Cardiovascular and Hemodynamic Contribution to Brain Aging

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Layout: Moein Mosleh
Cover design: Moein Mosleh, Behnam Sabayan
Published by: Drukkerij Mostert
ISBN: 978-94-90858-22-3

Financial support for printing of this thesis was kindly provided by the Leiden University Medical Centre, Leyden Academy on Vitality and Ageing, and Internationale Stichting Alzheimer Onderzoek (ISAO)

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PROEFSCHRIFT

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van de Rector Magnificus prof. mr. C. J. J. M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op woensdag 2 april 2014
klokke 16:15 uur

door

Behnam Sabayan
geboren te Shiraz, Iran
in 1984

Promotiecommisie

Promotoren:	Prof. dr. R. G. J. Westendorp	
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	Prof. dr. M. J. Daemen	(University of Amsterdam)
	Dr. L. J. Launer	(National Institute on Aging/ National Institute of Health, USA)

To my parents and Sanaz

And for the patients and participants
who gave me the opportunity to learn, think and practice.

"I prefer a short life with width to a narrow one with length."
– Avicenna, Persian Physician and Philosopher

Contents

1

General Introduction

Brain aging: demographic and biological perspectives

With a rapid rise in the number of old and very old individuals, the need for a comprehensive understanding of brain aging is growing[1]. Current data indicate that age-related disorders such as dementia and stroke impose a great burden on patients, families and health care systems[2,3]. The term "Brain Aging" denotes a constellation of morphological and neurophysiological alterations in the brain that ultimately leads to impairments in motor, cognitive and social skills[4]. A progressive accumulation of damaged molecules and impaired energy metabolism, in the absence or failure of healing mechanisms, has been implicated in the pathogenesis of brain aging[4,5]. Neuronal loss and diminished neuronal activity and connectivity are among the key features in an aging brain[6]. While these changes appear in many older individuals, recognition of factors that accelerate the process is crucial. Unique features of the brain like its metabolic demand and circulation might provide us with some clues for better understanding of the mechanisms leading to the accelerated brain aging. Strategies to slow down the process of brain aging might have implications in the treatment of age related disorders of the brain and contribute in better regulation of homeostasis which is a main function of the brain.

The brain: a vascular organ

Although the brain accounts for about 2% of body weight, it consumes about 20% of total body oxygen and 25% of total body glucose. High metabolic demand of the brain necessitates a remarkable fraction of cardiac output as well as a relatively constant level of blood flow[7]. Cerebral blood flow is tightly regulated by a harmonized function of the systemic and cerebrovascular circulations[8]. The heart provides a driving force for cerebral perfusion, extra-cranial vessels serve as a conduit for blood flow and send regulatory signals to the brain stem and intra-cranial vessels act in concert to maintain adequate cerebral blood flow despite fluctuations in systemic blood pressure[9]. In addition, there is a tight coordination between neuronal activity and blood flow within the brain parenchyma, known as functional hyperemia[10]. After a long-lasting exposure to cardiovascular risk factors, these regulatory mechanisms may fail to function properly which ultimately results in cerebral hypoperfusion[11]. Ultimately, chronic hypoperfusion might lead to neuronal energy crisis and impairs structural and functional integrity of the brain[12].

Cardiovascular and hemodynamic contribution to brain aging: missing links

Different lines of evidence from epidemiological, pathological and neuroimaging studies show that midlife cardiovascular risk factors are associated with impaired brain structure and function in old age[13,14]. Whether similar risk factors in late life have similar influence on the brain is a matter of debate. For instance, midlife hypertension has a well-established link with cerebrovascular events, cerebral small vessel disease and cognitive impairment[15]. Nevertheless, it is controversial whether all old and very old subjects benefit from lowering blood pressure for preservation of their brain function[16]. On one hand systemic hypertension might be seen as a risk factor that promotes brain vascular pathologies and on the other hand in older people who carry a great load of brain vascular pathologies higher blood pressure might be needed to overcome critical threshold for brain perfusion[17]. Cerebral perfusion is dependent on the normal function of the heart-brain axis[9]. During last couple of years several reports have shown that heart failure is closely linked with structural and functional features of brain aging[18]. Patients in advance stages of heart failure suffer from brain hypoperfusion and it has been shown that restoration of cardiac function, for example with cardiac transplantation, improves level of cerebral blood flow and cognition[19]. Whether older people free of heart failure but with sub-optimal cardiac function are also at a high risk for structural brain changes and cognitive impairment is yet to be determined[20]. Decline in cognitive performance is one of the key aspects of brain aging[21]. Cognitive impairment is common in old age and is strongly associated with covert brain vascular pathologies such as white matter hyperintensities, lacunar infarcts and cerebral microbleeds[22]. In line with this evidence, previous studies reported that about 10% of patients with first-time stroke suffer from pre-existing dementia[23]. Furthermore, it is frequently reported that subjects with dementia and cognitive impairment have lower cerebral blood flow which may predispose them to develop cerebral infarcts[24]. Further studies are needed to confirm whether assessment of cognitive performance in old age can be a tool to identify subjects at high risk for developing stroke. The brain acts as a central regulator of homeostasis by coordinating the physiology of extraneural tissues[4]. Therefore, it is possible that accelerated brain aging has detrimental effects not only on the brain function but also on the whole body and might affect survival of older people. Accordingly, it has been shown that structural and functional brain changes such as white matter hyperintensities, brain atrophy and cognitive impairment associate with shorter survival in old age[25-27]. However, role of cerebral perfusion, which is closely related to brain aging, in the maintenance of health and survival remained unknown.

Outline of this thesis

In the **second chapter** of this thesis, association between blood pressure and cognitive decline in a general population of the oldest old people is tested. The aim of **chapter three** is to answer the question whether the association between higher blood pressure and cerebrovascular events in very old people is dependent on the level of disability. **Chapter four** presents independent relationship of visit-to-visit blood pressure variability with cognitive impairment and manifestations of cerebral small vessel disease. **Chapters five, six** and **seven** are dedicated to the link between cardiac functioning and features of brain aging in two general populations of older people as well as in older subjects at high risk for cardiovascular disease. In a systematic review and meta-analysis (**chapter eight**), alterations of cerebral hemodynamics in patients with Alzheimers disease and vascular dementia were evaluated. **Chapter nine** compares the predictive value of cognitive impairment with Framingham stroke risk score in relation to future risk of stroke in the oldest old. **Chapter ten** expands current knowledge on the role of endothelial cells in regulation of cerebral blood flow. **Chapter eleven** presents new evidence on the relationship between level of cerebral blood flow and survival in old age. **Chapter twelve** summarizes key findings of this thesis and discusses them in the context of current knowledge about cardiovascular aspects of brain aging.

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High Blood Pressure and Resilience to Physical and Cognitive Decline

Manuscript based on this chapter has been published as:
Sabayan B, Oleksik AM, Maier AB, van Buchem MA, Poortvliet RK, de Ruijter W, Gussekloo J, de Craen AJ, Westendorp RG. High blood pressure and resilience to physical and cognitive decline in the oldest old: the Leiden 85-plus Study.
J Am Geriatr Soc. 2012;60(11):2014-9.

Summary

The aim of this chapter is to evaluate the association of various blood pressure measures at age 85 years with future decline in physical and cognitive function in the oldest old. From the Leiden 85-plus Study, five hundred and seventy two community-dwelling individuals aged 85 years were included. Blood pressure was measured at age 85 years during home visits. Activities of daily living (ADL) and Mini Mental State Examination (MMSE) were assessed at age 85 years and annually thereafter up to age 90 years. On average, participants were followed for 3.2 years. Cross-sectional and longitudinal analyses were performed using linear regression models. Systolic, diastolic, mean arterial blood pressure and pulse pressure were considered as the determinants. All analyses were adjusted for socio-demographic and cardiovascular factors. At age 85 years, higher systolic blood pressure and pulse pressure were associated with lower ADL disability scores (both p =0.01). Similarly, higher systolic blood pressure, diastolic blood pressure and mean arterial pressure were associated with higher MMSE scores (all $p<0.05$). From age 85 years onward, higher systolic blood pressure ($p<0.001$), mean arterial pressure (p=0.01) and pulse pressure (p=0.003) at age 85 years were associated with lower annual increases in ADL disability scores. Likewise, both higher systolic (p=0.03) and pulse pressure (p=0.008) at age 85 years were associated with lower annual declines in MMSE scores. Additional analyses showed that the association between high blood pressure and lower annual decline in MMSE score was most pronounced in subjects with high ADL disability. In the oldest old, higher systolic blood pressure and pulse pressure are associated with resilience to physical and cognitive decline, especially in those with pre-existing physical disability.

Introduction

Although high blood pressure is highly prevalent in the oldest old[1], the relationship between high blood pressure and adverse medical outcomes remains ambiguous[2]. In contrast to general expectation, it has been shown that low blood pressure, rather than high blood pressure carries the greatest mortality risk among the oldest old[3]. Low blood pressure may be a consequence of imminent heart failure, drug treatment or both[4]. On the other hand, high blood pressure may be reactive and can have a survival benefit while ensuring perfusion in critical organs[5]. High blood pressure in middle age has detrimental effects on physical and cognitive disabilities in later life[6,7]. Paradoxically, it has been suggested that in older people with physical disability, low blood pressure may lead to cerebral hypoperfusion and accelerated cognitive decline[8]. Since a considerable proportion of the oldest old individuals with physical disabilities have widespread vascular damage[9,10], high blood pressure might be a compensatory mechanism to maintain organ perfusion, function of body and brain, and ultimately prevention of physical and cognitive decline. Large randomized clinical trials such as Hypertension in the Very Elderly Trial (HYVET) showed no increased risk of cognitive impairment in very old subjects treated for hypertension[11]. However, these clinical trials generally included healthy participants, with low levels of co-morbidities and physical disability[12]. Therefore, observational studies are needed to address the trajectories of cognitive function dependent on blood pressure in unselected oldest old subjects with different levels of disability.

In the Leiden 85-plus Study, we have recruited a population-based sample of the oldest old with a wide variety of functional disability at age 85 years who were prospectively followed for five years for clinical outcomes. This allowed us to investigate the association between various blood pressure measures at age 85 years with future physical and cognitive decline in the oldest old. The underlying hypothesis under scrutiny is that older people with higher physical disability may benefit from higher blood pressure to preserve perfusion of the brain and to protect their cognitive function.

Methods

Study design and participants

The Leiden 85-plus Study is a population-based prospective follow-up study of inhabitants of Leiden, the Netherlands. Between 1997 and 1999, all inhabitants of 1912-1914 birth cohort (n = 705) were contacted in the month of their 85th birth-

day. There were no selection criteria on demographic features or health status. A total of 599 (397 women and 202 men) subjects agreed to participate (85%) and 572 of them for whom blood pressure and functionality measures were available, were included in this study. As described earlier, there was no significant difference between the demographic features and health status of those who participated and those who did not[13]. Participants were visited within one month after their 85th birthday at their homes where face-to-face interviews and neuropsychological testing were done. After age 85 years, all subjects were revisited annually until age 90 years for physical and cognitive assessment. On average, participants were followed for 3.2 years. The Medical Ethical Committee of the Leiden University Medical Centre approved the study, and informed consent was obtained from all the subjects.

Blood pressure

Blood pressure was measured at baseline in the seated position, using a mercury sphygmomanometer. During the home visits, two blood pressure measurements were done two weeks apart. Blood pressure measurements were recorded after at least 5 min of rest and no vigorous exercise in the preceding 30 min. The systolic blood pressure (SBP) was measured at Korotkoff sound 1, and the diastolic blood pressure (DBP) was measured at Korotkoff sound 5. The mean value of two measurements was calculated and used in further analyses. Mean arterial pressure (MAP) and pulse pressure (PP) were calculated as $1/3$(SBP) + $2/3$(DBP) and (SBP) - (DBP) respectively.

Physical and cognitive disability

To evaluate level of physical disability we used Groningen Activity Restriction Scale (GARS) which is a non-disease-specific instrument to measure disability in activities of daily living (ADL)[14]. In the GARS nine questions refer to ADL. Using four-category response for each question, a score of 9 indicates no disability while a score of 36 indicates highest disability in ADL. Based on the median of ADL scores, participants were categorized into two groups with high (ADL score ≥ 10) and low (ADL score < 10) physical disability. Disability in cognitive function was assessed in all participants using Mini-Mental State Examination (MMSE).

Demographic and clinical characteristics

Level of education was dichotomized into primary education and less versus more than primary education. All participants were interviewed about their

smoking habits and alcohol intake. Use of antihypertensive medication was extracted from pharmacy records. Antihypertensive medications were categorized into classes of calcium channel blockers, ACE inhibitors, beta blockers and diuretics. All participants were interviewed about their smoking habits and alcohol intake. Diabetes mellitus was considered present if diagnosed by the primary care physician, if the non-fasting glucose level was greater than 11 mmol/L or if a participant was taking antidiabetic medication. History of cardiovascular diseases including ischemic heart disease and peripheral vascular disease as well as stroke was obtained from general practitioners or nursing home physicians.

Statistical analysis

Since distribution of ADL and MMSE scores was skewed, summary statistics of them are reported as median and inter quartile range (IQR). In cross-sectional and longitudinal analyses, various measures of blood pressure were divided into three strata and baseline or changes in physical and cognitive scores were calculated in each stratum. Change in physical and cognitive disability were defined as the last ADL and MMSE scores minus first ADL and MMSE scores divided by observed years of follow up. To determine the p value for the trend over strata of blood pressure measures, linear regression analyses were performed using tertiles of blood pressure measures as determinants. We did our cross-sectional and longitudinal analyses in two steps. First, analyses were adjusted for sex and then we did further adjustment for level of education, smoking status, alcohol intake, history of stroke, types of antihypertensive medications, diabetes mellitus and history of cardiovascular diseases (including myocardial infarction, angina pectoris, intermittent claudication, vascular Surgery, congestive heart failure, arrhythmia). All longitudinal analyses were adjusted for baseline physical and cognitive scores in both steps. Finally, in a stratified analysis, we tested whether the association between blood pressure and cognitive decline was different in subjects with low and high physical disability at baseline. Interaction between level of physical disability and tertiles of blood pressure measures was tested by adding an interaction term in linear regression models. All analyses were carried out using SPSS software (version 17.0.0, SPSS Inc., Chicago, IL).

