclassification would have been differential and hence would have resulted only in random error. The relation between baseline periventricular and subcortical white matter lesions and its progression could, however, have been biased. Therefore, we blinded for scanning date in half of the study and assessed the effect of non-blinding. We concluded that non-blinding had not introduced bias.[22]

Confounding

Confounding results from confusion of the effect under study with that of an extraneous factor. A confounder is associated with both the determinant and the outcome and is not an intermediate factor in the causal pathway leading to the outcome variable under study. In our studies, we dealt with confounding by adjusting for potential confounders in multivariate regression models. Adjusting for an intermediate factor would result in an underestimation of effects.

In some instances a factor could be both a confounder and an intermediate factor. White matter lesion severity at baseline is related both to blood pressure at baseline and progression of white matter lesions and could therefore potentially confound the relationship between blood pressure and white matter lesion progression. Higher blood pressure most likely leads to white matter lesion progression through the continuation of a process that has resulted in the brain lesions at baseline, with possibly an acceleration or deceleration of the ongoing pathological process. Therefore, adjusting for base-

line white matter lesions would be equal to adjusting for a factor in the causal pathway, resulting in over adjusted estimates.[28]

Since white matter lesion and lacunar infarcts largely result from the same underlying pathological substrate, they should not be treated as confounders. Taking them together in one variable, as in the analyses with homocysteine, gives stronger and most likely better results. Whether lacunar brain infarcts and white matter lesions are related to incident stroke, is not purely an etiological question. The brain lesions per se will not cause stroke, but rather are indicators of a combination of exposure to stroke risk factors, the duration of this exposure, susceptibility to risk factors and possible interactions between these risk factors. The purpose of adjusting here is to estimate the independent association with incident stroke, which has a prognostic value and reflects, at least partly, the shared pathophysiology.

Causal inference

A precisely measured association between a factor and an outcome that is free of bias, does not imply that the observed association is a causal one. There has been much philosophical debate on causal inference which has not lead to one set of "hard-and-fast rules of evidence" to judge causation. Despite criticism, inductively oriented criteria are useful in evaluating causality.[29] Probably the most important criterion for causality is establishing a temporal relationship. Several of the studies (Chapter 3.2, 3.3 and 3.4.1) had a cross-sectional design, leaving the possibility that the proposed determinant is in fact the cause of the disease. A more biologically plausible explanation in these studies is that the determinants reflect their past levels, which are responsible for the association with the outcome. Other studies had a longitudinal design with repeated measurement of the determinant (Chapter 3.1 and 3.4.2). This reduced the possibility of inverse relations. However, since we do not know whether white matter lesions, lacunar infarcts and impaired vasomotor reactivity were already present in the past, inverse causality is still possible. Finally, we performed studies with a longitudinal design with repeated measurement of the outcome or with incident disease as the outcome (Chapter 4.1, 4.2 and 5), in which confusion of cause and effect is unlikely.

Among other criteria, in my opinion, causality should be further judged mainly on the basis of consistency of findings, their biological plausibility within the framework of current knowledge and on experimental data (i.e. randomized trials).

ETIOLOGY OF CEREBRAL SMALL VESSEL DISEASE

We studied the etiology of white matter lesions and lacunar infarcts on MRI scans. These MRI abnormalities correspond to a variety of pathological lesions. Lacunar infarcts are subcortical located ischemic infarcts of less than 20 mm in diameter.[30] Severe

irregular white matter lesions correspond to incomplete infarction with demyelination, loss of oligodendrocytes and nerve fibers, and reactive astrocytic gliosis. Small subcortical white matter lesions and smooth periventricular white matter lesions correspond to moderate changes with rarefaction and palor of myelin sheets, mild edema and widened Virchow-Robin spaces.[31-35]

In elderly people, these lesions result mostly from damage of the long penetrating medullary arteries and the short perforating arterial branches of the choroidal and striatal arteries that nourish the cerebral white matter and deep cerebral nuclei.[30-34,36-38] Damage of these vessels could lead to brain lesions by ischemia or by disturbances in the blood-brain barrier with leakage of fluid and macromolecules.[35,39,40] Since risk factors for lacunar infarcts and white matter lesions may be involved in either vessel damage or in the process from vessel damage to brain lesions, I will discuss them separately. Finally, I will briefly point at genetic factors in relation to cerebral small vessel disease.

Damage to small vessels

Different pathologies may affect the cerebral small vessels. The most frequently observed are micro-atheroma at the origin of perforating arterioles resembling large vessel atherosclerosis, concentric hyaline wall thickening of smaller distal arterioles with loss of smooth muscle cells, fibrinoid necrosis, amyloid angiopathy and elongation and tortuosity of long penetrating arterioles.[35,39]

These pathological features are all more common in older age. In our study, as in most other studies, age was strongly related to white matter lesions and lacunar infarcts. Since almost all people of 60 years and over have any white matter lesions on MRI (figure 2, Chapter 2), these lesions may to some extent be regarded to as "normal aging". Higher age is related to longer exposure to risk factors and their possible interactions. Furthermore, adaptive and compensating mechanism may fail with older age and hence increase susceptibility to disease.[41]

Higher blood pressure levels, higher homocysteine levels, and smoking were risk factors for cerebral small vessel disease in our study. These risk factors are involved in damage to vascular endothelial cells, which is the initial step in the process of atherosclerosis. Atherosclerosis principally occurs in large and medium-sized arteries but micro-atheroma may also be present in the most proximal parts of the small cerebral vessels. Inflammatory processes are an integral part of atherosclerosis. Increased CRP levels are found in different stages of atherosclerosis.[42,43] We observed that higher CRP levels are related to the presence and progression of small vessel disease independent of carotid atherosclerosis.

In people with chronic hypertension, vascular smooth muscle cells and elastic lamina of distal arterioles are replaced by fibrohyaline material and depositions of components of extracellular matrix, chiefly collagens.[44] Furthermore, cerebral arterioles may undergo structural remodeling, resulting in elongation and tortuosity of the arteries. This

remodeling may lead to widening of the perivascular or Virchow-Robin spaces, visible on MRI as "l'etat crible".[24,25,45]

We observed that plasma amyloid ß combined with the APOE ε4 allele is related to cerebral small vessel disease. Soluble amyloid ß can be internalized by smooth muscle cells and form insoluble fibrils, in particular in people who carry an APOE ε4 allele.[46-48] This fibrillized amyloid ß replaces smooth muscle cells, just like hyaline in reaction to hypertension, a process called cerebral amyloid angiopathy. These changes make the vessels stiff and fragile.[49] Cerebral white matter lesions and infarcts are common in patients with cerebral amyloid angiopathy.[50-52]

Endothelial cells of the cerebral vessels have, apart from providing a non-thrombogenic surface between blood and vessel wall, two other important functions, namely the blood-brain barrier and autoregulation of cerebral blood flow. Homocysteine and soluble amyloid ß may, next to cytotoxic effects, also directly impair cerebral autoregulatory function, by initiating an endothelial inflammatory response that interferes with the main vaso-active substances derived by the endothelium, nitric oxide and endothelin.[11,53-57] We found a relationship between impaired cerebral vasomotor reactivity and soluble amyloid ß levels assessed 6.5 years earlier, but not with levels concurrently assessed. These observations are more compatible with a cytotoxic effect of amyloid ß on smooth muscle cells than a direct vaso-active effect.

Women tended to have more small vessel disease than men. For atherosclerosis and myocardial infarction the reverse was found. Women selectively survive the period in which myocardial infarction is the main killer in men. Single risk factor exposure in women at midlife versus multiple risk factor exposure in men may underlie this difference. Postmenopausal hormonal and metabolic status may be a factor as well.[58,59] No interaction between risk factors and sex and cerebral small vessel disease was observed in our studies.

Consequences of damaged small vessels

Occlusion of arteries by localized micro-atheroma at the origin of perforating arterioles may be responsible for isolated, larger lacunar infarcts.[30,60] Inflammation may play a part in (micro-) atherosclerotic plaque rupture responsible for acute vessel occlusion, as in coronary syndromes.[42,43]

Critical stenosis and tortuous elongation of vessels result in increased vascular resistance. Loss of smooth muscle cells, stiffening of the vessels, and endothelial dysfunction lead to a disturbed autoregulation. Both could result in chronic or intermittent hypoperfusion and consequently in white matter lesions and probably multiple small lacunar infarcts. Periods of systemic hypotension due to aggressive blood pressure lowering treatment, orthostasis, cardiac failure, atrial fibrillation or dehydration may worsen cerebral hypoperfusion in people with a disturbed autoregulation. This in line with our observation that a decrease in blood pressure over time was associated with

more severe white matter lesions. Furthermore, a high blood pressure was not related to progression of white matter lesions and incident lacunar infarcts in people with severe white matter lesions or prevalent infarcts. Furthermore, a high blood viscosity due to inflammation or pro-trombotic agents increases vascular resistance and hence further decreases cerebral perfusion.[61-65]

Penetrating and medullary arterioles lack anastomoses. They are end-arteries that form an arterial border zone. Only the direct subcortical U-fibers that are spared from white matter lesions have a collateral circulation. Usually the border zone is located 3-10mm from the ventricular wall.[36,66] We observed stronger relationships between risk factors and periventricularly located white matter lesions than with subcortically located white matter lesions. The periventricular white matter may be particularly sensitive to autoregulation disturbances and low blood pressure.[67-69]

Oligodendrocytes are highly vulnerable to hypoperfusion.[66] A longer duration of hypoperfusion would be just enough for white matter lesions to progress. The oxygen extraction rate is increased in areas with severe white matter lesions as measured with positron emission tomography, reflecting compensation of altered cerebral blood flow.[70,71] We found that a lower arterial oxygen saturation was associated with periventricular white matter lesions. Low oxygen content in the blood may aggravate the consequences of hypoperfusion by failure to compensate decreased blood flow by increased oxygen extraction.[72]

Endothelial damage may result in loss of the blood-brain barrier and thus, in plasma protein extravasation and reactive astrogliosis, microgliosis and inflammation with secondary release of neuro-toxic molecules that might injure adjacent brain parenchyma.[40,73] Edema and inflammation are visible as white matter lesions on MRI.

The progression of white matter lesions from punctate to large confluent lesions is probably a continuous process, which has an exponential rather than a linear relation with time. People with no or only a few small lesions show no or minor progression within 3 years, whereas a large proportion of the people with large lesions at baseline show marked progression. The first small lesions probably indicate the start of small vessel damage. Growth and confluence of lesions is the combination of new vessel damage, continuation of existing hypoperfusion and failure of compensatory mechanisms. This is possibly further enhanced by inflammation and up-regulation of amyloid ß production as a consequence of ischemia.[74-77] Therefore, the development of white matter lesions will accelerate over time. The Austrian Stroke Prevention Study with follow-up MRI scans at 3 and 6 years after baseline showed such an exponential development of white matter lesions.[78]

Genetic factors

Monogenic disorders, such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) and various forms of familial cerebral

amyloidosis, that are both phenotypically characterized by cerebral small vessel disease, do not play a role in population-based studies in elderly people. These diseases are rare, their onset is for the most part well before the age of 60 years and their prognosis is poor.

Genetic polymorphisms may contribute to cerebral small vessel disease. Associations with polymorphisms in a variety of candidate genes have been investigated in relation to stroke.[79] In this thesis, we described an interaction between the APOE polymorphism and higher amyloid beta levels in relation to cerebral small vessel disease. We did not observe an association with the ACE I/D polymorphism and white matter lesions and infarcts,[80] although previous studies did.[81]

CLINICAL IMPLICATIONS AND TREATMENT

We found that people with silent (lacunar) brain infarcts and severe cerebral white matter lesions on MRI have more than a 3-fold increased risk of stroke. These people also have an increased risk of dementia, depression and death.[8,18,19,27,82-84] Furthermore, people with cerebral small vessel disease perform worse on cognitive tests and more often have depressive symptoms, gait disturbances and urine incontinence.[85-88] Accordingly, these lesions are not "silent" and lead to a poor prognosis. Several questions emerge. How can we prevent the development of these lesions and how should we treat people with lacunar infarcts and severe white matter lesions to prevent lesion progression and the occurrence of stroke, dementia, depression and death?

To prevent the development of cerebral small vessel disease, modifiable risk factors should be treated. We observed that people with hypertension have a higher risk of severe white matter lesions than people without. Moreover, people with successfully treated hypertension had a lower risk of severe white matter lesions than people with uncontrolled hypertension. People should be treated for hypertension as early as possible, since mid-life blood pressure and duration of hypertension are strong predictors of late life disease.[9,10,89,90] The optimal blood pressure level in elderly people may be different from that used in blood pressure lowering trials. These trials mostly include younger people. We found that in elderly people a strong decrease in diastolic blood pressure was related to more severe white matter lesions. Data from the Rotterdam Study show that a low diastolic blood pressure, among people that use blood pressure lowering drugs, is a risk factor for stroke.[91] The Syst-Eur trial indicated that blood pressure lowering by calcium-antagonists may prevent dementia in people over the age of 60 years with systolic hypertension.[92] However, the number of cases in the trial was very small, due to preliminary ending. Whether or not the protective effect was through blood pressure lowering is debated since another trial using diuretics and beta-blockers was negative.[93]

We found that an elevated homocysteine level is a risk factor for small vessel disease.

Homocysteine is also a risk factor for cognitive decline, stroke and dementia.[94-96] Trials have shown that vitamines B6 and B12 as well as folate supplements, reduce homocysteine levels.[97] New trials are underway to study the preventive effect of homocysteine lowering by vitamin suppletion on stroke.[98,99] An important advantage of this treatment is that it is unlikely to have side effects.

We observed that individuals who smoke have an increased risk of white matter lesions and those with diabetes have an increased risk of silent brain infarcts. No effects of hypercholesterolemia on white matter lesions or lacunar infarcts was found. Apart from specific goals in cerebral small vessel disease, prevention should be aimed at cardiovascular disease in general. For primary prevention of stroke, myocardial infarction and peripheral arterial disease, I refer to published guidelines.[100]

Whether people with present non-overt cerebral small vessel disease need extra medical attention is unknown. The clinical relevance of these lesions has not been recognized. General screening of the elderly population with MRI seems to be a very expensive and laborious way of identifying these people. However, in the future, it might be part of a "late-life check-up". In elderly people with cognitive dysfunction, depressive symptoms, gait disturbances or urine incontinence, screening for cerebral small vessel disease may be useful. What additional treatment could be effective to prevent stroke, depression, dementia and progression of cerebral small vessel disease in these people?

Aspirin is part of the standard therapy in TIA patients, who have a similar risk of stroke as people with silent brain infarcts or severe white matter lesions. TIAs, however, may apart from small vessel disease also be caused by large vessel atherosclerosis or atrial fibrillation.[101,102] It is uncertain whether people with cerebral small vessel disease benefit less from aspirin. Two trials showed a beneficial effect of anti-platelet therapy in people with lacunar infarction.[103,104] On the other hand, bleeding complication may be more common in people with small vessel disease, as observed in trials with oral anticoagulants.[105]

The PROGRESS trial shows that the combination therapy of an ACE-inhibitor and a diuretic gives a clear decrease in blood pressure and prevents stroke and myocardial infarction among both hypertensive and non-hypertensive patients with a history of stroke or TIA.[106] So blood pressure lowering treatment may be effective in prevention of stroke and myocardial infarction also in normotensives. It is however unknown whether the positive results of the combination therapy are generalizable to elderly people with cerebral small vessel disease.

Severe white matter lesions, reflecting areas of incomplete infarction, are in a way comparable with the penumbra of large territorial infarcts. In these people, cerebral perfusion is critical and depends solely on the systemic blood pressure. Aggressive blood pressure lowering strategies increase the risk of ischemia and may outweigh the beneficial effects of a lower blood pressure. Neuro-protection and vasodilatation in chronic hypoperfusion may be beneficial. Nimodipine, a calcium channel-blocker, has these

properties and prevents small vessel disease in experimental animals.[107] In humans, it has been proven protective in vasospasm after subarachnoid hemorrhage.[108] Further, in post-hoc subgroup analysis, nimodipine seems to have a beneficial effect on cognitive performance in people with dementia due to small vessel disease.[109] Memantine, an N-methyl-D-aspertate receptor antagonist, also has neuro-protective properties and seems to be effective and safe in those with mild-to-moderate vascular dementia.[110]

Statins (HMG-CoA reductase inhibitors) have, apart from their cholesterol lowering properties, characteristics that might be useful in people with cerebral small vessel disease. Statins reduce the levels of amyloid ß, stabilize atherosclerotic plaques, and may be anti-inflammatory and neuro-protective.[100,111] They also seem to increase cerebral blood flow and vasomotor reactivity by up-regulation of endothelium derived nitric oxide.[112,113]

Inflammatory processes seem to play a role in progression of white matter lesions as well as in atherosclerosis and Alzheimer's disease. Non-steroidal anti-inflammatory drugs are currently being studied for Alzheimer's disease and the initial results are positive.[114,115] They might be effective in the prevention of small vessel disease progression as well.

Until now, no randomized trial has specifically addressed the treatment of people with small vessel disease to prevent stroke, depression and dementia in late life. Because cerebral small vessel disease, stroke, depression and dementia are common disorders in elderly people, it should be involved in future research. Trials in patients with dementia probably due to small vessel disease (Subcortical Ischemic Vascular Dementia), are underway and the results may be generalizable to people with small vessel disease who are not yet demented.[109] However, in dementia, the damage may already be done and prevention may be too late.

FUTURE RESEARCH

The etiology of cerebral small vessel disease is complex. Risk factors for small vessel disease on MRI could be involved in either small vessel damage (e.g. hypertension) or in the process from damaged small vessel to brain tissue damage (e.g. hypotension). Hence different risk factors may have effects at different disease stages. Furthermore, different pathophysiological mechanisms may lead to small vessel damage (hypertension related versus amyloid angiopathy) and to brain tissue damage (ischemia versus disturbed blood brain barrier). The same white matter lesions on MRI could reflect different stages of tissue damage (mild edema versus demyelination and axonal loss). The relationship of these brain lesions with dementia, depression and stroke is even more complex. Future research should be aimed at unraveling this complexity.

More advanced imaging techniques are now available to study the progression

of white matter lesions. Repeated imaging with thinner slices in combination with a valid automated lesion detection tool could quantify the total change in lesion volume more precisely. Further, recent advances in MRI techniques could contribute greater insight into the underlying pathology in vivo than the conventional fast-spin echo MRI sequences. Diffusion-weighted MRI can differentiate between new and old lesions, magnetization-transfer MRI can assess the integrity of neuronal tracts and gradient echo sequences can detect microbleeds and distinguish them from ischemic lacunar infarcts. The correlation between these new MRI techniques and pathology should be studied.

Combinations of anatomical images and functional images with data on perfusion, oxygen extraction, glucose metabolism, integrity of the blood-brain barrier and localization of vasomotor reactivity impairment could give new insight into the occurrence of lesions at specific locations.[116] Furthermore, these combinations could help to elucidate the interaction and temporal relationship between blood pressure, autoregulation disturbances and hypoperfusion in the occurrence of severe white matter lesions.

In our studies, we were unable to assess the pathological status of the small vessels themselves. Photographs of retinal arteriolar and venular changes could be a good proxy for the cerebral small vessels.[19,117] Stratification on hypertensive retinal vasculopathy could provide new insight into the relation of blood pressure and white matter lesions.

Apart from structural changes of the cerebral small vessels, endothelial dysfunction or an endothelial inflammatory state may result in autoregulation disturbances and hence, in hypoperfusion. Both amyloid ß and homocysteine seem to have a negative influence on endothelial function by disturbing the balance between nitric oxide and endothelin.[11,53-55] The exact mechanism is unknown and warrants new research in relation to small vessel disease.

We described a potential interacting role of amyloid ß, APOE and ischemia. First, this relation needs confirmation in an independent sample. Second, it should be further studied in relation to the development of dementia. The combination of ischemic brain damage and amyloid ß and tau related neurodegeneration may result in earlier passage of the cognitive threshold of dementia than the two separately.[118] Additionally, ischemia may directly up-regulate amyloid precursor protein expression and increase amyloid ß production. On the other hand, vascular amyloid ß depositions may induce ischemia.[74,76,119] Imaging of parenchymal and vascular amyloid ß could help in studying this potential self strengthening mechanism.[120,121]

To understand the role of homocysteine in dementia, more insight is needed into how high homocysteine levels result in vascular and structural brain changes.[94,95,122] Vitamins and polymorphisms that play a role in methionine metabolism, probably underlie hyperhomocysteinemia. Their relative contribution to brain lesions, stroke and dementia is of potential interest for treatment strategies.

The predominance of severe small vessel disease in women needs further attention. Is it explained by selective survival? Or do postmenopausal metabolic and hormonal

status and genetic predisposition influence the risk of small vessel disease?[5,15,59]

Finally, the relation between different vascular and structural brain lesions on MRI with respect to a common pathophysiology and their temporal relation needs to be studied. Particularly, research is needed to clarify the complex interactions of ischemic brain infarcts, white matter lesions, general brain atrophy and temporal lobe atrophy in relation to dementia.[123-125] Not all people with lacunar infarcts or severe white matter lesions will become demented or develop a clinical manifest stroke. More knowledge is needed in order to identify those people who are at the highest risk. A larger MRI study, with more dementia and stroke cases, without selection due to an overextended follow-up, may provide new answers.