Results

A total of 382 (66.8%) participants were female. Sixty four percent had low education. Mean values of systolic blood pressure, diastolic blood pressure, mean arterial pressure and pulse pressure were 155mmHg, 77 mmHg, 103 mmHg and 78 mmHg respectively. The median ADL and MMSE scores were 10 and 26

respectively, reflecting low physical and cognitive disabilities in a considerable proportion of the participants. Table 1 shows the characteristics of participants at age 85 years in tertiles of systolic blood pressure. Subjects in lowest tertile of systolic blood pressure were more diabetic (p=0.05) and had lowest prevalence of alcohol consumption (p=0.04).

Table 1. Characteristics of the Study Participants at Age 85 Years in Tertiles of Systolic Blood Pressure.

	Tertiles of systolic blood pressure			
	110-146 mmHg (n= 186)	147-161 mmHg (n= 193)	162-215 mmHg (n= 193)	pValue†
Demographic factors				
Female, n (%)	125 (67.2)	125 (64.8)	132 (68.4)	0.79
Low education*, n (%)	107 (65.6)	122 (65.6)	113 (60.8)	0.32
Cardiovascular risk factors				
Ever smoking, n (%)	83 (45.6)	98 (52.4)	89 (46.6)	0.86
Regular alcohol use**, n (%)	77 (42.1)	105 (55)	101 (52.6)	0.04
BMI, mean (SD)	27.2 (4.6)	27 (4.0)	27.4 (4.6)	0.77
Diabetes mellitus, n (%)	32 (18.6)	32 (16.6)	23 (11.9)	0.05
Antihypertensive medication, n (%)	81 (43.5)	82 (42.7)	87 (45.3)	0.63
Co-morbidities				
Cardiovascular diseases***, n(%)	81 (45.3)	86 (46.5)	82 (43.9)	0.22
Stroke, n (%)	23 (12.4)	12 (6.3)	21 (10.9)	0.51
Parkinson, n (%)	6 (3.2)	6 (3.1)	3 (1.6)	0.30
Malignancy	27 (14.7)	40 (20.8)	34 (17.6)	0.46
Arthritis	62 (33.3)	61 (31.8)	61 (31.8)	0.77

Abbreviations: SD: standard deviation, BMI: body mass index

† Indicates p-value for trend over strata of systolic blood pressure

∗ Primary education or less.

∗∗ Self-reported alcohol consumption

∗∗∗ Including myocardial infarction, angina pectoris, intermittent claudication, vascular Surgery, congestive hear failure, arrhythmia

Table 2 shows cross-sectional findings on the association between various measures of blood pressure and level of physical and cognitive disability. At age 85 years, higher SBP and PP were associated with lower ADL disability scores (both p=0.01). Furthermore, higher SBP, DBP and MAP were associated with higher MMSE scores (all $p<0.01$).

Table 2. Physical and Cognitive Disability in Tertiles of Blood Pressure at Age 85 Years.

	Tertiles of blood pressure			
	Low	Middle	High	P for trend*
SBP (Range, mmHg)	(110-146)	(147-161)	(162-215)	
ADL disability score, median [IQR]	11 [9, 17]	9 [9, 12]	9 [9, 14]	0.01
MMSE score, median [IQR]	25 [18, 27]	26 [21, 29]	27 [24, 28]	0.001
DBP (Range, mmHg)	(43-71)	(72-80)	(81-115)	
ADL disability score, median [IQR]	11 [9, 16]	10 [9, 14]	9 [9, 13]	0.57
MMSE score, median [IQR]	25 [19, 27]	26 [22, 29]	27 [24, 28]	0.004
MAP (Range, mmHg)	(70-98)	(99-107)	(108-146)	
ADL disability score, median [IQR]	11 [9, 17]	10 [9, 13]	9 [9, 13]	0.08
MMSE score, median [IQR]	25 [18, 27]	26 [22, 29]	27 [24, 29]	< 0.001
PP (Range, mmHg)	(37-71)	(72-83)	(84-131)	
ADL disability score, median [IQR]	10 [9, 16]	9 [9, 14]	9 [9, 13]	0.01
MMSE score, median [IQR]	26 [19, 28]	26 [22, 28]	26 [24, 28]	0.06

Abbreviations: ADL: activities of daily living, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP; mean arterial pressure, PP: pulse pressure, IQR; inter quartile range, MMSE: mini mental state examination

$*$ Analyses were adjusted for sex, education, smoking status, alcohol intake, history of stroke, types of antihypertensive medications, diabetes mellitus and history of cardiovascular diseases

Table 3 presents longitudinal analyses from age 85 years onwards. Higher SBP ($p<0.001$), MAP (p=0.01) and PP (p=0.003) were associated with lower annual increases in ADL disability scores. Likewise, higher SBP (p=0.03) and PP (p=0.008) at age 85 years were associated with lower annual declines in MMSE scores.

To further explore our findings, we performed a stratified analysis that showed the association between high blood pressure and lower annual decline in MMSE scores to be most pronounced in subjects with high physical disability (figure 1). Among participants with high physical disability (ADL score $\geq$ 10) increase in tertile of SBP and PP was associated with a 0.39 and 0.41 point lower annual decline in MMSE scores respectively (p=0.04, p=0.03 respectively). Tests for interaction between blood pressure measures and physical disability were not statistically significant (all $p> 0.05$). In addition, we repeated the cross-sectional, longitudinal and stratified analyses in subjects who were not on antihypertensive medication. This sensitivity analysis showed findings similar to what we observed in the whole population (data not shown).

Table 3. Annual Change in Physical and Cognitive Disability from Age 85 Years Onwards in Tertiles of Blood Pressure

	Tertiles of blood pressure			
	Low	Middle	High	P for trend*
SBP (Range, mmHg)	(110-146)	(147-161)	(162-215)	
Δ ADL disability score, median [IQR]	1.8 [0.4, 3.7]	1.0 [0, 2.4]	0.5 [0, 1.6]	< 0.001
Δ MMSE score, median [IQR]	-1.0 [-2.0, 0]	-0.6 [-1.5, 0]	-0.4 [-1.2, 0]	0.03
DBP (Range, mmHg)	(43-71)	(72-80)	(81-115)	
Δ ADL disability score, median [IQR]	1.2 [0.4, 3.0]	1.0 [0, 2.7]	0.6 [0, 2.0]	0.13
Δ MMSE score, median [IQR]	-0.7 [-2.0, 0]	-0.6 [-1.6, 0]	-0.5 [-1.4, 0]	0.86
MAP (Range, mmHg)	(70-98)	(99-107)	(108-146)	
Δ ADL disability score, median [IQR]	1.4 [0.4, 3.6]	0.8 [0, 2.4]	0.6 [0, 2.0]	0.01
Δ MMSE score, median [IQR]	-0.8 [-2.0, 0]	-0.6 [-1.5, 0]	-0.4 [-1.4, 0]	0.83
PP (Range, mmHg)	(37-71)	(72-83)	(84-131)	
Δ ADL disability score, median [IQR]	1.6 [0.2, 3.7]	1.0 [0, 2.2]	0.7 [0, 2.0]	0.003
Δ MMSE score, median [IQR]	-0.8 [-2.2, 0]	-0.6 [-1.6, 0]	-0.3 [-1.0, 0]	0.008

Abbreviations: ADL: activities of daily living, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP; mean arterial pressure, PP: pulse pressure, IQR; inter quartile range
Δ: Indicates annual change in physical and cognitive scores calculated as (Last measure-First measure/Years of follow up)
∗ Analyses were adjusted for sex, education, smoking status, alcohol intake, history of stroke, types of antihypertensive medications, diabetes mellitus, history of cardiovascular diseases and baseline physical and cognitive scores

Discussion

In this study, we showed that higher levels of various blood pressure measures are associated with less physical and cognitive disabilities at age 85 years. Furthermore, in longitudinal analyses, we observed that higher SBP and PP at age 85 years are associated with lower physical and cognitive decline. The relation between high blood pressure and lower cognitive decline was most pronounced in those with preexisting physical disability. These associations were independent of cardiovascular risk factors and co-morbidities such as smoking, diabetes mellitus, coronary artery diseases, arrhythmias, peripheral vascular diseases and heart failure.

Our findings are consistent with previous observational studies in the oldest-old. In a recent study by Molander et al, among individuals aged 85 years and older, higher PP was associated with better cognition[15]. Furthermore, a similar study in Australian centenarians reported that higher SBP is associated with

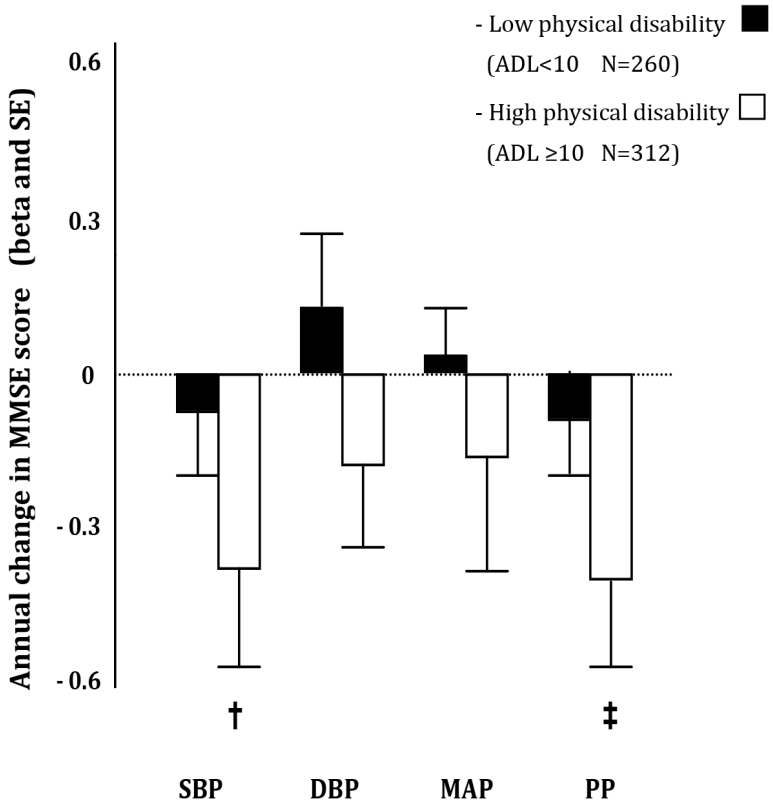

Figure 1. Annual change in MMSE (mini mental state examination) score for increase in tertiles of systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and pulse pressure (PP) in two groups of low and high physical disability measured by activities of daily living (ADL). † and ‡ show significant difference from unity (p=0.04 and p=0.03 respectively). Analyses were adjusted for sex, education, smoking status, alcohol intake, history of stroke, types of antihypertensive medications, diabetes mellitus, history of cardiovascular diseases and baseline mini mental state examination (MMSE) scores.

lower cognitive and physical disabilities[16]. In the third national health and nutrition examination in the United States, although hypertension was associated with poorer cognitive performance in younger subjects (aged less than 80 years), optimally-controlled blood pressure was associated with better cognitive function in those aged 80 years and older[17]. Similarly, in a study among individuals aged 85 years and over, five years follow up did not show a significant association between various measures of blood pressure measures at baseline and incidence of dementia[18].

One reason why the relationship between blood pressure and cognitive function differs between younger and oldest-old patients has been suggested by other authors: a higher blood pressure might be needed to overcome advanced arterial stiffness to ensure sufficient perfusion in critical organs[19]. Another possible reason for the observed findings is that those subjects that are most vulnerable to the adverse effects of high blood pressure have died earlier, leaving a subset who is resistant to these adverse effects. Alternatively, cognitive and physical disabilities may cause abnormalities in blood pressure regulation and decline in blood pressure[20,21]. An overall decline might be confounding both blood pressure and functional or cognitive decline. Although our longitudinal study helps to address some reverse causality, further controlled interventions in older patients with baseline disability are needed to fully answer questions posed by our results.

Clinical trials on the benefit of antihypertensive medication in very old subjects have shown mixed findings[22]. A recent meta-analysis by Bejan-Angoulvant et al shows that antihypertensive treatment in subjects 80 years and older reduces risk of cardiovascular events; however, it has no benecial effects on all cause or cardiovascular mortality[23]. This meta-analyses also showed that the mortality rate in trials were heterogeneous which might be explained by the heterogeneity in the population under study and/or an increase in mortality in trials where the participant received maximal allowable blood pressure lowering[24-26]. A limited number of studies evaluated the effect of antihypertensive treatment on cognitive function in very old subjects. In the hypertension in the very elderly trial (HYVET), antihypertensive treatment showed no significant benefit in reducing incidence of dementia[11]. However, the HYVET findings, when combined in a meta-analysis of other placebo-controlled, double-blind, trials of antihypertensive treatment in younger elderly people[27-29], a significant reduction in incident dementia was observed. It is worth pointing out that participants in HYVET were generally healthier than normal for their age, as shown by a low number of cardiovascular risk factors and co-morbidities which limits the extrapolation of these findings to the general population of the oldest old where physical and cognitive disability is common[12].

Physical disability has been linked to cerebrovascular pathologies in the brain[30].

In the presence of hypotension, regulatory mechanisms in the brain cannot effectively safeguard brain blood flow leading to decline in cerebral perfusion[31]. Consistent with this mechanism, in our study, those with poorest baseline physical function showed the strongest association between hypotension and greater cognitive decline.

In the coming years, the increasing number of the oldest old subjects with various levels of functional disability[32] highlights the necessity of an individualized approach for treatment of hypertension in this age group[33]. Further experimental and interventional studies, combined with imaging techniques, are warranted to further explore and substantiate the impact of physical disability on the association between systemic blood pressure and cognitive function. We also propose that future trials should test whether relaxation of blood pressure control in elderly with low or normal blood pressure can contribute in prevention of further physical and cognitive decline especially in elderly with advanced physical disability. This study has certain strengths. A relatively large group of the oldest old subjects, a long term follow up, low attrition rate and annual home visits to assess physical and cognitive disability can be put forward as the strengths of this study. However, there are limitations which are necessary to be considered when interpreting the results. First, our findings can be due to the fact that subjects who had lower blood pressure at age 85 years were already on the way down because of long histories of exposure to hypertension and co-morbidities. This decline in blood pressure could be concomitant with decline in physical and cognitive function after age 85 years raising the issue whether confounding by overall decline can explain our results. Nevertheless, our analyses show that subjects with low systolic blood pressure did not have higher prevalence of cardiovascular risk factors or co-morbidities except for diabetes mellitus. In addition, we performed all cross-sectional and longitudinal analyses adjusted for cardiovascular risk factors and co-morbidities at baseline which did not essentially change our estimates. As a second limitation, we used only ADL and MMSE to estimate physical and cognitive disability. Since there are no single criteria or definition for physical and cognitive disability, ADL and MMSE may not fully reflect the level physical and cognitive disabilities respectively. Furthermore, lack of information on neuroimaging and cardiac function of our participants, did not allow us to add further details on the causal interpretation of our findings.