References

1. Longstreth WT, Jr., Manolio TA, Arnold A, Burke GL, Bryan N, Jungreis CA, Enright PL, O'Leary D, Fried L. Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people. The Cardiovascular Health Study. Stroke. 1996;27:1274-82.
2. Longstreth WT, Jr., Bernick C, Manolio TA, Bryan N, Jungreis CA, Price TR. Lacunar infarcts defined by magnetic resonance imaging of 3660 elderly people: the Cardiovascular Health Study. Arch Neurol. 1998;55:1217-25.
3. Vermeer SE, Koudstaal PJ, Oudkerk M, Hofman A, Breteler MM. Prevalence and risk factors of silent brain infarcts in the population-based Rotterdam Scan Study. Stroke. 2002;33:21-5.
4. de Leeuw FE, de Groot JC, Achten E, Oudkerk M, Ramos LM, Heijboer R, Hofman A, Jolles J, van Gijn J, Breteler MM. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. J Neurol Neurosurg Psychiatry. 2001;70:9-14.
5. Sawada H, Udaka F, Izumi Y, Nishinaka K, Kawakami H, Nakamura S, Kameyama M. Cerebral white matter lesions are not associated with apoE genotype but with age and female sex in Alzheimer's disease. J Neurol Neurosurg Psychiatry. 2000;68:653-6.
6. Schmidt R, Fazekas F, Kapeller P, Schmidt H, Hartung HP. MRI white matter hyperintensities: three-year follow-up of the Austrian Stroke Prevention Study. Neurology. 1999;53:132-9.
7. Vermeer SE, Den Heijer T, Koudstaal PJ, Oudkerk M, Hofman A, Breteler MM. Incidence and risk factors of silent brain infarcts in the population-based Rotterdam Scan Study. Stroke. 2003;34:392-6.
8. Longstreth WT, Jr., Dulberg C, Manolio TA, Lewis MR, Beauchamp NJ, Jr., O'Leary D, Carr J, Furberg CD. Incidence, manifestations, and predictors of brain infarcts defined by serial cranial magnetic resonance imaging in the elderly: the Cardiovascular Health Study. Stroke. 2002;33:2376-82.
9. de Leeuw FE, de Groot JC, Oudkerk M, Witteman JC, Hofman A, van Gijn J, Breteler MM. Hypertension and cerebral white matter lesions in a prospective cohort study. Brain. 2002;125:765-72.
10. de Leeuw FE, de Groot JC, Oudkerk M, Witteman JC, Hofman A, van Gijn J, Breteler MM. A follow-up study of blood pressure and cerebral white matter le-

sions. Ann Neurol. 1999;46:827-33.
11. Hassan A, Hunt BJ, O'Sullivan M, Bell R, D'Souza R, Jeffery S, Bamford JM, Markus HS. Homocysteine is a risk factor for cerebral small vessel disease, acting via endothelial dysfunction. Brain. 2004;127:212-9.
12. Matsui T, Arai H, Yuzuriha T, Yao H, Miura M, Hashimoto S, Higuchi S, Matsushita S, Morikawa M, Kato A, Sasaki H. Elevated plasma homocysteine levels and risk of silent brain infarction in elderly people. Stroke. 2001;32:1116-9.
13. Hogervorst E, Ribeiro HM, Molyneux A, Budge M, Smith AD. Plasma homocysteine levels, cerebrovascular risk factors, and cerebral white matter changes (leukoaraiosis) in patients with Alzheimer disease. Arch Neurol. 2002;59:787-93.
14. Yamashita K, Kobayashi S, Yamaguchi S, Koide H. Cigarette smoking and silent brain infarction in normal adults. Intern Med. 1996;35:704-6.
15. Longstreth WT, Jr., Manolio TA, Arnold A, Burke GL, Bryan N, Jungreis CA, Enright PL, O'Leary D, Fried L. Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people. The Cardiovascular Health Study. Stroke. 1996;27:1274-82.
16. Liao D, Cooper L, Cai J, Toole J, Bryan N, Burke G, Shahar E, Nieto J, Mosley T, Heiss G. The prevalence and severity of white matter lesions, their relationship with age, ethnicity, gender, and cardiovascular disease risk factors: the ARIC Study. Neuroepidemiology. 1997;16:149-62.
17. Schmidt R, Fazekas F, Hayn M, Schmidt H, Kapeller P, Roob G, Offenbacher H, Schumacher M, Eber B, Weinrauch V, Kostner GM, Esterbauer H. Risk factors for microangiopathy-related cerebral damage in the Austrian stroke prevention study. J Neurol Sci. 1997;152:15-21.
18. Bernick C, Kuller L, Dulberg C, Longstreth WT, Jr., Manolio T, Beauchamp N, Price T. Silent MRI infarcts and the risk of future stroke: the cardiovascular health study. Neurology. 2001;57:1222-9.
19. Wong TY, Klein R, Sharrett AR, Couper DJ, Klein BE, Liao DP, Hubbard LD, Mosley TH. Cerebral white matter lesions, retinopathy, and incident clinical stroke. Jama. 2002;288:67-74.
20. Prins ND, van Dijk EJ, Vrooman HA, Oudkerk M, Hofman A, Koudstaal PJ, Breteler MMB. Progression of cerebral white matter lesions and the risk of stroke, dementia and depression. submitted.
21. Rothman K, Greenland S. Basic concepts. In: Rothman KJ and Greenland S. Modern Epidemiology. Philadelphia, PA: Lippincott Williams & Wilkins, second edition,1998.
22. Prins ND, van Straaten ECW, van Dijk EJ, Simoni M, Koudstaal PJ, Scheltens P, Breteler MMB, Barkhof F. Measuring progression of cerebral white matter lesions on MRI; A comparison of visual rating scales in relation to volumetric change. Neurology (in press).
23. Clarke R, Lewington S, Donald A, Johnston C, Refsum H, Stratton I, Jacques P, Breteler MM, Holman R. Underestimation of the importance of homocysteine as a risk factor for cardiovascular disease in epidemiological studies. J Cardiovasc Risk. 2001;8:363-9.
24. Bokura H, Kobayashi S, Yamaguchi S. Distinguishing silent lacunar infarction from enlarged Virchow-Robin spaces: a magnetic resonance imaging and pathological study. J Neurol. 1998;245:116-22.
25. Jungreis CA, Kanal E, Hirsch WL, Martinez AJ, Moossy J. Normal perivascular spaces mimicking lacunar infarction: MR imaging. Radiology. 1988;169:101-4.
26. Taylor WD, Steffens DC, MacFall JR, McQuoid DR, Payne ME, Provenzale JM, Krishnan KR. White matter hyperintensity progression and late-life depression

outcomes. Arch Gen Psychiatry. 2003;60: 1090-6.
27. Vermeer SE, Prins ND, den Heijer T, Hofman A, Koudstaal PJ, Breteler MM. Silent brain infarcts and the risk of dementia and cognitive decline. N Engl J Med. 2003;348:1215-22.
28. Weinberg CR. Toward a clearer definition of confounding. Am J Epidemiol. 1993;137:1-8.
29. Hill AB. The Environment and Disease: Association or Causation? Proc R Soc Med. 1965;58:295-300.
30. Fisher CM. Lacunes: small, deep cerebral infarcts. Neurology. 1965;15:774-784.
31. Brun A, Englund E. A white matter disorder in dementia of the Alzheimer type: a pathoanatomical study. Ann Neurol. 1986;19:253-62.
32. Fazekas F, Kleinert R, Offenbacher H, Schmidt R, Kleinert G, Payer F, Radner H, Lechner H. Pathologic correlates of incidental MRI white matter signal hyperintensities. Neurology. 1993;43:1683-9.
33. Scheltens P, Barkhof F, Leys D, Wolters EC, Ravid R, Kamphorst W. Histopathologic correlates of white matter changes on MRI in Alzheimer's disease and normal aging. Neurology. 1995;45:883-8.
34. van Swieten JC, van den Hout JH, van Ketel BA, Hijdra A, Wokke JH, van Gijn J. Periventricular lesions in the white matter on magnetic resonance imaging in the elderly. A morphometric correlation with arteriolosclerosis and dilated perivascular spaces. Brain. 1991;114 (Pt 2):761-74.
35. Roman GC, Erkinjuntti T, Wallin A, Pantoni L, Chui HC. Subcortical ischaemic vascular dementia. Lancet Neurol. 2002;1:426-36.
36. De Reuck J. The human periventricular arterial blood supply and the anatomy of cerebral infarctions. Eur Neurol. 1971;5: 321-34.
37. Van den Bergh R. Centrifugal elements in the vascular pattern of the deep intracerebral blood supply. Angiology. 1969;20: 88-94.
38. Furuta A, Ishii N, Nishihara Y, Horie A. Medullary arteries in aging and dementia. Stroke. 1991;22:442-6.
39. Pantoni L, Garcia JH. Pathogenesis of leukoaraiosis: a review. Stroke. 1997;28: 652-9.
40. Wardlaw JM, Sandercock PA, Dennis MS, Starr J. Is breakdown of the blood-brain barrier responsible for lacunar stroke, leukoaraiosis, and dementia? Stroke. 2003;34:806-12.
41. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a "set up" for vascular disease. Circulation. 2003;107:139-46.
42. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med. 1999;340:115-26.
43. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med. 1997;336:973-9.
44. Lammie GA. Hypertensive cerebral small vessel disease and stroke. Brain Pathol. 2002;12:358-70.
45. Braffman BH, Zimmerman RA, Trojanowski JQ, Gonatas NK, Hickey WF, Schlaepfer WW. Brain MR: pathologic correlation with gross and histopathology. 1. Lacunar infarction and Virchow-Robin spaces. AJR Am J Roentgenol. 1988;151:551-8.
46. Urmoneit B, Prikulis I, Wihl G, D'Urso D, Frank R, Heeren J, Beisiegel U, Prior R. Cerebrovascular smooth muscle cells internalize Alzheimer amyloid beta protein via a lipoprotein pathway: implications for cerebral amyloid angiopathy. Lab Invest. 1997;77:157-66.
47. Holtzman DM, Bales KR, Tenkova T, Fagan AM, Parsadanian M, Sartorius LJ, Mackey B, Olney J, McKeel D, Wozniak D, Paul SM. Apolipoprotein E isoform-dependent amyloid deposition and neuritic

degeneration in a mouse model of Alzheimer's disease. Proc Natl Acad Sci U S A. 2000;97:2892-7.
48. Holtzman DM, Fagan AM, Mackey B, Tenkova T, Sartorius L, Paul SM, Bales K, Ashe KH, Irizarry MC, Hyman BT. Apolipoprotein E facilitates neuritic and cerebrovascular plaque formation in an Alzheimer's disease model. Ann Neurol. 2000;47:739-47.
49. Vinters HV. Cerebral amyloid angiopathy. A critical review. Stroke. 1987;18: 311-24.
50. Gray F, Dubas F, Roullet E, Escourolle R. Leukoencephalopathy in diffuse hemorrhagic cerebral amyloid angiopathy. Ann Neurol. 1985;18:54-9.
51. Cadavid D, Mena H, Koeller K, Frommelt RA. Cerebral beta amyloid angiopathy is a risk factor for cerebral ischemic infarction. A case control study in human brain biopsies. J Neuropathol Exp Neurol. 2000;59:768-73.
52. Olichney JM, Hansen LA, Hofstetter CR, Grundman M, Katzman R, Thal LJ. Cerebral infarction in Alzheimer's disease is associated with severe amyloid angiopathy and hypertension. Arch Neurol. 1995;52:702-8.
53. Fassbender K, Mielke O, Bertsch T, Nafe B, Froschen S, Hennerici M. Homocysteine in cerebral macroangiography and microangiopathy. Lancet. 1999;353: 1586-7.
54. Fassbender K, Bertsch T, Mielke O, Muhlhauser F, Hennerici M. Adhesion molecules in cerebrovascular diseases. Evidence for an inflammatory endothelial activation in cerebral large- and small-vessel disease. Stroke. 1999;30:1647-50.
55. Thomas T, Thomas G, McLendon C, Sutton T, Mullan M. beta-Amyloid-mediated vasoactivity and vascular endothelial damage. Nature. 1996;380:168-71.
56. Paris D, Town T, Parker T, Humphrey J, Mullan M. A beta vasoactivity: an inflammatory reaction. Ann N Y Acad Sci. 2000;903:97-109.
57. Niwa K, Carlson GA, Iadecola C. Exogenous A beta1-40 reproduces cerebrovascular alterations resulting from amyloid precursor protein overexpression in mice. J Cereb Blood Flow Metab. 2000;20:1659-68.
58. Hak AE. Gender differences in cardiovascular disease. An epidemiologic study of endocrine factors. Thesis, 2002.
59. Thompson J, Khalil RA. Gender differences in the regulation of vascular tone. Clin Exp Pharmacol Physiol. 2003;30: 1-15.
60. Boiten J, Lodder J, Kessels F. Two clinically distinct lacunar infarct entities? A hypothesis. Stroke. 1993;24:652-6.
61. Schneider R, Ringelstein EB, Zeumer H, Kiesewetter H, Jung F. The role of plasma hyperviscosity in subcortical arteriosclerotic encephalopathy (Binswanger's disease). J Neurol. 1987;234:67-73.
62. Breteler MM, van Swieten JC, Bots ML, Grobbee DE, Claus JJ, van den Hout JH, van Harskamp F, Tanghe HL, de Jong PT, van Gijn J, et al. Cerebral white matter lesions, vascular risk factors, and cognitive function in a population-based study: the Rotterdam Study. Neurology. 1994;44:1246-52.
63. Tomimoto H, Akiguchi I, Ohtani R, Yagi H, Kanda M, Shibasaki H, Yamamoto Y. The coagulation-fibrinolysis system in patients with leukoaraiosis and Binswanger disease. Arch Neurol. 2001;58: 1620-5.
64. Tomimoto H, Akiguchi I, Wakita H, Osaki A, Hayashi M, Yamamoto Y. Coagulation activation in patients with Binswanger disease. Arch Neurol. 1999;56:1104-8.
65. Kario K, Matsuo T, Kobayashi H, Asada R, Matsuo M. 'Silent' cerebral infarction is associated with hypercoagulability, endothelial cell damage, and high Lp(a) levels in elderly Japanese. Arterioscler Thromb Vasc Biol. 1996;16:734-41.
66. Pantoni L, Garcia JH, Gutierrez JA.

Cerebral white matter is highly vulnerable to ischemia. Stroke. 1996;27:1641-6; discussion 1647.
67. Matsushita K, Kuriyama Y, Nagatsuka K, Nakamura M, Sawada T, Omae T. Periventricular white matter lucency and cerebral blood flow autoregulation in hypertensive patients. Hypertension. 1994;23:565-8.
68. Chamorro A, Pujol J, Saiz A, Vila N, Vilanova JC, Alday M, Blanc R. Periventricular white matter lucencies in patients with lacunar stroke. A marker of too high or too low blood pressure? Arch Neurol. 1997;54:1284-8.
69. Bakker SL, de Leeuw FE, de Groot JC, Hofman A, Koudstaal PJ, Breteler MM. Cerebral vasomotor reactivity and cerebral white matter lesions in the elderly. Neurology. 1999;52:578-83.
70. De Reuck J, Santens P, Strijckmans K, Lemahieu I. Cobalt-55 positron emission tomography in vascular dementia: significance of white matter changes. J Neurol Sci. 2001;193:1-6.
71. De Reuck J, Decoo D, Marchau M, Santens P, Lemahieu I, Strijckmans K. Positron emission tomography in vascular dementia. J Neurol Sci. 1998;154:55-61.
72. Miyamoto O, Auer RN. Hypoxia, hyperoxia, ischemia, and brain necrosis. Neurology. 2000;54:362-71.
73. Lin JX, Tomimoto H, Akiguchi I, Matsuo A, Wakita H, Shibasaki H, Budka H. Vascular cell components of the medullary arteries in Binswanger's disease brains: a morphometric and immunoelectron microscopic study. Stroke. 2000;31:1838-42.
74. Bennett SA, Pappas BA, Stevens WD, Davidson CM, Fortin T, Chen J. Cleavage of amyloid precursor protein elicited by chronic cerebral hypoperfusion. Neurobiol Aging. 2000;21:207-14.
75. Popa-Wagner A, Schroder E, Walker LC, Kessler C. beta-Amyloid precursor protein and ss-amyloid peptide immunoreactivity in the rat brain after middle cerebral artery occlusion: effect of age. Stroke. 1998;29:2196-202.
76. Kalaria RN, Bhatti SU, Lust WD, Perry G. The amyloid precursor protein in ischemic brain injury and chronic hypoperfusion. Ann N Y Acad Sci. 1993;695: 190-3.
77. Suenaga T, Ohnishi K, Nishimura M, Nakamura S, Akiguchi I, Kimura J. Bundles of amyloid precursor protein-immunoreactive axons in human cerebrovascular white matter lesions. Acta Neuropathol (Berl). 1994;87:450-5.
78. Schmidt R, Enzinger C, Ropele S, Schmidt H, Fazekas F. Progression of cerebral white matter lesions: 6-year results of the Austrian Stroke Prevention Study. Lancet. 2003;361:2046-8.
79. Hassan A, Markus HS. Genetics and ischaemic stroke. Brain. 2000;123 (Pt 9): 1784-812.
80. Sleegers K, den Heijer T, Van Dijk EJ, Hofman A, Koudstaal PJ, Breteler MM, Van Duijn CM. Angiotensin converting enzyme gene is associated with Alzheimer's disease in women. Am J Hum Genet. 2003;73:Abstract 1324.
81. Hassan A, Lansbury A, Catto AJ, Guthrie A, Spencer J, Craven C, Grant PJ, Bamford JM. Angiotensin converting enzyme insertion/deletion genotype is associated with leukoaraiosis in lacunar syndromes. J Neurol Neurosurg Psychiatry. 2002;72: 343-6.
82. Kuller LH, Lopez OL, Newman A, Beauchamp NJ, Burke G, Dulberg C, Fitzpatrick A, Fried L, Haan MN. Risk factors for dementia in the cardiovascular health cognition study. Neuroepidemiology. 2003;22:13-22.
83. O'Brien J, Desmond P, Ames D, Schweitzer I, Harrigan S, Tress B. A magnetic resonance imaging study of white matter lesions in depression and Alzheimer's disease. Br J Psychiatry. 1996;168: 477-85.

84. Inzitari D, Cadelo M, Marranci ML, Pracucci G, Pantoni L. Vascular deaths in elderly neurological patients with leukoaraiosis. J Neurol Neurosurg Psychiatry. 1997;62:177-81.
85. Kuller LH, Shemanski L, Manolio T, Haan M, Fried L, Bryan N, Burke GL, Tracy R, Bhadelia R. Relationship between ApoE, MRI findings, and cognitive function in the Cardiovascular Health Study. Stroke. 1998;29:388-98.
86. Whitman GT, Tang Y, Lin A, Baloh RW, Tang T. A prospective study of cerebral white matter abnormalities in older people with gait dysfunction. Neurology. 2001;57:990-4.
87. de Groot JC, de Leeuw FE, Oudkerk M, Hofman A, Jolles J, Breteler MM. Cerebral white matter lesions and depressive symptoms in elderly adults. Arch Gen Psychiatry. 2000;57:1071-6.
88. De Groot JC, De Leeuw FE, Oudkerk M, Van Gijn J, Hofman A, Jolles J, Breteler MM. Periventricular cerebral white matter lesions predict rate of cognitive decline. Ann Neurol. 2002;52:335-41.
89. Dufouil C, de Kersaint-Gilly A, Besancon V, Levy C, Auffray E, Brunnereau L, Alperovitch A, Tzourio C. Longitudinal study of blood pressure and white matter hyperintensities: the EVA MRI Cohort. Neurology. 2001;56:921-6.
90. Carmelli D, Swan GE, Reed T, Wolf PA, Miller BL, DeCarli C. Midlife cardiovascular risk factors and brain morphology in identical older male twins. Neurology. 1999;52:1119-24.
91. Voko Z, Bots ML, Hofman A, Koudstaal PJ, Witteman JC, Breteler MM. J-shaped relation between blood pressure and stroke in treated hypertensives. Hypertension. 1999;34:1181-5.
92. Forette F, Seux ML, Staessen JA, Thijs L, Birkenhager WH, Babarskiene MR, Babeanu S, Bossini A, Gil-Extremera B, Girerd X, Laks T, Lilov E, Moisseyev V, Tuomilehto J, Vanhanen H, Webster J, Yodfat Y, Fagard R. Prevention of dementia in randomised double-blind placebo-controlled Systolic Hypertension in Europe (Syst-Eur) trial. Lancet. 1998;352:1347-51.
93. Prevention of stroke by antihypertensive drug treatment in older persons with isolated systolic hypertension. Final results of the Systolic Hypertension in the Elderly Program (SHEP). SHEP Cooperative Research Group. Jama. 1991;265:3255-64.
94. Morris MS. Homocysteine and Alzheimer's disease. Lancet Neurol. 2003;2:425-8.
95. Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, Wilson PW, Wolf PA. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med. 2002;346:476-83.
96. Prins ND, Den Heijer T, Hofman A, Koudstaal PJ, Jolles J, Clarke R, Breteler MM. Homocysteine and cognitive function in the elderly: the Rotterdam Scan Study. Neurology. 2002;59:1375-80.
97. Graham IM, Daly LE, Refsum HM, Robinson K, Brattstrom LE, Ueland PM, Palma-Reis RJ, Boers GH, Sheahan RG, Israelsson B, Uiterwaal CS, Meleady R, McMaster D, Verhoef P, Witteman J, Rubba P, Bellet H, Wautrecht JC, de Valk HW, Sales Luis AC, Parrot-Rouland FM, Tan KS, Higgins I, Garcon D, Andria G, et al. Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project [see comments]. Jama. 1997;277:1775-81.
98. Spence JD, Howard VJ, Chambless LE, Malinow MR, Pettigrew LC, Stampfer M, Toole JF. Vitamin Intervention for Stroke Prevention (VISP) trial: rationale and design. Neuroepidemiology. 2001;20:16-25.
99. The VITATOPS (Vitamins to Prevent Stroke) Trial: rationale and design of an international, large, simple, randomised trial of homocysteine-lowering multi-

vitamin therapy in patients with recent transient ischaemic attack or stroke. Cerebrovasc Dis. 2002;13:120-6.