In conclusion, our results show that higher systolic blood pressure and pulse pressure are associated with resilience to physical and cognitive decline in the oldest old. Subjects with preexisting physical disability may benefit most from high blood pressure to preserve cognitive function.

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3

High Blood Pressure, Physical and Cognitive Function, and Risk of Stroke

Manuscript based on this chapter has been published as:
Sabayan B, van Vliet P, de Ruijter W, Gussekloo J, de Craen AJ, Westendorp RG. High blood pressure, physical and cognitive function, and risk of stroke in the oldest-old: The Leiden 85-plus Study.
Stroke. 2013;44(1):15-20

Summary

Epidemiological studies have shown mixed findings on the association between hypertension and stroke in the oldest-old. Heterogeneity of the populations under study may underlie variation of outcomes. The aim of this chapter is to examine whether level of physical and cognitive function moderates the association between blood pressure and stroke. We included 513 subjects aged 85 years old from the population based Leiden 85-plus Study. Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and pulse pressure (PP) were measured at baseline. Activities of daily living and Mini Mental State Examination were assessed to estimate level of physical and cognitive function, respectively. Five-year risk of stroke was estimated with Cox-regression analysis. In the entire cohort, there were no associations between various measures of blood pressure and risk of stroke except for the inverse relation between PP and stroke risk (HR: 0.80, 95 % CI: 0.66-0.98). Among subjects with impaired physical functioning, higher SBP (HR: 0.74, 95 % CI: 0.59-0.92), MAP (HR: 0.68, 95 % CI: 0.47-0.97) and PP (HR: 0.71, 95 % CI: 0.55-0.93) were associated with reduced risk of stroke. Likewise, among subjects with impaired cognitive functioning higher SBP was associated with reduced risk of stroke (HR: 0.80, 95 % CI: 0.65-0.98). In subjects with unimpaired cognitive functioning, higher DBP (HR: 1.98, 95 % CI: 1.21-3.22) and MAP (HR: 1.70, 95 % CI: 1.08-2.68) were associated with higher risk of stroke. Our findings suggest that impaired physical and cognitive function moderates the association between blood pressure and stroke.

Introduction

Clinical trials have shown mixed findings on the benefit of antihypertensive treatment in lowering risk of stroke in very old age[1]. It has been suggested that heterogeneity of the populations under study underlies variety of outcomes, but the explanatory biological variables have as yet remained uncertain[2, 3].

Inherent mechanisms in brain vessels keep cerebral blood flow constant despite variations in blood pressure[4]. Once advanced cerebrovascular damage is present, these mechanisms cannot effectively regulate level of cerebral blood flow[5, 6]. Therefore, low systemic blood pressure in the presence of cerebrovascular damage may lead to decline in cerebral blood flow which renders brain tissue vulnerable to ischemic events[7]. Both physical and cognitive impairment are common in very old age and are strongly associated with cerebrovascular pathologies and cerebral hypoperfusion[8-11]. Hence, low systemic blood pressure in subjects with physical and cognitive impairment might reduce cerebral blood flow and increase risk of ischemic stroke.

In the Leiden 85-plus Study, we have recruited a population-based sample of the oldest-old with a wide variety of functional impairments at age 85 years who were prospectively followed for clinical outcomes. This allowed us to examine whether very old subjects with different levels of physical and cognitive impairment show different associations between high blood pressure and risk of stroke. We hypothesized that higher blood pressure in the presence of physical and cognitive impairment is associated with lower risk of stroke.

Material and methods

Study design and participants

The Leiden 85-plus Study is a population-based prospective follow-up study of inhabitants of Leiden, the Netherlands. Between 1997 and 1999, all inhabitants of 1912-1914 birth cohort (n = 705) were contacted in the month of their 85th birthday. There were no selection criteria on demographic features or health status. A total of 599 (397 women and 202 men) subjects agreed to participate (85%). As described previously, there was no significant difference between the demographic features and health status of those who participated and those who did not[12]. Blood pressure measures and clinical data were available for 571 subjects. We excluded 58 subjects who had a clinically recognized stroke at baseline, leaving 513 subjects for this analysis. Participants were visited within one month after their 85th birthday at their homes where face-to-face interviews and clinical examinations were done. The Medical Ethical Committee of the Lei-

den University Medical Centre approved the study, and informed consent was obtained from all the subjects.

Blood pressure

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured at baseline, using a mercury sphygmomanometer, in seating position. During two home visits at age 85 years, two blood pressure measurements were done in two weeks apart. Blood pressure measurements were recorded after at least 5 min of rest and no vigorous exercise in the preceding 30 min. The mean value of the two measurements was calculated and used in further analyses. Mean arterial pressure (MAP) and pulse pressure (PP) were calculated as 1/3(SBP) + 2/3(DBP) and (SBP) - (DBP), respectively, using the mean blood pressure of the two visits.

Stroke

The primary outcome of this study was fatal plus non-fatal stroke. In the Netherlands detailed information on health status, emergency events and patients' hospitalizations are recorded with general practitioners. Occurrence of clinically recognized stroke during five years of follow up was assessed by annually interviewing general practitioners (for subjects living independently) or nursing home physicians (for subjects living in a nursing home). We used the World Health Organization definition of stroke of "rapidly developing clinical signs of focal (at times global) disturbance of cerebral functioning lasting >24 hours" to identify subjects with stroke events[13]. To assess fatal stroke, we obtained dates of deaths from the Dutch civic registry and specific data on causes of death from Statistics Netherlands, which assigns codes for all national death certificates according to the International Classification of Diseases and Related Disorders, 10th revision (ICD-10). Death due to stroke was classified as ICD-10 codes I60-I69[14].

Physical and cognitive function

To evaluate level of physical impairment in the participants we used Groningen Activity Restriction Scale (GARS) which is a non-disease-specific instrument to measure impairment in activities of daily living (ADL)[15]. In the GARS, nine questions refer to ADL. Using four-category response for each question, a score of 9 indicates no impairment while a score of 36 indicates highest impairment in ADL. Based on the median ADL scores (10 points), participants were categorized into two groups of impaired (ADL score $\geq$ 10) and unimpaired (ADL score = 9)

physical function. Impairment in cognitive function was assessed in all participants using Mini-Mental State Examination (MMSE)[16]. Similarly, based on the median MMSE scores (26 points), participants were categorized into two groups of impaired (MMSE score $\leq$ 26) and unimpaired (MMSE score $>$ 26) cognitive function.

Demographic and clinical characteristics

Level of education was dichotomized into primary education and less versus more than primary education. Use of antihypertensive medication was extracted from pharmacy records. All participants were interviewed about their smoking habits and alcohol intake. Diabetes mellitus was considered present if diagnosed by the primary care physician, if the nonfasting glucose level was greater than 11 mmol/L or if a participant was taking antidiabetic medication. History of cardiovascular diseases, including ischemic heart disease, intermittent claudication, vascular surgery, arrhythmia and heart failure, was obtained from general practitioners or nursing home physicians.

Statistical analysis

Since distribution of ADL and MMSE scores was skewed, summary statistics of them are reported as median and inter quartile range (IQR). Cox regression models were fitted with time-to-stroke as the outcome variable and each 10 mmHg increase in various blood pressure measures as the determinants. First, we performed our analyses adjusted for sex and then we did further adjustments for history of cardiovascular diseases, diabetes mellitus, smoking and use of antihypertensive medication in multivariate models. These variables were selected for multivariable analysis since they were correlated with level of blood pressure (data not shown) and have a well-established association with stroke[17]. Outputs of the Cox regression analyses were checked for violation of the proportional hazards and linearity assumptions. Furthermore, we performed a stratified analysis to test whether subjects with different levels of physical and cognitive function show different associations between blood pressure and stroke. Interaction between the level of physical and cognitive function with various blood pressure measures regarding the risk of stroke was tested by adding an interaction term in Cox regression models. All analyses were carried out using SPSS software (version 17.0.0, SPSS Inc., Chicago, IL).

Results

Table 1 summarizes the clinical characteristics of participants at age 85 years. Mean values of SBP, DBP, MAP and PP were 155mmHg, 77mmHg, 102mmHg and 78mmHg respectively. Median ADL and MMSE scores were 10 and 26, respectively. Clinical characteristics of the subjects with different levels of physical and cognitive function are separately presented in the supplement data (Table S-1 and Table S-2, http://stroke.ahajournals.org/content/44/1/15/suppl/DC1). Subjects with physical and cognitive impairment had lower levels of blood pressure measures in comparison to those with unimpaired physical and cognitive function (all $p<0.05$). In addition, subjects with cognitive impairment had higher prevalence of myocardial infarction (p=0.03) and heart failure (p=0.04) in comparison to those with unimpaired cognitive function.

Table 1. Clinical characteristics of 513 participants at age 85 years

Characteristics	Value
Socio-demographics	
Female, n (%)	344 (67.1)
Low education*, n (%)	312 (63.9)
Ever smoking, n (%)	242 (48.2)
Regular alcohol intake†, n (%)	262 (51.6)
Cerebrovascular risk factors	
History of CVD‡, n (%)	218 (44)
Heart failure, n (%)	63 (12.3)
History of DM, n (%)	79 (16)
BMI, mean (SD)	27.2(4.5)
Total cholesterol, mmol/L mean (SD)	5.7 (1.1)
Triglycerides, mmol/L mean (SD)	1.6 (0.8)
Systolic blood pressure, mmHg mean (SD)	155 (18)
Diastolic blood pressure, mmHg mean (SD)	77 (10)
Pulse pressure, mmHg mean (SD)	78 (15)
Mean arterial pressure, mmHg mean (SD)	102 (11)
Antihypertensive medication, n (%)	222 (43.3)
Functionality measures	
ADL, median [IQR]	10 [9,13]
MMSE, median [IQR]	26 [23,28]

∗ Primary education and less.
†Self-reported alcohol consumption
‡ Including myocardial infarction, angina pectoris, intermittent claudication, congestive heart failure and arrhythmia
Abbreviations: CVD: cardiovascular diseases, DM: diabetes mellitus, BMI: body mass index, SD: standard deviation, IQR: interquartile range, ADL: activities of daily living, MMSE: mini mental state examination

During five years of follow up, 58 of the 513 (11.3%) subjects developed stroke, of which 27 (46.5%) cases were fatal. Risk of stroke per each 10 mmHg increase in various blood pressure measures in the whole population is presented in table 2. The proportional hazards and linearity assumptions of the Cox regression analyses were confirmed after checking the outputs. In the sex adjusted models, there was no association between different measures of blood pressure and risk of stroke (all p>0.05). Further adjustment for socio-demographic and cardiovascular factors did not essentially change the estimates except for the association between higher PP and lower risk of all stroke (Hazard ratio [HR]: 0.80, 95 % CI: 0.66-0.98). In a sensitivity analysis, we tested the association between blood pressure measures and risk of stroke in subjects who were not on antihypertensive medication. In this sensitivity analysis we also found no association between different measures of blood pressure and risk of stroke (Table S-3 http://stroke.ahajournals.org/content/44/1/15/suppl/DC1).

Table 2. Risk of stroke for each 10mmHg increase in systolic blood pressure, diastolic blood pressure, mean arterial pressure and pulse pressure

	Crude model			**Adjusted model**		
	HR	(95% CI)	P-value	HR	(95% CI)	P-value
Fatal and non-fatal stroke						
SBP	0.91	(0.78-1.06)	0.21	0.86	(0.73-1.02)	0.09
DBP	1.03	(0.78-1.37)	0.82	1.04	(0.76-1.40)	0.81
MAP	0.93	(0.73-1.19)	0.57	0.89	(0.68-1.17)	0.41
PP	0.86	(0.72-1.03)	0.10	0.80	(0.66-0.98)	0.03

Crude Model: Adjusted for sex
Adjusted Model: Analyses were adjusted for sex, cardiovascular diseases, diabetes mellitus, antihypertensive medication, smoking
Abbreviations: HR: Hazard ratio, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, PP: pulse pressure

Figure 1 shows the associations between various measures of blood pressure and risk of stroke dependent on level of physical and cognitive function. Significant interactions were present between SBP, DBP, MAP and PP and level of physical function regarding the risk of stroke (all p for interaction <0.05). Similarly, we observed significant interactions between SBP, DBP and MAP and level of cognitive function regarding the risk of stroke (all p for interaction <0.05). Interactions were most pronounced for those subjects who had both impaired physical and cognitive function (n=144) when compared to those who had neither of these disabilities (n=180). To explore whether use of more than one blood pressure value in our analyses changed our results, we performed a sensitivity analysis in which the association of mean blood pressure measures at age 85 and 86 years with stroke events from age 86 years onwards was investigated. This

sensitivity analysis did not change the effect estimates (data not shown).

Table 3 shows multivariable adjusted analyses on the associations between various measures of blood pressure and risk of all strokes, stratified for level of physical and cognitive function at baseline. In subject with unimpaired physical functioning, no significant association was found between various measures of blood pressure and risk of stroke (all $p>0.05$), albeit virtually all HRs were above unity, indicating higher risk of stroke with increasing blood pressure. Conversely, in subjects with impaired physical functioning, higher SBP (HR: 0.74, 95 % CI: 0.59-0.92), MAP (HR: 0.68, 95 % CI: 0.47-0.97) and PP (HR: 0.71, 95 % CI: 0.55-0.93) were associated with reduced risk of stroke. In subjects with unimpaired cognitive functioning, higher measures of blood pressure were associated with increased risk of stroke, which was statistically significant for DBP and MAP (HR: 1.98, 95% CI: 1.21-3.22 and 1.70, 95% CI: 1.08-2.68, respectively). In contrast, in subjects with impaired cognitive function, higher measures of blood pressure were associated with lower risk of stroke, which was statistically significant for SBP (HR: 0.80, 95 % CI: 0.65-0.98). To test whether the outcomes of this stratified analysis were dependent on specific MMSE cut-offs, we repeated our analyses using a MMSE cut-off at 20 points and found similar outcomes (data not shown).

Table 3. Risk of stroke for each 10mmHg increase in systolic blood pressure, diastolic blood pressure, mean arterial pressure and pulse pressure stratified for level of physical and cognitive function

	Unimpaired			**Impaired**		
	HR	(95% CI)	P-value	HR	(95% CI)	P-value
Physical function		n= 251			n= 262	
SBP	1.11	(0.88-1.41)	0.38	0.74	(0.59-0.92)	0.007
DBP	1.45	(0.94-2.25)	0.07	0.81	(0.53-1.22)	0.31
MAP	1.32	(0.91-1.93)	0.14	0.68	(0.47-0.97)	0.03
PP	1.00	(0.73-1.35)	0.98	0.71	(0.55-0.93)	0.01
Cognitive function		n= 247			n= 266	
SBP	1.21	(0.90-1.61)	0.21	0.80	(0.65-0.98)	0.03
DBP	1.98	(1.21-3.22)	0.006	0.83	(0.57-1.21)	0.33
MAP	1.70	(1.08-2.68)	0.02	0.74	(0.52-1.03)	0.08
PP	0.96	(0.66-1.39)	0.82	0.80	(0.63-1.01)	0.06

Hazard ratio (HR) was measured for each 10 mmHg increase in blood pressure measures

Abbreviations: HR: Hazard ratio, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, PP: pulse pressure

Analyses were adjusted for sex, cardiovascular diseases, diabetes mellitus, antihypertensive medication, smoking

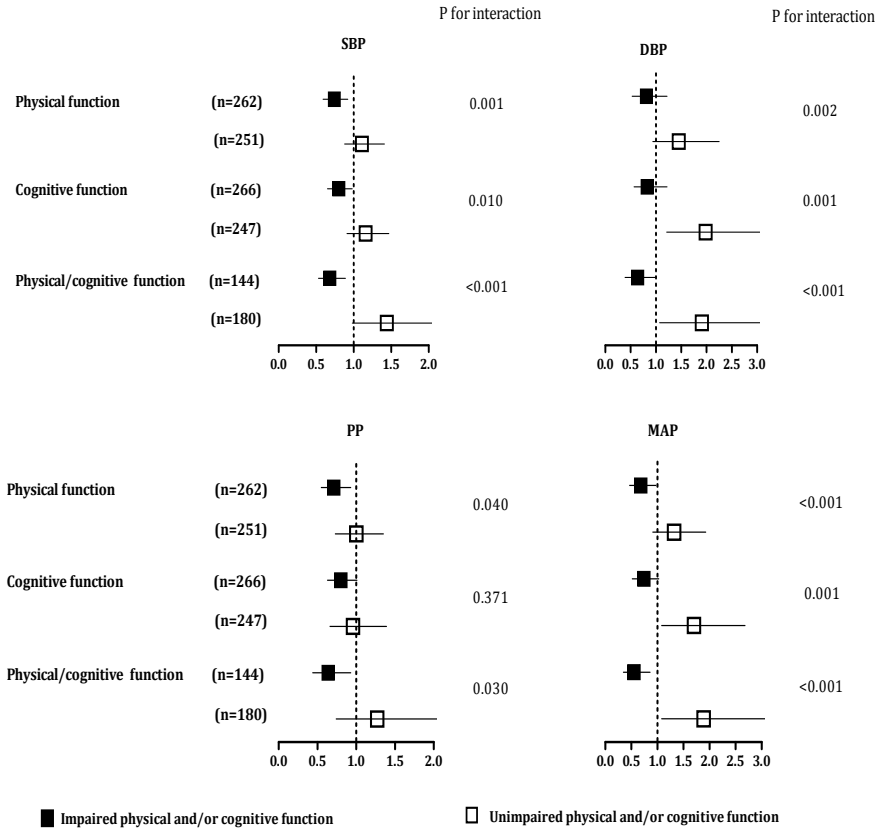

Figure 1. Hazard ratios (HRs) and 95% confidence interval on the associations between blood pressure and risk of stroke dependent on level of physical and cognitive function. Unimpaired physical function was defined as ADL score = 9 and impaired physical function as ADL score $\geq$ 10. Unimpaired cognitive function was defined as MMSE score $>$ 26 and impaired cognitive function as MMSE score $\leq$ 26. Subjects with unimpaired physical and cognitive function had both ADL score = 9 and MMSE score $>$ 26. Whereas subjects with impaired physical and cognitive function had both ADL score $\geq$ 10 and MMSE score $\leq$ 26. HRs were estimated for each 10 mmHg increase in blood pressure adjusted for sex, cardiovascular diseases, diabetes mellitus, antihypertensive medication, smoking.
Abbreviations: SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, PP: pulse pressure, ADL: activities of daily living, MMSE: mini mental examination.

Discussion

The main findings of this study are twofold. First, high blood pressure is not associated with increased risk of stroke in a general population of the oldest-old. Second, in subjects with physical and/or cognitive impairment, high blood pressure might be paradoxically associated with lower risk of stroke.

Although hypertension has been recognized as a strong risk factor for stroke, current evidence shows that the predictive value of high blood pressure for stroke attenuates with age[18]. In line with this evidence, we found that high blood pressure is not associated with increased risk of stroke in the oldest-old. Furthermore, our observations suggest that in very old age high blood pressure in the presence of physical and cognitive impairment, reflecting high load of brain vascular pathologies, may preserve cerebral perfusion and lower risk of stroke. This observation might be explained in different ways. First, lower blood pressure in subjects with physical and cognitive impairment might be related to better treatment of hypertension in high risk subjects. Furthermore, low blood pressure related risk of stroke in subjects with previous functional impairment can be due to the fact that structural heart disease and/or poor cardiac functioning are associated with both low blood pressure and risk of stroke[19]. This might partially explain different associations between blood pressure measures and stroke in subjects with different levels of cognitive function, since subjects with cognitive impairment had higher prevalence of myocardial infarction and heart failure. However, subjects with physical impairment did not have more cardiovascular pathologies at age 85 years compared to the subjects with unimpaired physical function. Additionally, the observed associations in this study did not essentially change after adjustment for antihypertensive medications and most known risk factors for cardiovascular disease, such as sex, smoking, diabetes mellitus and history of cardiovascular diseases.

The alternative explanation can be that low blood pressure may lead to insufficient cerebral perfusion, particularly in the presence of advance cerebrovascular damage[20]. It is well-established that both physical and cognitive impairment are associated with structural and functional alterations in the brain vasculature leading to impaired cerebral perfusion[21, 22]. In this setting, our findings may imply that higher blood pressure in the presence of advanced brain vascular damage can play a compensatory role to improve cerebral perfusion and thereby lower risk of ischemic events, especially low-flow infarcts[23]. However, we are not able to make a causal inference solely from our observational data and further experimental and interventional studies are needed to examine this hypothesis.

Although the number of oldest-old subjects is rapidly increasing and prevalence of hypertension as well as functional impairment is high in this age group[24], clinical guidelines on antihypertensive therapy in very old subjects with different

co-morbidities and functional impairment are not clear[25]. This ambiguity might stem from the fact that clinical trials have generally enrolled healthy and competent older participants while very old individuals with multiple morbidities and cognitive impairment have participated less in such studies[26]. Previously, it has been shown that results of the published trials are not always applicable to the treatment of older frail patients with multiple co-morbid conditions and concomitant functional impairment[27]. Findings of current study may support further investigations to optimize benefits of blood pressure control in very old patients based on level of functional impairment.

Our study has certain strengths and major limitations. As a strength, this study was conducted in a relatively large population of the oldest-old people with long-term follow-up period. As a major limitation, we did not have neuroimaging data to identify subjects with unrecognized cerebrovascular pathologies at baseline. Similarly, we were not able to determine type, load and location of stroke events during follow-up period. However, it has been reported that the majority of strokes in subjects aged 80 years and older are of the ischemic type[28, 29]. Another limitation of this study is that we used only ADL and MMSE to estimate level of physical and cognitive impairment. Since there is no single criterion or definition for physical and cognitive impairment, ADL and MMSE may not fully reflect level of physical and cognitive impairment, respectively. Detection of outcomes by interviewing general practitioners might be the other potential limitation of this study. This method could result in detection bias explaining the differential blood pressure effect in subjects with different levels of functional impairments. In addition, it was possible that general practitioners had different diagnostic sensitivity in patients with and without physical and cognitive impairment.

In conclusion, our findings suggest that impaired physical and cognitive functioning moderates the traditional association between higher blood pressure and increased risk for stroke. We found that in subjects with physical and cognitive impairment, a higher blood pressure was associated with lower risk of stroke. Given the observational design of this study and the mentioned limitations, caution should be used in interpretation of our results as we cannot make a causal inference from our findings. However, these findings merit further interventional and experimental studies, combined with imaging techniques, to test whether less intensive control of blood pressure in patients with physical and cognitive impairment, can contribute to better cerebral perfusion and prevention of subsequent cerebrovascular events.

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4

Visit-to-visit Blood Pressure Variability and Impaired Brain Structure and Function

Manuscript based on this chapter has been published as:
Sabayan B*, Wijsman LW*, Foster-Dingley JC, Stott DJ, Ford I, Buckley BM, Sattar N, Jukema JW, van Osch MJ, van der Grond J, van Buchem MA, Westendorp RG, de Craen AJ, Mooijaart SP. Association of visit-to-visit variability in blood pressure with cognitive function in old age: prospective cohort study.
BMJ. 2013 29;347:f4600.
∗ The authors had equal contribution

Summary

The aim of this chapter is to investigate the association between visit-to-visit blood pressure variability and cognitive function in elderly subjects. In this prospective cohort study, 5461 participants at risk of cardiovascular disease with mean age of 75.3 years were included. Blood pressure was measured every three months during an average period of 3.2 years. Blood pressure variability was defined as the standard deviation of visit-to-visit blood pressure measurements. Four domains of cognitive function including selective attention, processing speed, immediate and delayed memory were assessed. In an MRI substudy of 553 participants, structural brain volumes, cerebral microbleeds, infarcts and white matter hyperintensities were measured. Subjects with higher visit-to-visit systolic blood pressure variability had worse performance on all cognitive tests, including attention (mean difference high vs. low thirds) 3.08 seconds (95% confidence interval (CI) 0.85; 5.31); processing speed -1.16 digits coded (95% CI -1.69; -0.63), immediate memory -0.27 pictures remembered (95% CI -0.41; -0.13) and delayed memory -0.30 pictures remembered (95% CI -0.49; -0.11). Furthermore, higher systolic and diastolic blood pressure variability was associated with lower hippocampal volume and cortical infarcts, and higher diastolic blood pressure variability was associated with cerebral microbleeds (all $p<0.05$). All associations were adjusted for average blood pressure and cardiovascular risk factors. Higher visit-to-visit blood pressure variability independent of average blood pressure associates with impaired cognitive function in old age.

Introduction

Visit-to-visit blood pressure variability independent of average blood pressure is related to cerebrovascular damage[1]. It has been shown that higher visit-to-visit blood pressure variability increases risk of stroke and antihypertensive medications which decrease both blood pressure variability and mean blood pressure more effectively reduce risk of stroke[2]. In addition, observational studies have demonstrated associations of blood pressure variability, independent of average blood pressure, with white matter hyperintensities, carotid artery intima media thickness and atherosclerosis in elderly subjects[3-5].

There is a well-established relationship between increased blood pressure variability and end organ damage[6]. Recent evidence indicates that higher visit-to-visit blood pressure variability is linked with microvascular damage, endothelial injury and disturbances in vascular smooth muscle functioning[7 8]. Indicators of cerebral small vessel disease including white matter hyperintensities, cortical microinfarcts and cerebral microbleeds are implicated in the pathogenesis of cognitive impairment[9-11]. Several pathological, observational and experimental studies showed that disruption of the blood brain barrier due to microvascular damage results in neuronal injury and accelerates neuronal loss and brain atrophy[12]. Hence, higher blood pressure variability might potentially lead to cognitive impairment through changes in the brain structures and development of cerebral small vessel disease.

The aim of this study was to investigate the association of visit-to-visit blood pressure variability independent of average blood pressure with cognitive function in older subjects at high risk of cardiovascular disease. Possible explanations behind this association were additionally investigated in an MRI substudy.

Methods

Study design and participants

The data in this study were obtained from the PROSPER study, a randomized, double-blind, placebo-controlled trial designed to investigate the effect of pravastatin in prevention of vascular events in elderly men and women with pre-existing cardiovascular disease or risk factors thereof. This trial included 5,804 individuals aged 70-82 years old who were enrolled from three collaborating centers in Ireland, Scotland and the Netherlands. Approximately 50% of the participants showed evidence of cardiovascular disease including stable angina, intermittent claudication, stroke (type of stroke, hemorrhagic or ischemic, was unknown), transient ischemic attack, myocardial infarction and vascular surgery.

The rest of participants had one or more major cardiovascular risk factors, defined as hypertension, cigarette smoking or diabetes mellitus. The primary outcome of the PROSPER study was the combined endpoint of definite or suspect death from coronary heart disease, non-fatal myocardial infarction and fatal or non-fatal stroke during a mean follow-up period of 3.2 years. In the present study we included 5,461 participants for whom data on blood pressure variability and cognitive function were available. Additionally, participants from the Netherlands were invited to participate in an MRI substudy. Participants were included from both pravastatin and placebo groups as we previously reported that treatment with pravastatin did not influence cognitive function, structural brain volumes or indicators of cerebral small vessel disease[13-15]. The institutional ethics committees of the three collaborating centers approved the study and all participants gave written informed consent.

Blood pressure measurements

Systolic and diastolic blood pressure were measured at baseline and repeated every three months. Blood pressure was measured in sitting position using a fully automatic electronic sphygmomanometer (Omron M4®). All measurements were performed in the same clinical setting. The average values of these blood pressure measurements were calculated and used in the analyses. Visit-to-visit blood pressure variability was defined as the standard deviation of blood pressure measurements during the study period. We only report the blood pressure variability by using the standard deviation. Variance and coefficient of variation, which are two other measures of variability, are strongly correlated with the standard deviation (data are presented in supplemental table S-1 http://www.bmj.com/content/suppl/2013/07/29/bmj.f4600.DC1) and they showed similar associations with cognitive and MRI outcomes (data not shown).