100. Goldstein LB, Adams R, Becker K, Furberg CD, Gorelick PB, Hademenos G, Hill M, Howard G, Howard VJ, Jacobs B, Levine SR, Mosca L, Sacco RL, Sherman DG, Wolf PA, del Zoppo GJ. Primary prevention of ischemic stroke: A statement for healthcare professionals from the Stroke Council of the American Heart Association. Circulation. 2001;103:163-82.
101. Kappelle LJ, van Latum JC, Koudstaal PJ, van Gijn J. Transient ischaemic attacks and small-vessel disease. Dutch TIA Study Group. Lancet. 1991;337:339-41.
102. Kappelle LJ, van Latum JC, van Swieten JC, Algra A, Koudstaal PJ, van Gijn J. Recurrent stroke after transient ischaemic attack or minor ischaemic stroke: does the distinction between small and large vessel disease remain true to type? Dutch TIA Trial Study Group. J Neurol Neurosurg Psychiatry. 1995;59:127-31.
103. Bousser MG, Eschwege E, Haguenau M, Lefaucconnier JM, Thibult N, Touboul D, Touboul PJ. "AICLA" controlled trial of aspirin and dipyridamole in the secondary prevention of athero-thrombotic cerebral ischemia. Stroke. 1983;14:5-14.
104. Gent M, Blakely JA, Easton JD, Ellis DJ, Hachinski VC, Harbison JW, Panak E, Roberts RS, Sicurella J, Turpie AG. The Canadian American Ticlopidine Study (CATS) in thromboembolic stroke. Lancet. 1989;1:1215-20.
105. Gorter JW. Major bleeding during anticoagulation after cerebral ischemia: patterns and risk factors. Stroke Prevention In Reversible Ischemia Trial (SPIRIT). European Atrial Fibrillation Trial (EAFT) study groups. Neurology. 1999;53:1319-27.
106. Randomised trial of a perindopril-based blood-pressure-lowering regimen among 6,105 individuals with previous stroke or transient ischaemic attack. Lancet. 2001;358:1033-41.
107. de Jong GI, Jansen AS, Horvath E, Gispen WH, Luiten PG. Nimodipine effects on cerebral microvessels and sciatic nerve in aging rats. Neurobiol Aging. 1992;13:73-81.
108. Ohman J, Heiskanen O. Effect of nimodipine on the outcome of patients after aneurysmal subarachnoid hemorrhage and surgery. J Neurosurg. 1988;69:683-6.
109. Inzitari D, Erkinjuntti T, Wallin A, Del Ser T, Romanelli M, Pantoni L. Subcortical vascular dementia as a specific target for clinical trials. Ann N Y Acad Sci. 2000;903:510-21.
110. Orgogozo JM, Rigaud AS, Stoffler A, Mobius HJ, Forette F. Efficacy and safety of memantine in patients with mild to moderate vascular dementia: a randomized, placebo-controlled trial (MMM 300). Stroke. 2002;33:1834-9.
111. Fassbender K, Simons M, Bergmann C, Stroick M, Lutjohann D, Keller P, Runz H, Kuhl S, Bertsch T, von Bergmann K, Hennerici M, Beyreuther K, Hartmann T. Simvastatin strongly reduces levels of Alzheimer's disease beta -amyloid peptides Abeta 42 and Abeta 40 in vitro and in vivo. Proc Natl Acad Sci U S A. 2001;98:5856-61.
112. Endres M, Laufs U, Huang Z, Nakamura T, Huang P, Moskowitz MA, Liao JK. Stroke protection by 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors mediated by endothelial nitric oxide synthase. Proc Natl Acad Sci U S A. 1998;95:8880-5.
113. Sterzer P, Meintzschel F, Rosler A, Lanfermann H, Steinmetz H, Sitzer M. Pravastatin improves cerebral vasomotor reactivity in patients with subcortical small-vessel disease. Stroke. 2001;32:2817-20.
114. Breitner JC. NSAIDs and Alzheimer's disease: how far to generalise from tri-

als? Lancet Neurol. 2003;2:527.
115. Zandi PP, Anthony JC, Hayden KM, Mehta K, Mayer L, Breitner JC. Reduced incidence of AD with NSAID but not H2 receptor antagonists: the Cache County Study. Neurology. 2002;59:880-6.
116. Rossini PM, Altamura C, Ferretti A, Vernieri F, Zappasodi F, Caulo M, Pizzella V, Del Gratta C, Romani GL, Tecchio F. Does cerebrovascular disease affect the coupling between neuronal activity and local haemodynamics? Brain. 2004;127: 99-110.
117. Kwa VI, van der Sande JJ, Stam J, Tijmes N, Vrooland JL. Retinal arterial changes correlate with cerebral small-vessel disease. Neurology. 2002;59:1536-40.
118. Snowdon DA, Greiner LH, Mortimer JA, Riley KP, Greiner PA, Markesbery WR. Brain infarction and the clinical expression of Alzheimer disease. The Nun Study. Jama. 1997;277:813-7.
119. Kalaria RN. Small vessel disease and Alzheimer's dementia: pathological considerations. Cerebrovasc Dis. 2002;13 Suppl 2:48-52.
120. Petrella JR, Coleman RE, Doraiswamy PM. Neuroimaging and early diagnosis of Alzheimer disease: a look to the future. Radiology. 2003;226:315-36.
121. Agdeppa ED, Kepe V, Petri A, Satyamurthy N, Liu J, Huang SC, Small GW, Cole GM, Barrio JR. In vitro detection of (S)-naproxen and ibuprofen binding to plaques in the Alzheimer's brain using the positron emission tomography molecular imaging probe 2-(1-[6-[(2-[(18) F]fluoroethyl)(methyl)amino]-2-naphthyl]ethylidene)malono nitrile. Neuroscience. 2003;117:723-30.
122. Seshadri S, Wolf PA. Homocysteine and the brain: vascular risk factor or neurotoxin? Lancet Neurol. 2003;2:11.
123. Mungas D, Jagust WJ, Reed BR, Kramer JH, Weiner MW, Schuff N, Norman D, Mack WJ, Willis L, Chui HC. MRI predictors of cognition in subcortical ischemic vascular disease and Alzheimer's disease. Neurology. 2001;57:2229-35.
124. Fein G, Di Sclafani V, Tanabe J, Cardenas V, Weiner MW, Jagust WJ, Reed BR, Norman D, Schuff N, Kusdra L, Greenfield T, Chui H. Hippocampal and cortical atrophy predict dementia in subcortical ischemic vascular disease. Neurology. 2000;55:1626-35.
125. Pohjasvaara T, Mantyla R, Salonen O, Aronen HJ, Ylikoski R, Hietanen M, Kaste M, Erkinjuntti T. How complex interactions of ischemic brain infarcts, white matter lesions, and atrophy relate to poststroke dementia. Arch Neurol. 2000;57:1295-300.

7 Summary
Samenvatting

Summary

Cerebral white matter lesions and lacunar brain infarcts are frequently seen on magnetic resonance imaging (MRI) scans of elderly people. Damage of small cerebral blood vessels, also known as cerebral small vessel disease, is involved in the etiology of these lesions. There is accumulating evidence that these lesions are associated with an increased risk of gait disturbances, cognitive decline and depression at late-life. Eventually, they may either by themselves or in combination with neuro-degenerative changes, lead to dementia. In addition to clinically manifest infarcts and hemorrhages, white matter lesions and lacunar infarcts are frequently observed on brain images, suggesting a common etiology. A better understanding of the etiology of cerebral small vessel disease could contribute to the prevention of stroke, dementia and also of more subtle limitations in daily living within a rapidly increasing elderly population.

To gain more insight in the etiology of cerebral small vessel disease in elderly people, we used data from the Rotterdam Scan Study, a large ongoing population-based cohort study among 1,077 people aged 60 to 90 years. All participants underwent brain MRI in 1995 to1996, of whom 668 underwent a second MRI more than three years later. All MRI scans were rated for the presence, severity and progression of cerebral white matter lesions and lacunar brain infarcts. We distinguished periventricularly and subcortically located white matter lesions.

In **chapter 2** we review studies on the frequency of cerebral small vessel disease on CT and MRI scans in healthy elderly people, as well as in patients with dementia and stroke. The frequency varies largely among studies due to differences in methodology and risk factor profiles.

In **chapter 3** we describe the relationship between several risk factors and cerebral small vessel disease. In **chapter 3.1** we studied, within a large European collaborative study, the relationship with blood pressure, change in blood pressure, hypertension and treatment of hypertension. Higher systolic and diastolic blood pressure levels were asso-

ciated with more severe periventricular and subcortical white matter lesions. The same relationship was found for blood pressure levels assessed concurrently with the MRI and blood pressure levels assessed 6 years earlier. Both a clear increase and decrease in diastolic blood pressure over time was strongly related with periventricular white matter lesions. People who were successfully treated for hypertension had a lower risk of severe white matter lesion compared to those who had uncontrolled hypertension.

In **chapter 3.2** we show the relationship between homocysteine and cerebral small vessel disease. Homocysteine is a potential modifiable risk factor for stroke and dementia. People within the group with the 20 percent highest homocysteine levels had a 3 times increased risk of either silent brain infarcts or severe white matter lesions compared to people within the group with the 20 percent lowest levels.

Chapter 3.3 showed that people with COPD or lower arterial oxygen saturation had more severe periventricular white matter lesions, but not more subcortical white matter lesions or lacunar infarcts.

Chapter 3.4 reports on the role of plasma amyloid β (Aβ) and APOE ε4 in relation to lacunar infarcts and white matter lesions and impaired cerebral autoregulation. Plasma $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels were strongly associated with white matter lesions and lacunar brain infarcts in people who carry an APOE ε4 allele, while in non-carriers there were no or only weak associations (**chapter 3.4.1**). Impaired cerebral vasomotor reactivity, as measured with transcranial Doppler, was related to higher plasma $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels measured on average 6.5 years earlier, but not to concurrent Aβ levels. Furthermore, a decrease in plasma $A\beta_{1\text{-}40}$ levels over time was associated with a higher prevalence of impaired vasomotor reactivity (**chapter 3.4.2**).

In **chapter 4** we describe the progression of white matter lesions and the risk factors associated with this progression. In **chapter 4.1** we report that one third of the people in our study had progression of white matter lesion within 3 years. In total 10 percent had marked progression. Higher age, higher blood pressure, smoking and the presence of severe white matter lesion and lacunar brain infarcts at baseline were related to progression of white matter lesions. Women had a higher risk of marked subcortical white matter lesion progression than men. Higher CRP levels were associated with baseline severity and progression of white matter lesions, and in particular with marked lesion progression. Higher CRP levels showed a non-significant trend toward more lacunar infarcts at baseline and new lacunar infarcts during follow-up (**chapter 4.2**).

Finally, we compare the risk of stroke between people with and without cerebral small vessel disease on MRI in **chapter 5**. Elderly people with silent brain infarcts or severe white matter lesions had a more than 3-fold-increased risk of stroke, which could not be explained by established stroke risk factors.

In **chapter 6** I reflect on the main findings in the context of current knowledge and give suggestions for future research.

On the basis of our studies and current literature we presume that white matter

lesions and lacunar brain infarct develop as follows: cardiovascular risk factors, in particular hypertension, cause atherosclerosis-like changes and narrowing of the lumen of small cerebral vessels. Aβ may be involved in cerebral small vessel damage as well. Narrowing of the vessel lumen and the inability of the damaged vessels to regulate the cerebral blood pressure results in chronic ischemia of the cerebral white matter. This ischemia is made worse by drops in systemic blood pressure and possibly by low arterial oxygen saturation. The ischemic changes in the white matter are visible on MRI as white matter lesions. A total occlusion of the small cerebral vessels results in a lacunar brain infarct.

Successful treatment of hypertension and lowering of homocysteine levels probably helps to prevent cerebral small vessel disease. People with cerebral small vessel disease have an increased risk of stroke and dementia. It is yet unknown whether medication additional to that used for treatment of these risk factors is effective to prevent stroke and dementia.

Samenvatting

Cerebrale wittestofafwijkingen en lacunaire herseninfarcten worden vaak gezien op "magnetic resonance imaging (MRI) scans" van oudere mensen. Beschadiging van de kleine bloedvaten van de hersenen, ook wel cerebrale microangiopathie genoemd, speelt een rol bij het ontstaan van deze afwijkingen. In toenemende mate zijn er aanwijzingen dat deze afwijkingen gerelateerd zijn aan een verhoogd risico op cognitieve achteruitgang, loopstoornissen en depressie op latere leeftijd. Uiteindelijk kunnen ze, al dan niet in combinatie met neuro-degeneratieve veranderingen, leiden tot dementie. Wittestofafwijkingen en lacunaire herseninfarcten worden ook vaak gezien op afbeeldingen van de hersenen van mensen die kort geleden een beroerte hebben gehad. Dit zou op een gemeenschappelijke oorzaak kunnen wijzen. Een beter begrip van het ontstaan van cerebrale microangiopathie, kan bijdragen aan het voorkomen van beroerte, dementie en ook van meer subtiele achteruitgang in het dagelijks functioneren van een steeds groter wordende groep ouderen.