Cognitive function

The Mini-Mental State Examination (MMSE) was used to evaluate global cognitive function at baseline; a cutoff score of 24 points or more (out of 30) was used as an inclusion criterion to exclude subjects with poor cognitive function at baseline. In the present study we used data on cognitive function assessed at the end of the study, after a mean follow up time of 3.1 years, by a cognitive test battery consisting of four different tests. The Stroop-Colour-Word-Test was used to test selective attention and reaction time of the participants. The participants were asked to read a color name which was displayed in a color different from the color it actually names. The outcome parameter was total number of seconds to complete the test; a higher score therefore indicates worse performance. General

cognitive speed was tested by the Letter-Digit Coding Test. The participants had to match certain digits with letters according to a provided key. The outcome variable was the total number of correct entries in 60 seconds, and therefore higher scores represented better performance. The Picture-Word Learning Test was used to assess immediate and delayed memory performance. Fifteen pictures were presented at the participants, and they were asked to recall as many pictures as possible in three trials. After 20 minutes they were asked to repeat the test to measure their delayed recall. The outcome parameter is the accumulated number of correct recalled pictures, immediate and after 20 minutes. Higher scores thus indicate better performance. A detailed description of the cognitive tests and the procedures has been published previously[16].

MRI substudy

Of the 1,100 Dutch participants in the PROSPER study, 646 consented to participate in the MRI substudy. Forty of the 646 original study participants died during follow-up period. From the remaining 606 participants, an MRI was performed at the end of follow up period. Visit-to-visit blood pressure measurements and MRI data were available for a total number of 553 subjects. Details of individually MRI scan parameters have been published previously[13].

All imaging was performed on an MR system operating at field strength of 1.5 Tesla (Philips Medical Systems, Best, the Netherlands). Gray and white matter volumes were calculated by SIENAX technique. In short, SIENAX starts by extracting brain and skull images from the single whole-head input data. The brain image is then afne-registered to Montreal Neurological Institute (MNI) 152 space (by using the skull image to determine the registration scaling), done primarily to obtain the volumetric scaling factor to be used as normalization for head size. Next, tissue-type segmentation with partial volume estimation is carried out to calculate total volume of brain tissue (including separate estimates of volumes of gray matter, white matter, peripheral gray matter, and ventricular cerebrospinal fluid)[17]. The algorithm FIRST (FMRIBs Integrated Registration and Segmentation Tool) was applied to estimate the volume of hippocampus. In addition, volume of six other subcortical regions including nucleus accumbens, globus pallidus, amygdala, putamen, caudate nucleus and thalamus were estimated. FIRST is part of FSL (FMRIB's Software Library) and performs both registration and segmentation of the mentioned subcortical regions[18]. To assess cerebral microbleeds, all MRI scans were read in consensus by two experienced raters who were blinded to subjects' clinical history. Cerebral microbleeds were defined as focal areas of signal loss on T2*-weighted gradient echo pulse sequence ("blooming effect") that are invisible or smaller on T2-weighted MRI [19]. For each subject the number and location (cortical, subcortical, and in-

fratentorial) of the cerebral microbleeds were recorded. Segmentation of white matter hyperintensities volume was performed automatically using software for Neuro-Image Processing in Experimental Research (SNIPER), an in-house developed program for image processing[20]. This segmentation was based on the T2-weighted and FLAIR images. Cerebral infarcts were defined as parenchymal defects seen on FLAIR images with the same signal intensity as CSF and a surrounding rim of high signal intensity following a vascular distribution.

Demographic and clinical characteristics

Demographic, medical and anthropometric data of the participants were recorded at baseline. A fasting venous blood sample was taken for biochemical and hematological assessment. Apolipoprotein E phenotype was determined on plasma samples by Western blotting [21].

Statistical analysis

Characteristics of the study participants are reported as mean (standard deviation) for continuous variables and frequency (percentage) for categorical variables. The correlation between blood pressure variability and average blood pressure was assessed by calculating the Pearson's Correlation Coefficient. Linear regression models were used to assess the association of blood pressure variability and average blood pressure with cognitive function. Dependent variables were the mean scores of the cognitive tests. In the tables these scores are presented in thirds of systolic and diastolic blood pressure and blood pressure variability. In the MRI substudy, we used logistic regression models to estimate the odds ratio and 95% confidence interval of the presence of microbleeds or infarcts in different thirds of blood pressure variability as well as average blood pressure. Multivariable linear regression models were used to test the association between blood pressure variability and average blood pressure with volume of white matter hyperintensities and structural brain volumes. P-values in all the analyses were calculated using systolic and diastolic blood pressure variability as continuous variables. We performed our analyses in three steps. In the first step, crude analyses were performed, in which we only adjusted for cognitive test version where appropriate. In the second step, we added age, sex, education and country as covariates to investigate the potential influence of these factors on the associations (model 1). In the final model (model 2), we further adjusted the analyses for the following potential confounders: cardiovascular diseases and risk factors (history of vascular disease, history of hypertension, history of diabetes mellitus, smoking status, cholesterol levels, body mass index), average blood pressure, statin treatment and apo E genotype. We adjusted the analyses

of systolic blood pressure variability with cognitive function and MRI outcomes for average systolic blood pressure. The analyses of diastolic blood pressure variability with cognitive function and MRI outcomes were adjusted for average diastolic blood pressure. Since the associations did not essentially change in different models, results of the second model are presented in the manuscript and results from the other models are presented as supplementary data (tables S-2 and S-3 http://www.bmj.com/content/suppl/2013/07/29/bmj.f4600.DC1). All analyses were performed using SPSS software (version 20.0.0, SPSS Inc., Chicago, IL).

Results

Table 1 shows the characteristics of participants in the whole group and MRI substudy. Blood pressure was measured in an average number of 12.7 and 12.9 visits in the whole group and MRI substudy respectively. Average systolic and diastolic blood pressure over the period of blood pressure measurements was 153.1 mmHg and 82.5 mmHg respectively. Mean values of standard deviation of systolic and diastolic blood pressure during this period were 14.8 mmHg and 7.1 mmHg respectively. There was a weak but statistically significant correlation between average systolic blood pressure and standard deviation of systolic blood pressure measurements ($r=0.20$, $p<0.001$). Similarly, average diastolic blood pressure was weakly but statistically significantly correlated with standard deviation of diastolic blood pressure measurements ($r=0.12$, $p<0.001$).

Table 2 shows the association of systolic and diastolic visit-to-visit blood pressure variability with cognitive function. Higher systolic and diastolic blood pressure variability was associated with worse performance on the Stroop test (both $p<0.001$), Letter-Digit Coding test (both $p<0.001$), immediate Picture-Word Learning test (both $p<0.001$) and delayed Picture-Word Learning test (both $p=0.001$). All associations were independent of average blood pressure and cardiovascular diseases and risk factors, as all analyses were adjusted for these factors. Mean cognitive scores (95% confidence intervals) in each third of systolic and diastolic blood pressure variability are presented in Figure 1. Data on the association of blood pressure variability with cognitive function from crude and minimally adjusted models are shown in supplemental material (table S-2 and S-3 http://www.bmj.com/content/suppl/2013/07/29/bmj.f4600.DC1). Furthermore, we found a significant association of higher average systolic and diastolic blood pressure with worse performance in different domains of cognitive function (all $p<0.05$), except for the association between higher average systolic blood pressure and performance on the Picture-Word Learning tests ($p>0.05$) (data presented in the supplemental material table S-4 http://www.bmj.com/content/suppl/2013/07/29/bmj.f4600.DC1).

Table 1. Table 1. Characteristics of study participants in whole group and MRI substudy

	All (n=5,461)	MRI substudy (n=553)
Demographics		
Number of visits, mean (SD)	12.7 (2.4)	12.9 (1.5)
Age, years, mean (SD)	75.3 (3.3)	74.9 (3.2)
Female, n (%)	2,822 (51.7)	241 (43.6)
Age left school, years, mean (SD)	15.1 (2.1)	15.5 (2.9)
Vascular risk factor		
History of hypertension, n (%)	3,399 (62.2)	341 (63.1)
History of diabetes mellitus, n (%)	576 (10.5)	91 (16.5)
History of stroke or TIA, n (%)	606 (11.1)	89 (16.1)
History of myocardial infarction, n (%)	714 (13.1)	67 (12.1)
History of vascular disease, n (%)	2,404 (44.0)	240 (43.4)
Current smoker, n (%)	1,433 (26.2)	115 (20.8)
Body mass index , kg/m^2, mean (SD)	26.9 (4.2)	26.7 (3.6)
Total cholesterol, mmol/L, mean (SD)	5.7 (0.9)	5.7 (0.8)
Blood pressure, mean (SD)		
Systolic blood pressure, mmHg*	153.1 (16.1)	156.1 (16.4)
Diastolic blood pressure, mmHg*	82.5 (7.5)	85.1 (7.3)
Variability in systolic blood pressure, mmHg**	14.8 (5.0)	13.9 (4.6)
Variability in diastolic blood pressure, mmHg**	7.1 (2.9)	7.4 (2.3)
Cognitive function, mean (SD)***		
Stroop test score, seconds	69.4 (31.6)	56.9 (23.3)
Letter-Digit Coding test score, digits coded	21.8 (8.0)	26.3 (7.4)
PLTi score, pictures remembered	9.2 (2.2)	10.1 (2.2)
PLTd score, pictures remembered	9.8 (3.1)	11.1 (3.0)
MRI features		
Grey matter, ml, mean (SD)	—	590 (44)
White matter, ml, mean (SD)	—	768 (38)
Hippocampus, ml, mean (SD)	—	7.5 (1.1)
Micro-bleeds, n (%)	—	124 (24.0)
Infarcts, n (%)	—	180 (33.6)
Cortical	—	65 (12.1)
Lacunar	—	112 (21.0)
WMH volume, ml, mean (SD)	—	7.2 (1.1)

Abbreviations: SD, standard deviation; n, number; TIA, transient ischemic attack; PLTi, Picture-Word Learning

Test immediate; PLTd, Picture-Word Learning Test delayed; WMH, white matter hyperintensity.

∗ defined as the mean of all blood pressure measurements during follow-up.

∗∗ defined as the standard deviation of all blood pressure measurements during follow-up.

∗∗∗ defined as the cognitive test score at the end of follow-up.

∗∗∗∗ defined as the sum of the z-score of each cognitive test, averaged across all available cognitive tests.

Table 2. Cognitive function in thirds of visit-to-visit blood pressure variability

	Third of visit-to-visit blood pressure variability			
	Low (n=1820)	Middle (n=1821)	High (n=1820)	P-value
Systolic blood pressure				
Range of SD, mmHg	0.7-12.2	12.3-16.2	16.3-64.4	
Stroop, seconds	68.46 (0.79)	68.75 (0.79)	71.54 (0.82)	<0.001
LDCT, digits coded	22.40 (0.19)	21.82 (0.19)	21.24 (0.19)	<0.001
PLTi, pictures remembered	9.37 (0.05)	9.28 (0.05)	9.10 (0.05)	<0.001
PLTd, pictures remembered	10.00 (0.07)	9.89 (0.07)	9.70 (0.08)	0.001
Diastolic blood pressure				
Range of SD, mmHg	0-6.5	6.6-8.5	8.6-33.1	
Stroop, seconds	68.28 (0.79)	68.89 (0.79)	71.34 (0.80)	<0.001
LDCT, digits coded	22.35 (0.19)	21.93 (0.19)	21.27 (0.19)	<0.001
PLTi, pictures remembered	9.41 (0.05)	9.22 (0.05)	9.13 (0.05)	<0.001
PLTd, pictures remembered	10.01 (0.07)	9.88 (0.07)	9.74 (0.07)	0.001

Abbreviations: SD, standard deviation; n, number; LDCT, Letter-Digit Coding test; PLTi, Picture-Word Learning Test immediate; PLTd, Picture-Word Learning Test delayed.

Data are adjusted values of the mean (standard error) of each cognitive function test.

Adjustments were made for age, sex, body mass index, Statin treatment, apo E genotype, country, education, test version where appropriate, smoking, cholesterol level, history of vascular diseases, history of hypertension, history of diabetes mellitus, and average blood pressure measures.

Table 3. Structural brain volumes in three groups of visit-to-visit blood pressure variability

	Three groups of visit-to-visit blood pressure variability			
	Low	Middle	High	P-value
Systolic blood pressure	(n = 194)	(n = 210)	(n = 149)	
Range of SD, mmHg	0.7-12.2	12.3 - 16.2	16.3 - 64.4	
Grey matter	593 (3)	590 (3)	589 (3)	0.21
White matter	770 (3)	770 (3)	765 (3)	0.19
Hippocampus	7.6 (0.07)	7.6 (0.07)	7.4 (0.08)	0.01
Diastolic blood pressure	(n = 178)	(n=184)	(n=191)	
Range of SD, mmHg	0 - 6.5	6.6 - 8.5	8.6 - 33.1	
Grey matter	591 (3)	594 (3)	587 (3)	0.18
White matter	768 (3)	772 (3)	764 (3)	0.62
Hippocampus	7.6 (0.07)	7.5 (0.07)	7.4 (0.07)	0.01

Data are structural brain volumes presented in mean (standard error) ml.

Abbreviations: SD, standard deviation.

Analyses were adjusted for age, sex, body mass index, Statin treatment, smoking, cholesterol level, history of vascular diseases, history of hypertension, history of diabetes mellitus, and average blood pressure measures.

Table 3 shows the association of systolic and diastolic visit-to-visit blood pressure variability with structural brain volumes. Higher systolic and diastolic blood pressure variability was associated with lower hippocampal volume (both p=0.01). There was no association between blood pressure variability and volume of the other brain structures (all p>0.05), except for the association between higher systolic blood pressure variability and lower amygdala and putamen volumes (both p=0.04). Again, all analyses were adjusted for average systolic and diastolic blood pressure, which themselves did not associate with structural brain volumes (all p>0.05) (table S-6 http://www.bmj.com/content/suppl/2013/07/29/bmj.f4600.DC1).

Table 4. Microbleeds, infarcts and white matter hyperintensities in three groups of visit-to-visit blood pressure variability

	Three groups of visit-to-visit blood pressure variability			
	Low	Middle	High	P-value
Systolic blood pressure	(n=207)	(n=191)	(n=137)	
Range of SD, mmHg	0.7-12.2	12.3-16.2	16.3-64.4	
Microbleeds, OR (95% CI)	1(ref)	1.13 (0.69-1.85)	1.30 (0.77-2.21)	0.39
Infarcts, OR (95% CI)	1(ref)	0.95 (0.61-1.48)	1.26 (0.78-2.04)	0.40
Cortical	1(ref)	1.34 (0.68-2.64)	2.22 (1.09-4.54)	0.02
Lacunar	1(ref)	0.79 (0.48-1.31)	0.84 (0.48-1.46)	0.97
WMH volume, ml, mean (SE)	8.12 (1.02)	7.34 (1.08)	7.79 (1.19)	0.98
Diastolic blood pressure	(n=215)	(n=166)	(n=154)	
Range of SD, mmHg	0-6.5	6.6-8.5	8.6-33.1	
Microbleeds, OR (95% CI)	1(ref)	1.75 (1.05-2.91)	1.77 (1.06-2.96)	0.01
Infarcts, OR (95% CI)	1 (ref)	0.99 (0.63-1.56)	1.32 (0.84-2.06)	0.43
Cortical	1(ref)	1.87 (0.93-3.76)	2.19 (1.10-4.37)	0.02
Lacunar	1(ref)	0.95 (0.57-1.60)	1.17 (0.70-1.95)	0.75
WMH volume, ml, mean (SE)	7.65 (1.05)	8.27 (1.11)	7.93 (1.10)	0.55

Abbreviations: SD, standard deviation; n, number; OR: odds ratio, CI: confidence interval, WMH: white matter hyperintensity, SE: standard error.

Analyses were adjusted for age, sex, body mass index, statin treatment, smoking, cholesterol level, history of vascular diseases, history of hypertension, history of diabetes mellitus, and average blood pressure measures.

Data for microbleeds, infarcts and white matter hyperintensities were available for 535 participants

Table 4 shows the association between visit-to-visit blood pressure variability and cerebral microbleeds, infarcts and white matter hyperintensities. Higher systolic and diastolic blood pressure variability was associated with higher risk of cortical infarcts (both p=0.02). Prevalence of cortical infarcts in participants with low, middle and high systolic blood pressure variability was 9.2%, 12.0%

and 16.2% respectively. Prevalence of cortical infarcts in participants with low, middle and high diastolic blood pressure variability was 7.9%, 13.3% and 16.2% respectively. Furthermore, higher diastolic blood pressure variability was associated with higher risk of all type microbleeds (p=0.01) as well as subcortical microbleeds (p=0.004). Prevalence of microbleeds in participants with low, middle and high systolic blood pressure variability was 21.2%, 23.9% and 28.4% respectively. Prevalence of cortical infarcts in participants with low, middle and high diastolic blood pressure variability was 17.8%, 27.5% and 28.9% respectively. Systolic and diastolic blood pressure variability was not associated with white matter hyperintensities (both $p>0.05$). We found no association of average systolic and diastolic blood pressure with cerebral microbleeds, infarcts and white matter hyperintensities (all $p>0.05$) (data presented in the supplemental material table S-6). We performed four sensitivity analyses to explore whether the association between visit-to-visit blood pressure variability and the studied outcomes could be affected by (1) participants with a history of clinical stroke/transient ischemic attack (n=606) and cardiovascular disease (n=2404); (2) participants with new or change in antihypertensive therapy during the study period (n=2733); (3) participants who developed vascular events (n=872) or arrhythmia (n=506) during the study period; (4) participants with high average blood pressure (defined as average systolic blood pressure of $\geq$ 140 mmHg and diastolic blood pressure of $\geq$ 80 mmHg during the study period) (n=830). These sensitivity analyses showed that the results did not materially change. In an overall sensitivity analysis we excluded all participants with previously mentioned conditions (n=4654) and results remained essentially unchanged (data not shown).

Discussion

The main findings of this study are threefold. First, higher visit-to-visit systolic and diastolic blood pressure variability is associated with worse performance in different domains of cognitive function. Second, higher systolic and diastolic blood pressure variability is associated with lower hippocampal volume and risk of cortical infarcts, and higher diastolic blood pressure variability is associated with risk of cerebral microbleeds. Third, these associations are independent of various cardiovascular risk factors in particular average systolic and diastolic blood pressure.

Although hypertension is a well-established risk factor for cardiovascular diseases[22], increasing evidence indicates that the predictive value of conventional blood pressure measurement for cardiovascular diseases attenuates with increasing age[23 24]. Recent studies have shown that higher visit-to-visit blood

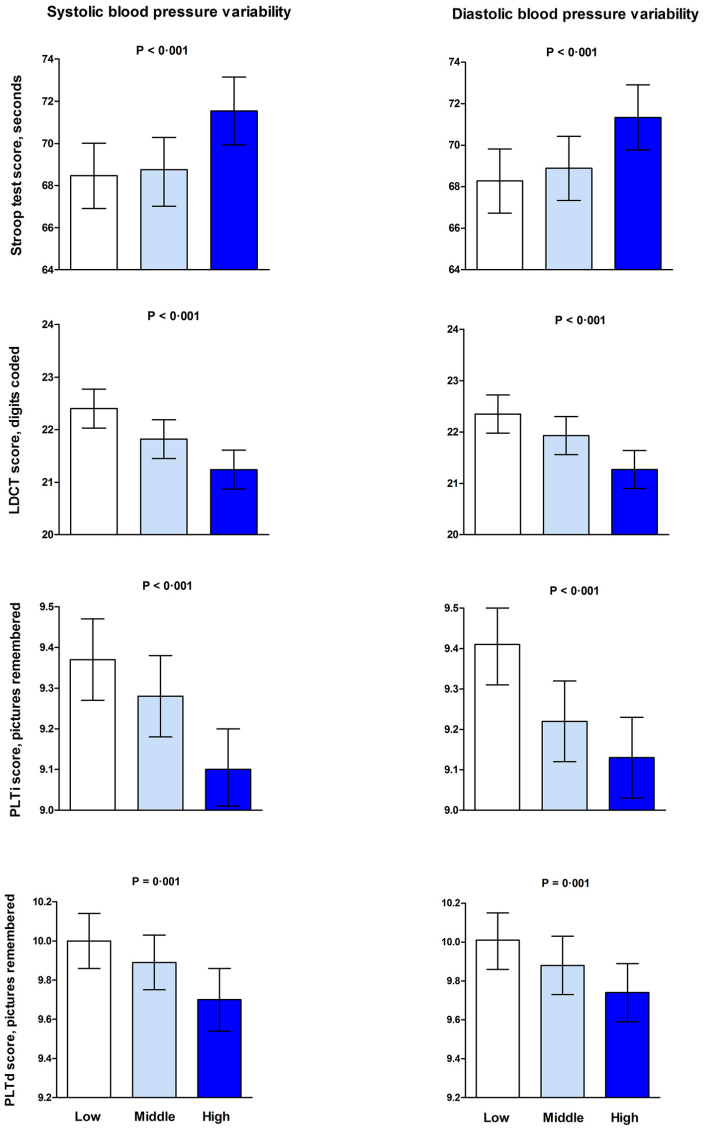

Figure 1. Stroop test, Letter-Digit Coding Test (LDCT), immediate Picture-Word Learning Test (PLTi) and delayed Picture-Word Learning Test (PLTd) scores in low, middle and high thirds of systolic and diastolic visit-to-visit blood pressure variability. Bars represent mean and 95% confidence interval. All analyses were adjusted for age, sex, body mass index, Statin treatment, apo E genotype, country, education, test version where appropriate, smoking, cholesterol level, history of vascular diseases, history of hypertension, history of diabetes mellitus, and average blood pressure measures.

pressure variability increases risk of cardiovascular events[25], stroke [1 24] and carotid artery atherosclerosis [26] in older subjects, independent of average blood pressure. Given the link between neuro-vascular dysfunction and cognitive impairment [27], a recent study on 201 elderly participants at high risk of cardiovascular disease showed that high visit-to-visit blood pressure variability during 12 months was associated with worse performance in MMSE and global deterioration scale[28]. Consistent with this finding, by using a population of over five thousand participants and over three years of blood pressure measurements, we showed that high visit-to-visit systolic and diastolic blood pressure variability associates with worse performance in different domains of cognitive function including selective attention, processing speed, immediate verbal memory and delayed verbal memory.

The magnitude of associations in this study, reflected as differences in cognitive scores between top and bottom thirds of systolic and diastolic blood pressure variability, are comparable with the observed differences in cognitive function between groups of apolipoprotein E (Apo E) genotype on cognitive function[29]. Apo E4 genotype is a well-recognized risk factor for development of dementia in later life and it has been shown that subjects who carry this risk factor have a four times higher risk of developing late onset Alzheimer's disease[30]. This similar differences in cognitive test scores in Apo E groups and blood pressure variability implies that the observed associations can be considered clinically relevant. Different explanations can be proposed for the observed association between high visit-to-visit blood pressure variability and impaired cognitive function. First, both blood pressure variability and cognitive impairment could stem from a common cause, without being causally related themselves. Cardiovascular risk factors are the most likely candidate[31]. Nevertheless, we reported our analyses adjusted for different cardiovascular risk factors and we performed a sensitivity analysis, by separately excluding subjects with history of cardiovascular diseases, which did not change our estimates, although we accept that residual confounding could remain from unmeasured CVD risk factors. As a second explanation, high visit-to-visit blood pressure variability might reflect a long term hemodynamic instability in the systemic circulation which puts stress on the vascular endothelium[7, 32]. This hemodynamic stress may lead to endothelial dysfunction and micro-vascular damage with consequent alterations in brain structure and function[33]. Thirdly, exaggerated fluctuations in systemic blood pressure could result in repeated episodes of cerebral hypoperfusion causing neuronal injury and cell death particularly in vulnerable brain regions such as the hippocampus[4]. In line with latter explanations, we found that higher visit-to-visit blood pressure variability is related to lower hippocampal volume and presence of cerebral microbleeds and cortical infarcts. Given the well-described association of hippocampal atrophy [34] and cerebral small vessel

disease [10] with cognitive impairment, our findings may suggest that decreased hippocampal volume, cerebral microbleeds and cortical infarcts are potential pathogenic mechanisms behind the association between visit-to-visit blood pressure variability and cognitive impairment.

Current evidence on the association of blood pressure variability with structural brain damage and cerebral small vessel disease mainly comes from studies that focused on ambulatory blood pressure variability rather than visit-to-visit blood pressure variability. These studies showed that higher ambulatory blood pressure variability associates with brain atrophy and white matter lesions[35-37]. In the present study, we only observed the association of visit-to-visit blood pressure variability with lower hippocampal volume, cerebral microbleeds and cortical infarcts. This might imply that different measures of blood pressure variability carry different predictive values for brain outcomes[24]. Data on the association between visit-to-visit blood pressure variability and manifestations of small vessel diseases are scarce. Consistent with our findings, a recent study showed that higher visit-to-visit blood pressure variability in subjects with a history of ischemic stroke was associated with progression of cerebral microbleeds but not with white matter lesions[38]. It is worth pointing out that it is still unclear whether higher blood pressure variability is a cause or consequence of brain pathologies. It has been suggested that higher blood pressure variability itself could originate from previously established brain pathologies disturbing central autonomic control[39]. While clinical trials have shown conflicting findings on the benefit of antihypertensive therapy on reducing risk of dementia, calcium channel blockers, the most effective drug class to reduce blood pressure variability[40], showed significant efficacy in lowering risk of vascular cognitive impairment[41]. This might highlight potential clinical implications of blood pressure variability reducing agents in lowering risk of brain vascular pathologies and cognitive impairment in old age. Collectively, we are not able to make a causal inference from our observation and future long-term investigations are warranted to examine whether strategies to reduce blood pressure variability can effectively decrease risk of cognitive impairment as well as brain vascular pathologies.

The major strengths of this study include a large sample size and application of an extended standardized cognitive test battery to assess cognitive function. In addition, availability of neuroimaging data provided us with a unique opportunity to investigate potential biological pathways linked to the association between visit-to-visit blood pressure variability and cognitive function. However, this study has certain limitations that should be kept in mind when evaluating the results. First, we included elderly participants at risk of cardiovascular diseases with relatively preserved cognitive function (MMSE $\geq$ 24 points), which might limit the extrapolation of our findings to a general population of elderly.

However, this restriction has likely resulted in a homogeneous study population who are among the main target groups for preventing cognitive decline[42]. Second, the outcomes of this study were evaluated at one time point and long-term longitudinal studies are needed to test whether lowering blood pressure variability could lead to decelerated cognitive decline and lower load of brain pathologies. Third, due to the limited number of participants in the MRI sub-study, we had limited power in several outcome measures. This means that absence of significant associations for several outcome measures should be interpreted with caution. There are reports indicating that higher visit-to-visit blood pressure variability is related to a higher risk of stroke and cerebrovascular damage[1], however, the exact mechanisms behind these associations are still unclear. This issue needs to be addressed in future MRI studies with larger number of participants. Fourth, although we adjusted our analyses for different potential confounding factors, there still might be some other confounders that we did not consider in our analyses. Future studies investigating the determinants of visit-to-visit blood pressure variability might help to better understand the association between visit-to-visit blood pressure variability and neurocognitive outcomes.

In conclusion, our findings suggest that higher visit-to-visit blood pressure variability independent of average blood pressure is associated with worse cognitive performance in older subjects at high risk of cardiovascular disease. Changes in hippocampal volume and occurrence of cortical infarcts and cerebral microbleeds might be candidate pathogenic mechanisms behind this association. This observation merits further interventional studies to address whether reducing blood pressure variability can decrease risk of cognitive impairment in old age.