Om meer inzicht te krijgen in het ontstaan van cerebrale microangiopathie bij oudere mensen hebben we onderzoek gedaan binnen de Rotterdam Scan Study. Dit is een groot prospectief onderzoek onder 1077 mensen van 60 tot 90 jaar uit de algemene bevolking. Alle deelnemers ondergingen een MRI-scan van de hersenen in 1995-1996 en bij 668 van hen volgde een tweede MRI-scan meer dan drie jaar later. Alle MRI-scans werden beoordeeld op aanwezigheid, ernst en progressie van wittestofafwijkingen en lacunaire herseninfarcten. Onderscheid werd gemaakt in periventriculair en subcorticaal gelokaliseerde wittestofafwijkingen.

In **hoofdstuk 2** geven we een overzicht van studies waarin is gekeken naar de frequentie van voorkomen van cerebrale microangiopathie op CT- en MRI-scans bij zowel gezonde mensen als bij patiënten met een beroerte of dementie. De gerapporteerde frequentie varieerde enorm tussen de verschillende studies ten gevolge van verschillen in gebruikte onderzoeksmethodologie en risicoprofielen.

In **hoofdstuk 3** beschrijven we de relatie tussen verschillende risicofactoren en cerebrale microangiopathie. In **hoofdstuk 3.1** bestuderen we binnen een groot Europees samenwerkingsverband de relatie met bloeddruk, veranderingen in bloeddruk, hypertensie en de behandeling van hypertensie. Hogere systolische en diastolische bloeddrukwaarden waren geassocieerd met ernstigere periventriculaire en subcorticale wittestofafwijkingen. Deze associatie was hetzelfde zowel voor de bloeddruk gemeten ten tijde van de MRI-scan, als voor de bloeddruk gemeten zes jaar eerder. Zowel een duidelijke toename als een duidelijke afname in diastolische bloeddruk correspondeerde met een toename van het risico op ernstige periventriculaire wittestofafwijkingen. Mensen met succesvol behandelde hypertensie hadden een lager risico op ernstige wittestofafwijkingen dan mensen met niet-succesvol behandelde hypertensie.

In **hoofdstuk 3.2** laten we de relatie tussen homocysteïne spiegels en cerebrale microangiopathie zien. Homocysteïne is een mogelijk behandelbare risicofactor voor beroerte en dementie. Mensen in de groep met de 20 procent hoogste homocysteïne waarden hadden een drie keer verhoogd risico op stille herseninfarcten of wittestofafwijkingen vergeleken met mensen uit de groep met de 20 procent laagste waarden.

In **hoofdstuk 3.3** tonen we dat mensen met COPD of een lage arteriële zuurstofsaturatie een verhoogd risico hebben op ernstige periventriculaire wittestofafwijkingen, maar niet op subcorticale wittestofafwijkingen of lacunaire infarcten.

Hoofdstuk 3.4 geeft de rol van plasma amyloïd β (Aβ) en APOE ε4 weer in relatie tot lacunaire herseninfarcten, wittestofafwijkingen en gestoorde cerebrale autoregulatie. Plasma $A\beta_{1\text{-}40}$ en $A\beta_{1\text{-}42}$ spiegels waren sterk gerelateerd aan wittestofafwijkingen en lacunaire infarcten bij mensen die drager zijn van het APOE ε4 allel. Bij mensen die geen drager zijn van dit allel werd geen of alleen een zwakke relatie gevonden (**hoofdstuk 3.4.1**). Gestoorde cerebrale vasomotor-reactiviteit gemeten met transcraniële Doppler, was geassocieerd met hogere plasma $A\beta_{1\text{-}40}$ en $A\beta_{1\text{-}42}$ spiegels gemeten 6.5 jaar eerder, maar niet met spiegels die ten tijde van de transcraniële Doppler-meting waren gemeten. Verder vonden we dat een daling in de plasma $A\beta_{1\text{-}40}$ spiegels geassocieerd was met het vaker voorkomen van een gestoorde vasomotor-reactiviteit (**hoofdstuk 3.4.2**).

In **hoofdstuk 4** beschrijven we hoe de frequentie en ernst van wittestofafwijkingen veranderen in de tijd en welke risicofactoren gerelateerd zijn aan die veranderingen. Eén derde van de mensen vertoonde progressie van wittestofafwijkingen in een periode van ruim drie jaar. In totaal 10 procent had ernstige progressie. Een hogere leeftijd, hogere bloeddruk, roken en de aanwezigheid van ernstige wittestofafwijkingen en lacunaire infarcten aan het begin van de studie waren gerelateerd aan progressie van wittestofafwijkingen. Vrouwen hadden meer progressie van subcorticale wittestofafwijkingen dan mannen (**hoofdstuk 4.1**). Mensen met een hogere bloedspiegel van het ontstekingseiwit C-reactief-proteïne hadden ook meer progressie van wittestofafwijkingen. Ze hadden mogelijk ook meer lacunaire infarcten, deze resultaten waren echter niet significant (**hoofdstuk 4.2**).

Hoofdstuk 5 beschrijft het verband tussen stille, meestal lacunaire, herseninfarcten, wittestofafwijkingen en de kans op beroerte. Mensen met deze afwijkingen hebben een ruim drie keer verhoogd risico op een beroerte. Dit werd niet verklaard door de aanwezigheid van reeds bekende risicofactoren voor beroerte.

In **hoofdstuk 6** worden de belangrijkste bevindingen in de context van de bestaande literatuur besproken en worden suggesties voor verder onderzoek gedaan.

Op basis van onze studies en bestaande literatuur veronderstellen wij dat wittestofafwijkingen en lacunaire herseninfarcten als volgt ontstaan: cardiovasculaire risicofactoren en met name hypertensie veroorzaken atherosclerose-achtige afwijkingen en lumen vernauwing van de kleine bloedvaten van de hersenen. Ook Aβ kan een rol spelen in het beschadigen van de kleine cerebrale bloedvaten. Als gevolg van de vaatvernauwing en het onvermogen van de beschadigde bloedvaten om de cerebrale bloeddruk te reguleren, ontstaat er chronische ischemie van de cerebrale witte stof. Deze ischemie wordt verergerd door systemische bloeddrukdalingen en mogelijk ook door een lage arteriële zuurstof-saturatie. De ischemische veranderingen in de witte stof leiden tot het MRI-beeld van wittestofafwijkingen. Een totale afsluiting van de kleine bloedvaten kan resulteren in een lacunair herseninfarct.

Het succesvol behandelen van hoge bloeddruk en het verlagen van homocysteïne spiegels helpt waarschijnlijk bij het voorkomen van cerebrale microangiopathie. Mensen met cerebrale microangiopathie hebben een verhoogd risico op dementie en beroerte. Het is nog onbekend of bij deze mensen, naast het behandelen van risicofactoren, het geven van extra medicatie zinvol is ter voorkoming van deze ziekten.

Appendix

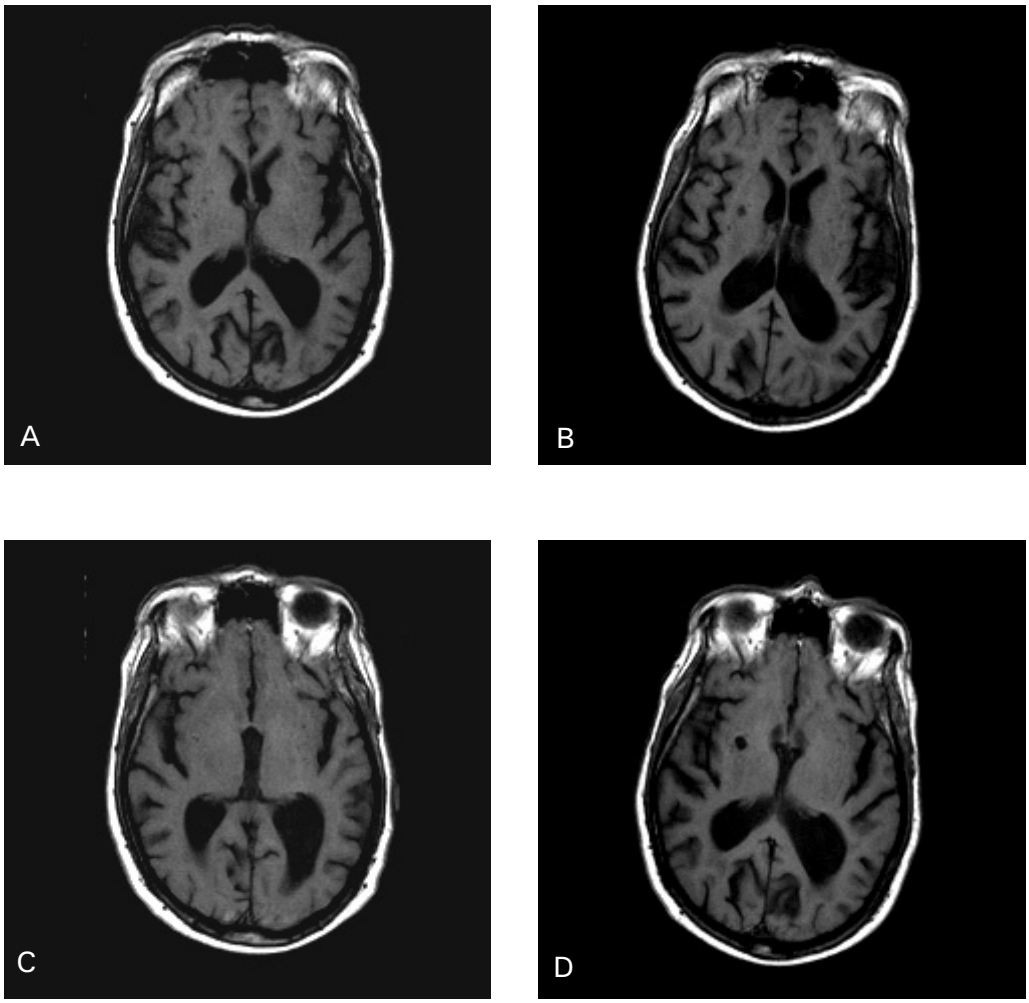

Figure 1. Incident lacunar infarct. A,C: T1-weighted spin echo MR image at baseline. B,D: T1-weighted spin echo MR image at follow-up with hypointense lesion in right putamen.