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5

Cardiac Function and Features of Brain Aging

Manuscript based on this chapter has been submitted as:
Sabayan B, van Buchem MA, Sigurdsson S, Zhang Q, Harris TB, Gudnason V, Arai AE, Launer LJ. Cardiac Hemodynamics and Brain Ageing: The AGES-Reykjavik Study

Summary

The aim of this chapter is to investigate the association between cardiac hemodynamics and features of brain ageing in community dwelling older subjects. Data are from 931 subjects (b. 1907-35) who participated in a sub-study of the Age, Gene/Environment Susceptibility (AGES)-Reykjavik Study. Cardiac hemodynamics including left ventricular stroke volume (LVSV) and cardiac output (CO) were measured using cardiac MRI. We assessed parenchymal brain volumes, performance in three domains of cognition and presence of mild cognitive impairment or dementia. Each 10 ml lower LVSV was associated with 5.0 ml (95% CI 2.6-7.4) lower total parenchymal brain volume and 4.4 ml (95% CI 2.5-6.3) lower gray matter volume. Likewise, each unit (L/min) lower CO was associated with 4.4 ml (95% CI 1.0-7.9) lower total brain parenchymal volume and 4.5 ml (95% CI 1.8-7.3) lower gray matter volume. Lower LVSV was associated with worse performance in memory ($p=0.040$), processing speed ($p=0.003$) and executive functioning ($p<0.001$). Lower CO was associated with worse performance in processing speed ($p=0.014$) and executive functioning ($p<0.001$). A decrease in each 10 ml of LVSV and each unit of CO associated with higher risk of mild cognitive impairment or dementia (OR: 1.25, 95% CI 1.02-1.561 and OR: 1.43, 95% CI 1.05-1.96 respectively). All these associations were independent of cardiovascular diseases and risk factors such as hypertension, diabetes mellitus and body mass index. Sub-optimal cardiac functioning, as reflected in lower levels of stroke volume and cardiac output, is associated with structural and functional features of brain ageing.

Introduction

Structural and functional integrity of the brain depends on adequate and constant supply of oxygen and nutrient through cerebral blood flow[1]. Chronic cerebral hypoperfusion has been shown to play a key role in development of age-related brain pathologies in animal models[2]. Animal models with decreased cerebral blood flow carry a higher risk for neuronal injury and death, blood brain barrier disruption, cerebrovascular pathologies, and cognitive deficit[3-6].

Congestive heart failure has a well-established association with decline in cerebral blood flow[7]. Hence, patients with heart failure have been described as human models for studying the impact of cerebral hypoperfusion on abnormal brain ageing[8]. Different lines of evidence indicate that patients with heart failure are at a higher risk for structural and functional brain abnormalities and interventions to improve cardiac functioning might lead to neurocognitive benefits in these patients[9-11]. Recently, it has been debated whether early disturbances in cardiac functioning, as reflected in cardiac hemodynamics, should also be considered as a risk factor for abnormal brain ageing[12]. Given the scarcity of data on the link between suboptimal changes in cardiac functioning and features of brain ageing in elderly community dwelling subjects, we aimed to investigate the association of cardiac hemodynamics with structural and functional features of brain ageing in a community-based cohort of older subjects.

Methods

Study population

Participants were from the ICELAND MI (Imaging Cardiac Evaluation to Locate Areas of Necrosis and Detect MI) cohort, which is a substudy of the Age, Gene/Environment Susceptibility (AGES)-Reykjavik Study (n=5764)[13]. The AGES-Reykjavik study is a randomly selected population-based cohort of men and women born between 1907 and 1935 in Iceland. This study was approved by the National Bioethics Committee in Iceland that acts as the institutional review board for the Icelandic Heart Association and by the National Institute on Ageing intramural institutional review board. Participants in the AGES-Reykjavik study were enrolled from January 2004 to January 2007. Details of inclusion procedure for the AGES-Reykjavik study have been reported previously[14]. To be eligible for the sub-study participants should be free of contraindications for MRI scanning and gadolinium contrast injection. All participants gave informed consent for this sub-study.
The overall study goals and study design of the ICELAND MI study have been

described previously[13]. Participants in the ICELAND MI cohort were recruited in two phases. The first phase involved random recruitment and a second phase recruited all eligible and willing participants with diabetes. Of those who underwent cardiac MRI (n = 970), 34 participants had nondiagnostic cardiac MRI scans due to arrhythmia or inability to hold breath (n = 14), claustrophobia (n = 7), inability to gate cardiac images (n = 3), technical issues with reconstruction and data transfer (n = 9), or artifact from spinal implants (n = 1). For five participants data on the brain outcomes was not available leaving a final cohort of 931 participants (including 671 who were randomly selected and 260 with diabetes) for this analysis.

Cardiac MRI and hemodynamic parameters

Cardiac magnetic resonance scans were performed on a study-dedicated 1.5-T Signa Twinspeed system (GE Healthcare) using a 4-element cardiac phased array coil. Typical cine steady-state free precession (SSFP) scan parameters resulted in pixel dimensions of 1.8 $\times$ 2.1 mm, a slice thickness of 8mm with a 3-mm gap, and 30 images per cycle. Standard long-axis and short-axis views were obtained to evaluate global and regional function. End-systolic phase was determined as the minimal cross-sectional area of a midventricular slice. Left ventricular end diastolic volume (LVEDV) and Left ventricular end systolic volume (LVESV) were computed by the summation of disks method. Left ventricular stroke volume (LVSV) in ml was calculated by subtracting LVESV from LVEDV. Left ventricular ejection fraction (LVEF) in % was calculated as LVSV/LVED $\times$ 100 and cardiac output (CO) in L/min was calculated as LVSV/1000 $\times$ heartbeat per minute.

Brain Imaging

All the participants underwent a high-resolution brain MRI on the same 1.5-T system. The imaging protocol has been described previously[14, 15] and included 3D spoiled-gradient recalled T1-weighted, fast spin echo proton density/T2-weighted, fluid-attenuated inversion recovery (FLAIR) and echo-planar imaging gradient echo T2*-weighted sequences. All images were acquired to give full brain coverage with slices angled parallel to the anterior commissure-posterior commissure line in order to give reproducible image views in the oblique-axial plane. Total brain, white and grey matter, and white matter hyperintensity volumes were computed automatically with the AGES-Reykjavik/Montreal Neurological Institute pipeline, which accommodates full brain coverage including cerebellum and brainstem, includes multispectral images (T1-weighted 3D spoiled-gradient recalled sequence, FLAIR and proton density/T2-weighted fast spin echo sequences), and is high throughput and with minimal editing. Data

on manifestations of cerebral small vessel disease including white matter hyperintensities, infarcts and cerebral microbleeds (CMB) were obtained. The segmentation pipeline, its components and accuracy has been described in detail elsewhere[16]. White matter hyperintensities were considered present in regions where signal intensity was higher than that of normal white and grey matter on both T2-weighted and FLAIR images. Infarcts and CMB were evaluated qualitatively according to a standardized protocol that required a neuroradiologist to identify the parenchymal defects and radiographers who entered additional data on slice, location, and image characteristics. Infarcts were defined as a defect of the brain parenchyma with signal intensity equal to cerebrospinal fluid on all pulse sequences (FLAIR, T2-weighted, proton density weighted). CMB were defined as focal areas of signal void within the brain parenchyma that (1) are visible on T2*-weighted GRE-EPI images, (2) are smaller or invisible on T2-weighted FSE images ("blooming effect"), (3) are not abutting a parenchymal defect, and (4) do not show any other structure in the area of signal void. Using these criteria, CMB can be differentiated from areas of signal void based on vascular flow voids (which do not show the "blooming effect"), from past larger hematomas associated with parenchymal defects, and from cavernomas. Areas of symmetric hypointensities of the globus pallidus and putamen, likely to represent calcification or nonhemorrhagic iron deposits, were excluded.

Cognitive function and depressive symptoms

A battery of six different neurocognitive tests was administered to all participants. From these tests, three cognitive domain composite scores were calculated: (1) the memory composite score included the immediate and delayed recall of a modified version of the California Verbal Learning Test[17]; (2) the speed of processing composite included the Figure Comparison Test[18], the Digit Symbol Substitution Test[19], and the Stroop Test[20] part 1 and 2; and (3) the executive function composite included a short version of the Cambridge Neuropsychological Test Automated Battery Spatial Working Memory test, the Digits Backward test[19], and the Stroop test part 3. Composite measures were computed by converting raw scores on each test to standardized Z-scores and averaging the Z-scores across the tests in each composite. Inter-rater reliability for all tests was excellent (Spearman correlations range, 0.96-0.99). Cognitive impairment was considered to be present if subjects had either mild cognitive impairment or dementia. Mild cognitive impairment and dementia case ascertainment was a 3-step process described previously[21]. Briefly, all subjects were screened on cognitive function with the Mini-Mental State Examination[22] and Digit Symbol Substitution Test. Those with positive screen results were administered a diagnostic battery of neuropsychological tests and, among them, those with positive

screen results were examined by a neurologist and a proxy interview was administered. A consensus diagnosis, according to international guideline, was made by a panel that included a geriatrician, neurologist, neuropsychologist, and neuroradiologist. Depressive symptoms were assessed using the 15-item Geriatrics Depression Scale (GDS-15)[23]. The GDS-15 is a well-established screening tool to detect depression in elderly people and consists of yes and no questions. Higher scores in the GDS-15 indicate greater number of depressive symptoms.

Other covariates

Level of education and smoking status were assessed by questionnaire. Diabetes was defined as a history of diabetes, use of glucose-modifying medication, or fasting blood glucose of 7 mmol/L. Hypertension was defined as measured systolic blood pressure 140 mm Hg or diastolic blood pressure 90 mm Hg, or self-reported doctor's diagnosis of hypertension, or using antihypertensive medications. Prevalent coronary heart disease was defined as self-reported history of coronary artery disease or coronary artery bypass surgery or angioplasty or angina pectoris on the Rose Angina Questionnaire, or evidence on ECG of possible or probable myocardial infarction. The diagnosis of atrial fibrillation was made by a twelve lead electrocardiogram (ECG) performed during the AGES-Reykjavik study comprehensive examination. Additionally, hospital discharge diagnosis codes from all hospitals in Reykjavik from January 1987 till the day of the study examination were reviewed for the diagnosis of atrial fibrillation (ICD-9 code 427.9 or ICD-10 code I48).

Statistical analyses

Characteristics of the study participants are reported as mean (standard deviation) for continuous variables and number (percentage) for categorical variables. GDS score is reported as median (interquartile range) since it was not distributed normally. The association between hemodynamic parameters and continues outcomes was estimated with linear regression models, and the association between hemodynamic parameters and dichotomous outcomes was estimated with logistic regression models. We performed our analyses in two steps. In the first step all the analyses were adjusted for age and sex. In the second step all analyses were adjusted for age, sex, education, smoking status, hypertension, diabetes mellitus, prevalent coronary heart disease, total cholesterol, body mass index, atrial fibrillation and systolic blood pressure. For the association between hemodynamic parameters and brain volumes, analyses were additionally adjusted for intracranial volume. All the probability values were calculated by using cardiac hemodynamics as continuous variables. Analysis of covariance was used

to calculate the adjusted means and standard errors for the brain volumes and cognitive scores in the tertiles of hemodynamic parameters. All analyses were carried out using SPSS software (version 20.0.0, SPSS Inc., Chicago, IL).

Results

Mean age of participants was 75.9 years and 47.7% of them were male. Average values for LVEF, LVSV and CO were 60.5%, 62.1 ml and 4.0 L/min respectively (Table 1).

Table 1. Characteristics of the study participants: AGES-RS ICELAND-MI

Characteristics	Values
Number of participants	931
Socio-demographics	
Age, mean (SD)	75.9 (5.2)
Male, n (%)	444 (47.7)
Low education*, n (%)	206 (22.2)
Current smoker, n (%)	105 (11.3)
Cardiovascular risk factors	
Hypertension, n (%)	770 (82.7)
Diabetes mellitus, n (%)	330 (35.4)
Coronary heart disease, n (%)	208 (22.3)
Atrial fibrillation, n (%)	138 (14.8)
Systolic blood pressure, mmHg, mean (SD)	143.4 (19.7)
Diastolic blood pressure, mmHg, mean (SD)	74.0 (9.5)
Total cholesterol, mmol/L, mean (SD)	5.5 (1.2)
Body mass index, kg/m^2, mean (SD)	27.6 (4.3)
Cardiac hemodynamics	
Left ventricular end diastolic volume, ml, mean (SD)	105.0 (29.8)
Left ventricular end systolic volume, ml, mean (SD)	43.0 (23.3)
Left ventricular ejection fraction, %, mean (SD)	60.5 (9.8)
Left ventricular stroke volume, ml, mean (SD)	62.1 (15.2)
Cardiac output**, L/min, mean (SD)	4.0 (1.0)
Brain outcomes	
Total brain tissue volume, ml, mean (SD)	1082.0 (104.8)
Grey matter volume, ml, mean (SD)	678.0 (64.8)
White matter volume, ml, mean (SD)	384.3 (48.2)
Memory composite score, point, mean (SD)	0.07 (0.9)
Processing speed composite score, point, mean (SD)	0.10 (0.8)
Executive function composite score, point, mean (SD)	0.04 (0.7)
Geriatric depression scale score, point, median (IQR)	2 (1-3)
Cognitive impairment***, n (%)	81 (8.8)
White matter lesion volume, ml, mean (SD)	19.7 (18.2)
Infarcts, n (%)	311 (33.4)
Microbleeds, n (%)	110 (11.9)

∗ Primary school education or less

∗∗ Data for cardiac output was available for 922 participants.

∗∗∗ Presence of mild cognitive impairment or dementia

Abbreviations: SD; standard deviation, n; number, IQR; inter quartile range,

As presented in table 2, multivariate analyses showed that each 10 ml lower LVSV was associated with 5.0 ml (95% CI 2.6-7.4) lower total brain parenchymal volume and 4.4 ml (95% CI 2.5-6.3) lower gray matter volume. Likewise, each unit (L/min) lower CO was associated with 4.4 ml (95% CI 1.0-7.9) lower total brain parenchymal volume and 4.5 ml (95% CI 1.8-7.3) lower gray matter volume. Lower LVSV and CO were not associated with white matter volume and we found no association between LVEF and any of the brain volumes we examined (all $p<0.05$). Figure 1 shows adjusted mean values of the brain volumes in tertiles of LVEF, LVSV and CO.

In multivariate analyses lower LVEF was associated with worse performance in executive function ($p<0.001$) (Table 3). Lower LVSV was associated with worse performance on memory ($p=0.04$), processing speed ($p=0.003$) and executive function ($p<0.001$) tests. Likewise, lower CO was associated with worse scores in processing speed ($p=0.014$) and executive function ($p<0.001$). Figure 2 shows adjusted mean values of the cognitive scores in tertiles of LVEF, LVSV and CO.