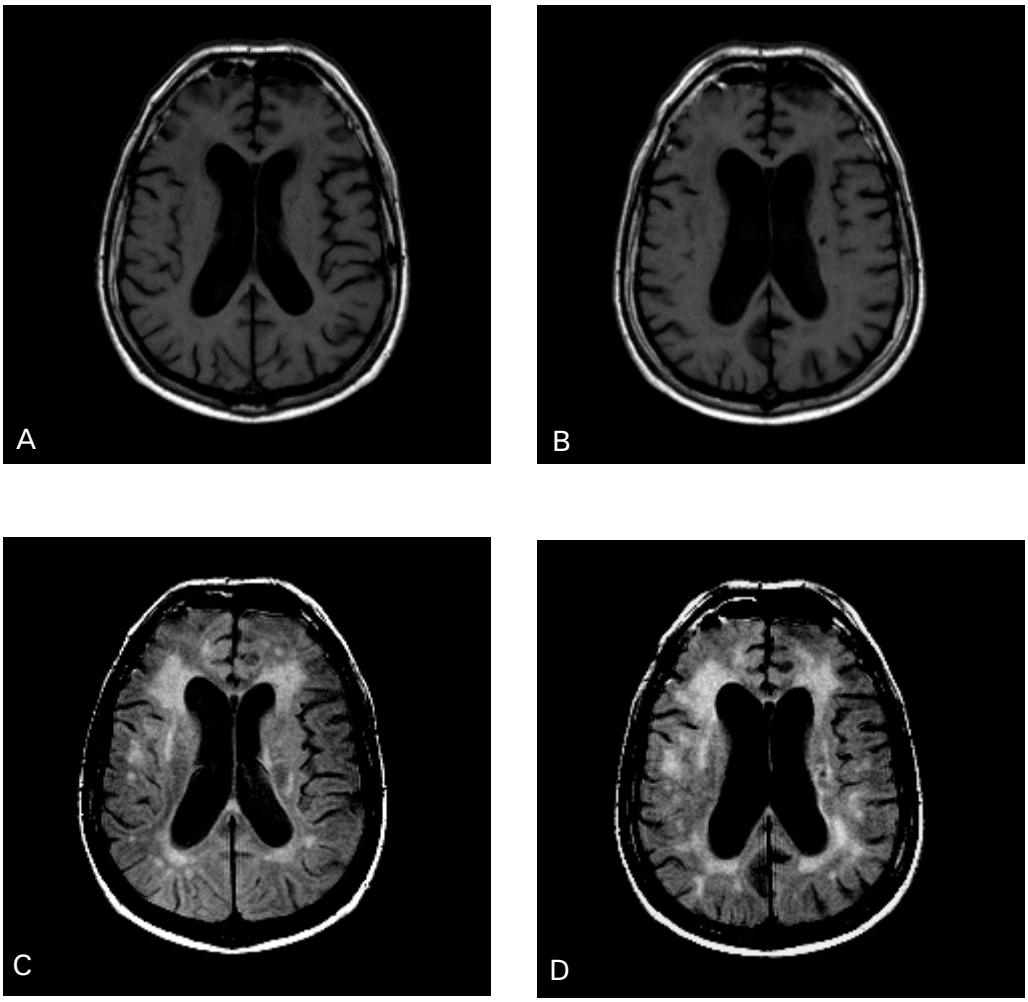

Figure 2. Incident lacunar infarct and progression of white matter lesions. A: T1-weighted spin echo MR image at baseline. B: T1-weighted spin echo MR image at follow-up with new hypointense lesion in the left periventricular white matter C: PD-weighted spin echo image at baseline. D: PD-weighted spin echo image at follow-up with more hyperintense lesions in both the periventricular as the subcortical region.

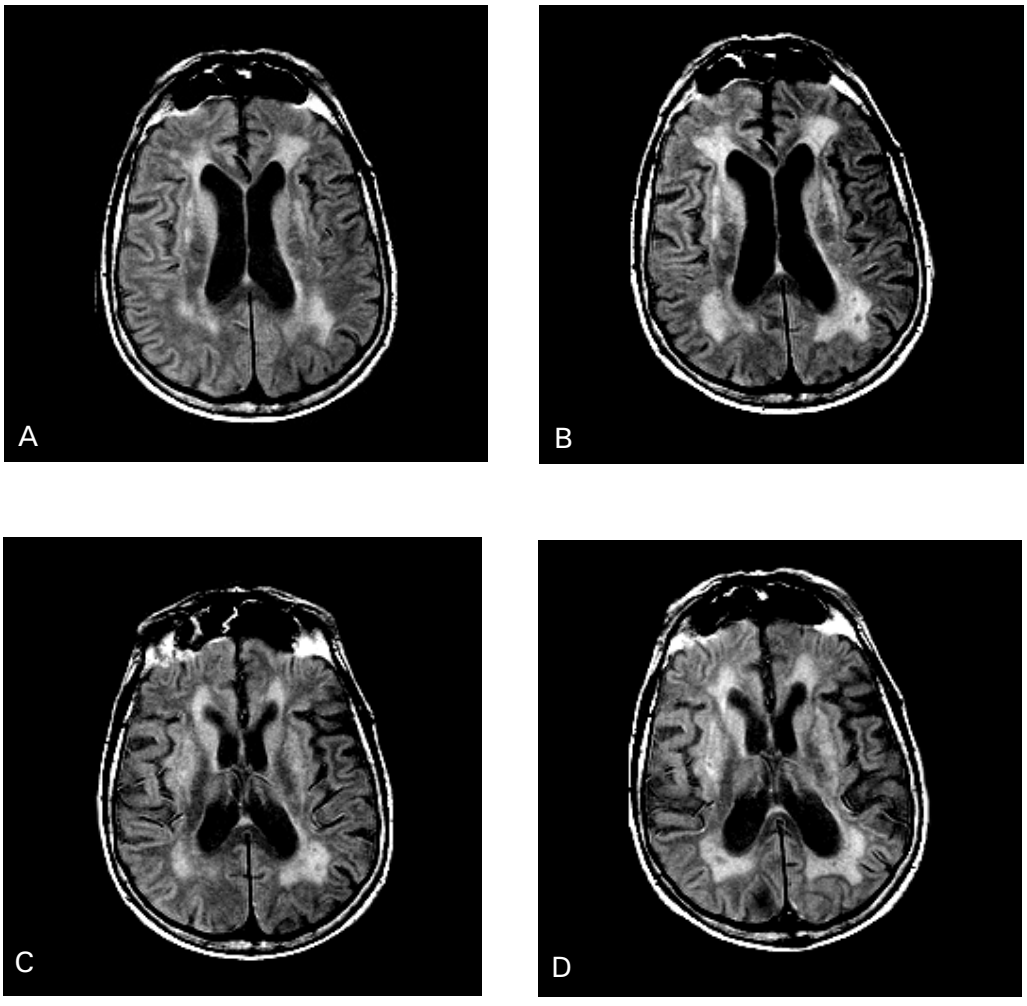

Figure 3. Progression of white matter lesions in periventricular and subcortical region. A,C: PD-weighted spin echo image at baseline. B,D: PD-weighted spin echo image at follow-up with extensive hyperintense lesions ranging from the border of the lateral ventricle to the subcortical U-fibers.

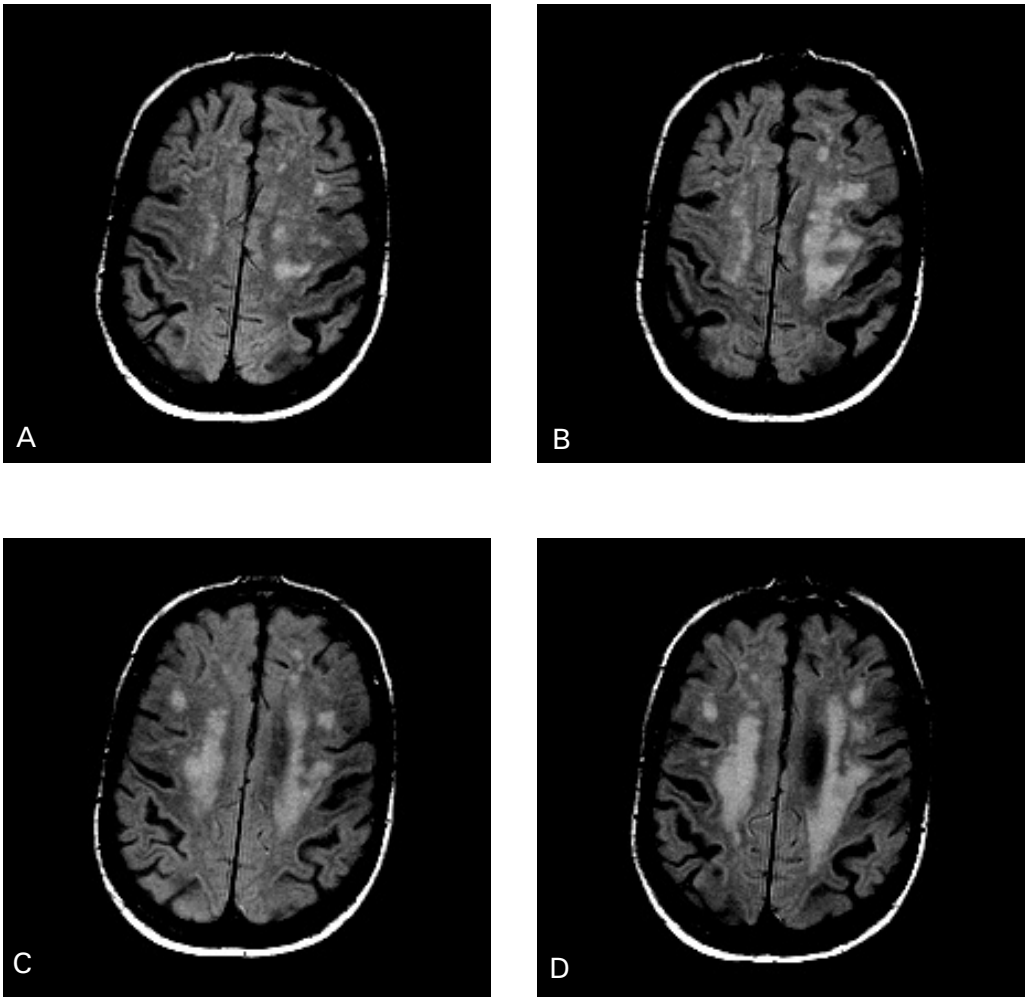

Figure 4. Progression of white matter lesions in the periventricular and subcortical regions. A,C,E,G,I,K: PD-weighted spin echo image at baseline. B,D,F,H,J,L: PD-weighted spin echo image at follow-up.

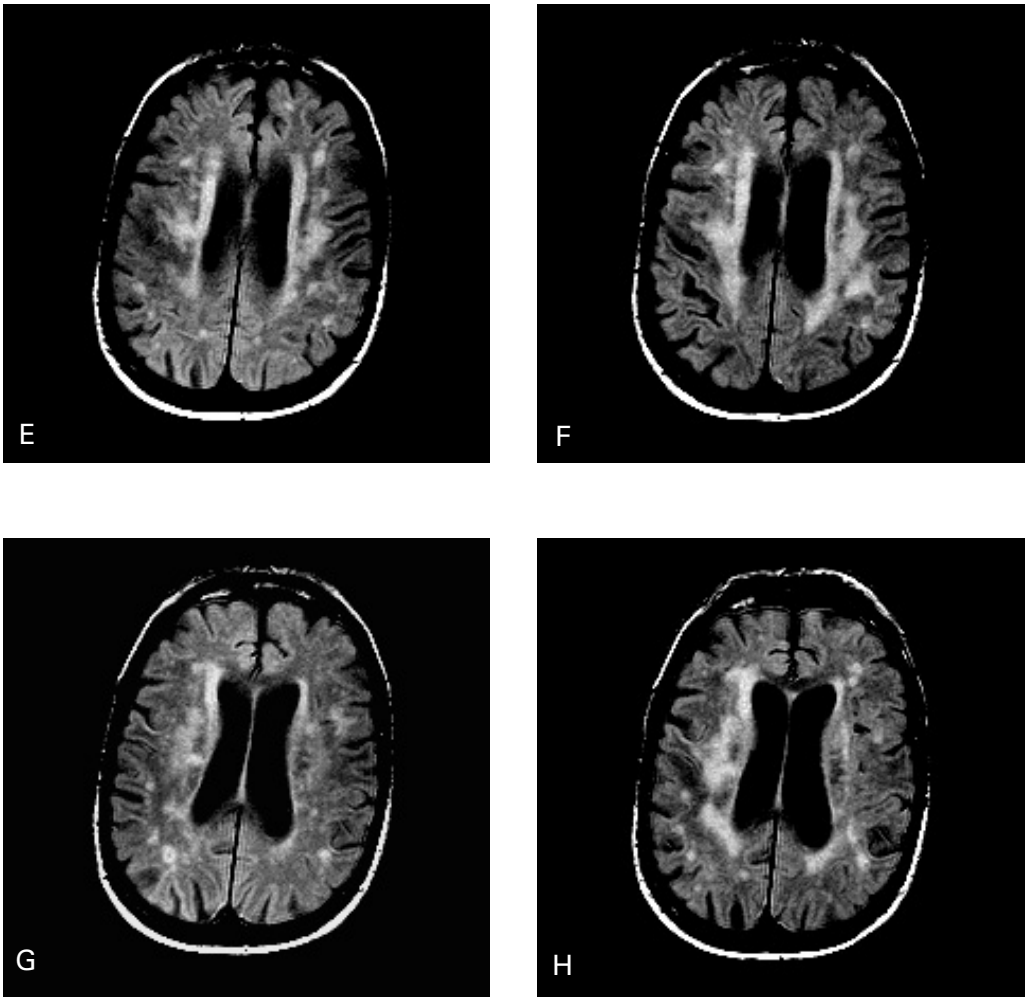

Figure 4. Cont'd

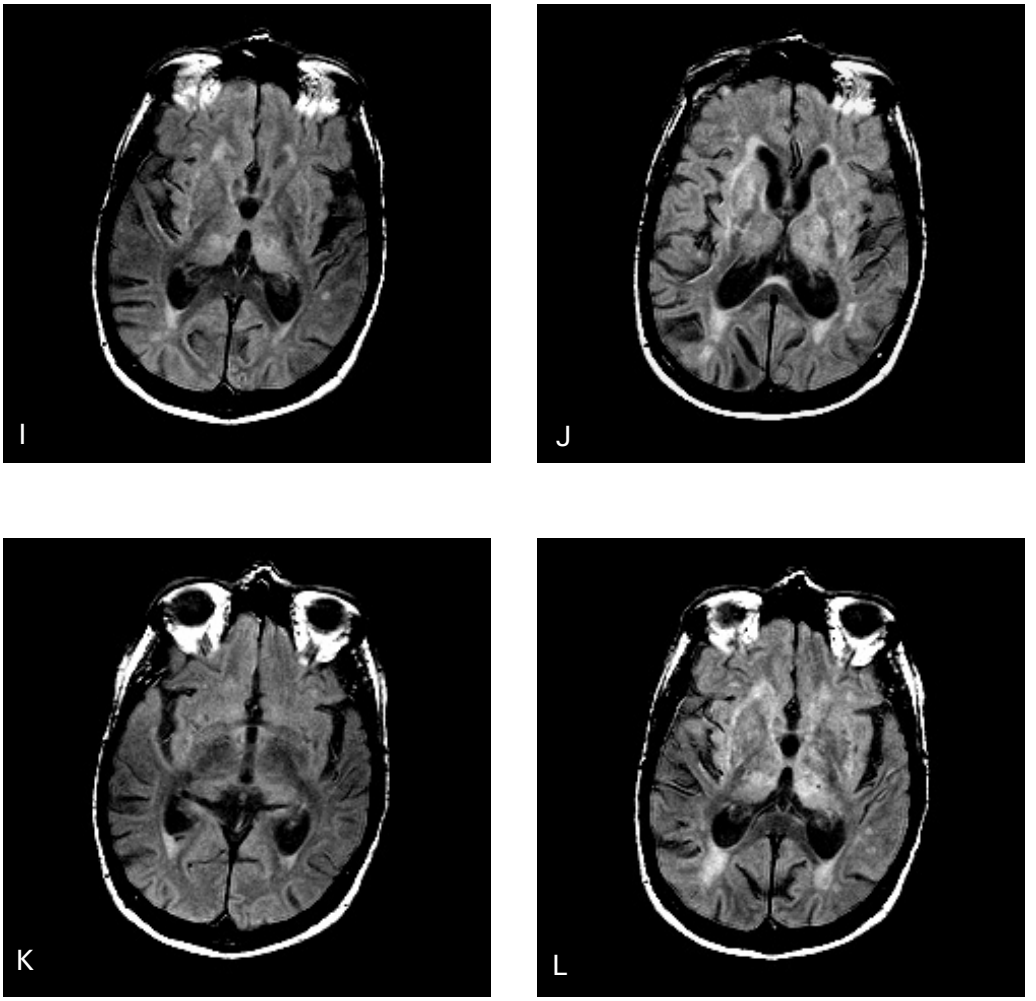

Figure 4. Cont'd

Epiloog

Veel mensen hebben bijgedragen aan de totstandkoming van dit proefschrift. Voordat ik een aantal van hen persoonlijk wil bedanken, wil ik de deelnemers aan de Rotterdam Scan Studie, de ERGO studie, de EPOZ studie en de CASCADE studie heel hartelijk dank zeggen. Zij hebben door hun geheel vrijwillige deelname de in dit proefschrift beschreven studies mogelijk gemaakt. In dit woord van dank wil ik ook hun huisartsen betrekken, die belangeloos hun medewerking verleenden.

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List of publications

Van Dijk EJ, Hupperts RMM, Van der Jagt M, Bijvoet HWC, Hasan D. Diagnosis of perimesencephalic nonaneurysmal subarachnoid hemorrhage with computed tomography. J Stroke Cerebrovasc Dis. 2001;10:247-251

Van Dijk EJ, Prins ND, Vermeer SE, Koudstaal PJ, Breteler MM. Frequency of white matter lesions and silent lacunar infarcts. J Neural Transm Suppl. 2002:25-39.

Vermeer SE, van Dijk EJ, Koudstaal PJ, Oudkerk M, Hofman A, Clarke R, Breteler MM. Homocysteine, silent brain infarcts, and white matter lesions: The Rotterdam Scan Study. Ann Neurol. 2002;51:285-9

Vermeer SE, Hollander M, van Dijk EJ, Hofman A, Koudstaal PJ, Breteler MMB. Silent brain infarcts, white matter lesions and the risk of stroke. Stroke 2003;34:1126-1129.

Van Straaten ECW, Scheltens P, Knol D, Van Buchem MA, Van Dijk EJ, Hofman PA, Karas G, Kjartansson O, De Leeuw FJ, Prins N, Schmidt R, Visser MC, Weinstein HC, Barkhof F. Operational definitions for the NINDS-AIREN criteria for vascular dementia. Stroke 2003:34;1907-1912

Den Heijer T, Vermeer SE, van Dijk EJ, Prins ND, Koudstaal PJ, Hofman A, Breteler MM. Diabetes mellitus and hippocampal and amygdalar atrophy on MRI of nondemented elderly. Diabetologia 2003;46:1604-1610.

Van Dijk EJ, Vermeer SE, De Groot JC, van de Minkelis J, Prins ND, Oudkerk M, Hofman A, Koudstaal PJ, Breteler MMB. Arterial oxygen saturation, COPD and cerebral small vessel disease. J Neurol Neurosurg Psych (in press).

Van Dijk EJ, Hupperts RMM, Van der Jagt M, Bijvoet HWC, Hasan D. Diagnosis of perimesencephalic nonaneurysmal subarachnoid hemorrhage with computed tomography. J Stroke Cerebrovasc Dis. 2001;10:247-251

Van Dijk EJ, Prins ND, Vermeer SE, Koudstaal PJ, Breteler MM. Frequency of white matter lesions and silent lacunar infarcts. J Neural Transm Suppl. 2002:25-39.

Vermeer SE, van Dijk EJ, Koudstaal PJ, Oudkerk M, Hofman A, Clarke R, Breteler MM. Homocysteine, silent brain infarcts, and white matter lesions: The Rotterdam Scan Study. Ann Neurol. 2002;51:285-9

Vermeer SE, Hollander M, van Dijk EJ, Hofman A, Koudstaal PJ, Breteler MMB. Silent brain infarcts, white matter lesions and the risk of stroke. Stroke 2003;34:1126-1129.

Van Straaten ECW, Scheltens P, Knol D, Van Buchem MA, Van Dijk EJ, Hofman PA, Karas G, Kjartansson O, De Leeuw FJ, Prins N, Schmidt R, Visser MC, Weinstein HC, Barkhof F. Operational definitions for the NINDS-AIREN criteria for vascular dementia. Stroke 2003:34;1907-1912

Den Heijer T, Vermeer SE, van Dijk EJ, Prins ND, Koudstaal PJ, Hofman A, Breteler MM. Diabetes mellitus and hippocampal and amygdalar atrophy on MRI of nondemented elderly. Diabetologia 2003;46:1604-1610.

Van Dijk EJ, Vermeer SE, De Groot JC, van de Minkelis J, Prins ND, Oudkerk M, Hofman A, Koudstaal PJ, Breteler MMB. Arterial oxygen saturation, COPD and cerebral small vessel disease. J Neurol Neurosurg Psych (in press).

Van Dijk EJ, Prins ND, Vermeer SE, Hofman A, Van Duijn C, Koudstaal PJ, Breteler MMB. Amyloid ß, APOE, brain infarcts and white matter lesions. Ann Neurol (in press)

Prins ND, van Straaten ECW, van Dijk EJ, Simoni M, Koudstaal PJ, Scheltens P, Breteler MM, Barkhof F. Measuring progression of cerebral white matter lesions on MRI; A comparison of visual rating scales in relation to volumetric change. Neurology (in press).

Prins ND, van Dijk EJ, Den Heijer T, Vermeer SE, Koudstaal PJ, Oudkerk M, Hofman A, Breteler MMB. Cerebral white matter lesions and the risk of dementia. The Rotterdam Scan Study. Arch Neurol (in press).

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Ewoud van Dijk was born on November 11th, 1974 in Voorschoten, the Netherlands. He attended secondary school at the Vlietland College in Leiden and graduated in 1993. That year he started medical school at the University of Maastricht. During his studies, he worked as a student assistant at the department of Anatomy & Embryology (Prof.dr. J. Drukker) and at the department of Neurology (Dr. R.M. Hupperts). He graduated from medical school in August 1999 (cum laude). In that month he started to work at the department of Neurosurgery at the Erasmus Medical Center in Rotterdam (Prof.dr. C.J. Avezaat). In June 2000, he started the research project described in this thesis in the Neuroepidemiology group (Prof.dr. M.M.B. Breteler) of the Department of Epidemiology & Biostatistics (Prof.dr. A. Hofman) in collaboration with the department of Neurology (Prof.dr. P.J. Koudstaal) of the Erasmus Medical Center. In 2003, he obtained a Master of Science in Clinical Epidemiology at the Netherlands Institute for Health Sciences. He started his training as a neurologist at the department of Neurology at the Erasmus Medical Center (Prof.dr. P.A.E. Sillevis Smitt), March 1st, 2004.