In age and sex adjusted analyses, lower LVEF, LVSV and CO were associated with depressive symptoms (Table 4). However, further adjustment for cardiovascular risk factors attenuated the significance of the associations. We observed that each 10 ml lower LVSV was associated with 1.25-fold (95% CI 1.02-1.51) higher risk of cognitive impairment and each unit lower CO was associated with 1.43-fold (95% CI 1.05-1.96) higher risk of cognitive impairment. All these associations were independent of socio-demographic and cardiovascular factors. We found no associations between cardiac hemodynamic parameters and manifestations of cerebral small vessel disease. To test whether the association of cardiac hemodynamic parameters with brain volumes and cognitive functioning was the same in the groups of randomly selected subjects and diabetic patients, we performed a stratified analysis and found similar associations (all p for interactions <0.05). In a sensitivity analysis, to test whether observed associations were not dependent on subjects with very low cardiac functioning, we excluded subjects in the lowest decile of the cardiac hemodynamic parameters and the associations did not essentially change (data not shown).

Table 2. Cardiac hemodynamic measures and brain volumes: AGES-RS ICELAND-MI

		Total brain parenchyma		Gray matter		White matter	
		Beta* (95% CI)	P value	Beta* (95% CI)	P value	Beta* (95% CI)	P value
LVEF	Model 1	-4.1 (-10.0, 1.8)	0.17	-4.0 (-7.7, -0.3)	0.03	-1.8 (-3.5, 2.0)	0.58
	Model 2	-2.3 (-5.8, 1.2)	0.19	-2.1 (-4.9, 0.7)	0.14	-0.4 (-2.3, 1.5)	0.67
LVSV	Model 1	-8.9 (-12.8, -5.0)	<0.001	-7.1 (-9.5, -4.6)	<0.001	-1.8 (-3.6, 0.5)	0.06
	Model 2	-5.0 (-7.4, -2.6)	<0.001	-4.4 (-6.3, -2.5)	<0.001	-1.1 (-2.4, 0.2)	0.09
CO	Model 1	-10.8 (-16.4, -5.2)	<0.001	-9.2 (-12.8, -5.7)	<0.001	-1.4 (-4.1, 1.6)	0.29
	Model 2	-4.4 (-7.9, -1.0)	0.012	-4.5 (-7.3, -1.8)	0.001	-0.6 (-1.9, 1.8)	0.94

Model 1- Adjusted for age and sex

Model 2- Adjusted for age, sex, education, current smoker, intracranial volume, history of hypertension, diabetes mellitus, systolic blood pressure, prevalent coronary heart disease, total cholesterol, body mass index, atrial fibrillation

∗ Betas represent change in the brain volumes for each 10 % lower LVEF, 10 ml lower LVSV and one L/min lower CO.

Abbreviation: LVEF; Left ventricular ejection fraction, LVSV: Left ventricular stroke volume, CO; Cardiac output

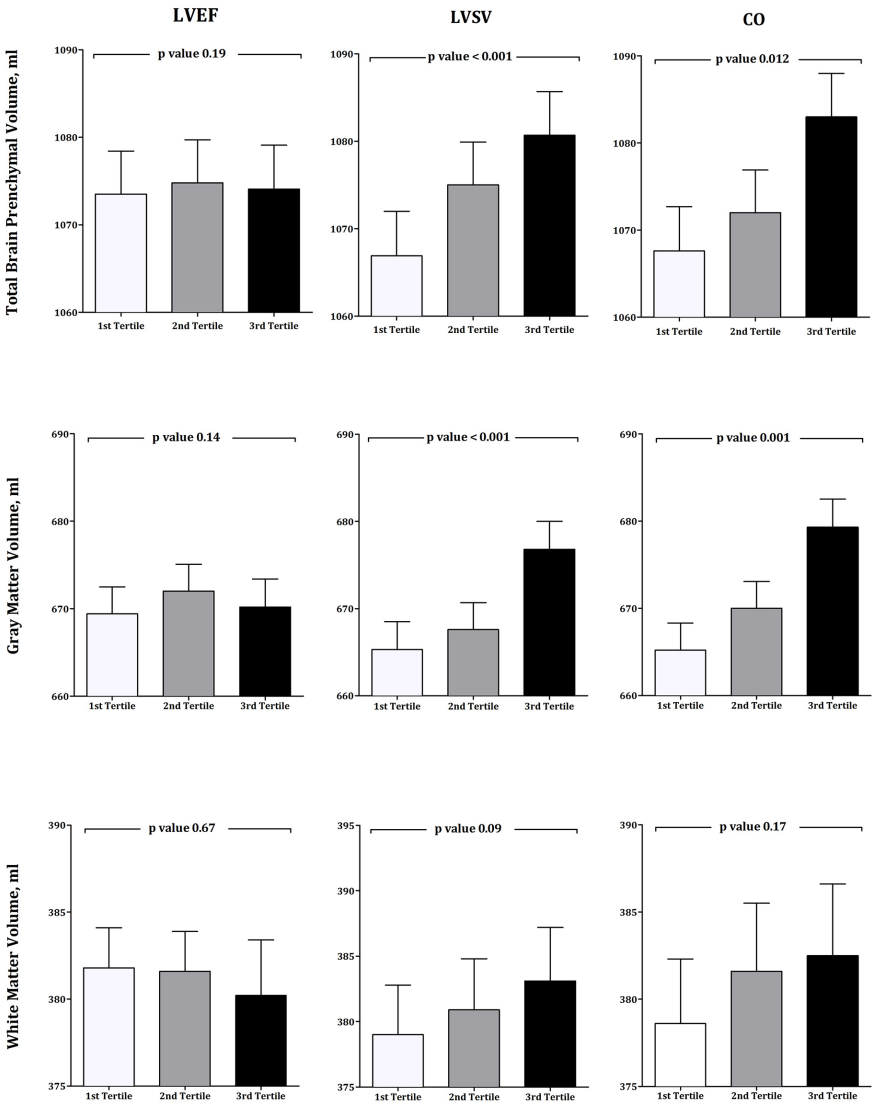

Figure 1: Brain tissue volumes in tertiles of left ventricular ejection fraction (LVEF), left ventricular stroke volume (LVSV) and cardiac output (CO). Bars represent adjusted means and standard errors. Analyses were adjusted for age, sex, education, current smoker, intracranial volume, history of hypertension, diabetes mellitus, systolic blood pressure, prevalent coronary heart disease, total cholesterol, body mass index and atrial fibrillation. Ranges for tertiles of LVEF are low: 15.2-58.4%, middle: 58.5-65.2% and high: 65.3-85.3%. Ranges for tertiles of LVSV are low: 20.3-55.3ml, middle: 55.4-66.5ml and high: 66.6-166.9ml. Ranges for tertiles of CO are low:1.6-3.4L/min, middle: 3.5-4.2L/min and high: 4.3-11.7L/min.

Table 3. Cardiac hemodynamic measures and cognitive functioning: AGES-RS ICELAND-MI

		Memory		Processing Speed		Executive Function	
		Beta* (95% CI)	P value	Beta* (95% CI)	P value	Beta* (95% CI)	P value
LVEF	Model 1	-0.01 (-0.06, 0.05)	0.78	-0.04 (-0.10, -0.01)	0.028	-0.09 (-0.13, -0.04)	<0.001
	Model 2	0.00 (-0.05, 0.06)	0.91	-0.03 (-0.08, 0.01)	0.13	-0.08 (-0.13, -0.04)	<0.001
LVSV	Model 1	-0.04 (-0.08, -0.01)	0.015	-0.06 (-0.09, -0.03)	0.001	-0.06 (-0.09, -0.03)	<0.001
	Model 2	-0.04 (-0.07, 0.00)	0.04	-0.05 (-0.08, -0.02)	0.003	-0.06 (-0.09, -0.03)	<0.001
CO	Model 1	-0.04 (-0.09, 0.01)	0.12	-0.07 (-0.10, -0.02)	0.004	-0.08 (-0.12, -0.03)	< 0.001
	Model 2	-0.03 (-0.08, 0.03)	0.32	-0.06 (-1.0, -0.11)	0.01	-0.08 (-0.12, -0.03)	<0.001

Model 1- Adjusted for age and sex

Model 2- Adjusted for age, sex, education, current smoker, history of hypertension, diabetes mellitus, systolic blood pressure, prevalent coronary heart disease, total cholesterol, body mass index, atrial fibrillation

∗ Betas represent change in the brain volumes for each 10% lower LVEF, 10 ml lower LVSV and one L/min lower CO

Abbreviation: LVEF; Left ventricular ejection fraction, LVSV: Left ventricular stroke volume, CO; Cardiac output

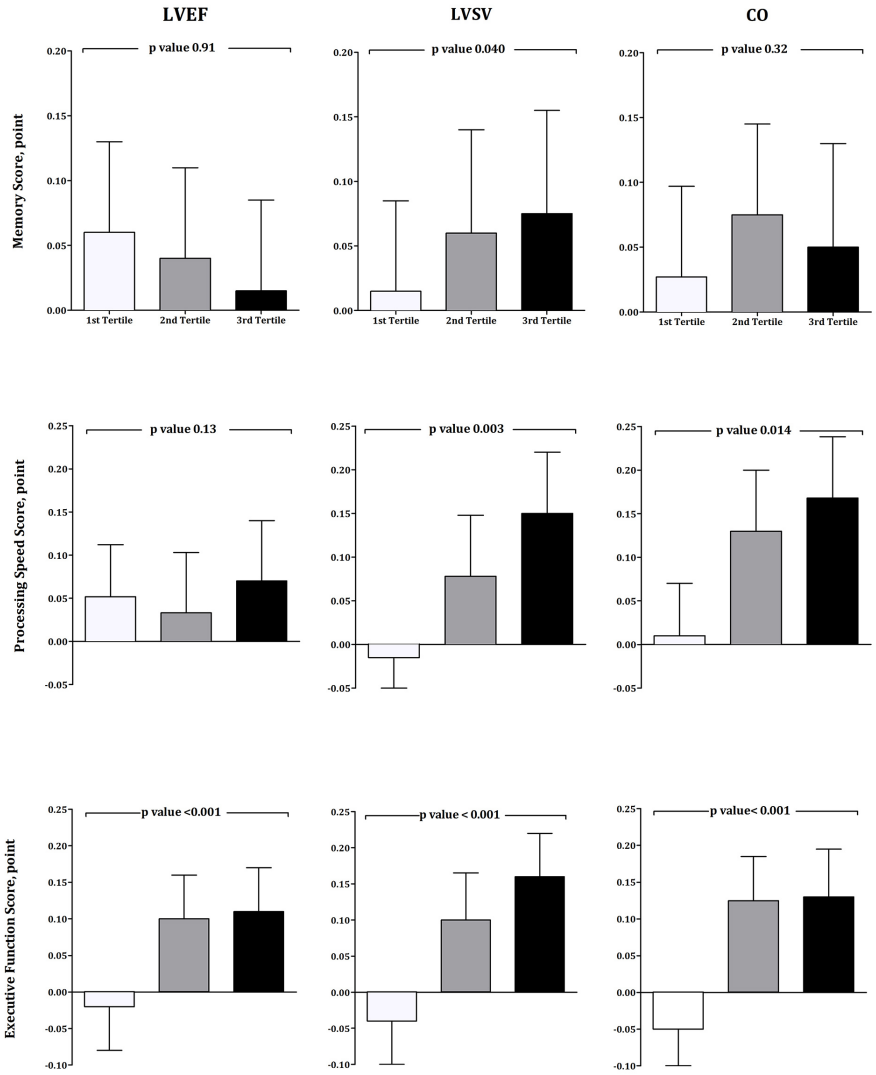

Figure 2: Cognitive function in tertiles of left ventricular ejection fraction (LVEF), left ventricular stroke volume (LVSV) and cardiac output (CO). Bars represent adjusted means and standard errors. Analyses were adjusted for age, sex, education, current smoker, history of hypertension, diabetes mellitus, systolic blood pressure, prevalent coronary heart disease, total cholesterol, body mass index and atrial fibrillation. Ranges for tertiles of LVEF are low: 15.2-58.4%, middle: 58.5-65.2% and high: 65.3-85.3%. Ranges for tertiles of LVSV are low: 20.3-55.3ml, middle: 55.4-66.5ml and high: 66.6-166.9ml. Ranges for tertiles of CO are low:1.6-3.4L/min, middle: 3.5-4.2L/min and high: 4.3-11.7L/min.

Table 4. Association of cardiac hemodynamics with depression and cognitive impairment: AGES-RS ICELAND-MI

		Depression		Cognitive impairment†	
		Beta* (95% CI)	P value	OR* (95% CI)	P value
LVEF	Model 1	0.16 (0.02, 0.30)	0.03	1.16 (0.99, 1.47)	0.09
	Model 2	0.08 (-0.06, 0.23)	0.25	1.07 (0.84, 1.37)	0.56
LVSV	Model 1	0.10 (0.00, 0.19)	0.05	1.28 (1.06, 1.59)	0.004
	Model 2	0.07 (-0.03, 0.17)	0.16	1.25 (1.02, 1.51)	0.033
CO	Model 1	1.5 (0.15, 2.90)	0.029	1.56 (1.18, 2.08)	0.003
	Model 2	1.1 (-0.33, 2.50)	0.13	1.43 (1.05, 1.96)	0.025

Model 1- Adjusted for age and sex
Model 2- Adjusted for age, sex, education, current smoker, history of hypertension, diabetes mellitus, systolic blood pressure, prevalent coronary heart disease, total cholesterol, body mass index, atrial fibrillation
† Defined as mild cognitive impairment or dementia
∗ Betas and odds ratios are presented for each 10% lower LVEF, 10 ml lower LVSV and one L/min lower CO
Abbreviation: LVEF; Left ventricular ejection fraction, LVSV: Left ventricular stroke volume, CO; Cardiac output

Discussion

Findings of this study suggest that sub-optimal cardiac functioning, as reflected in cardiac hemodynamics, is associated with lower brain volumes and cognitive performance. We observed that community-dwelling older subjects with low stroke volume and cardiac output are at a higher risk for brain parenchymal loss and cognitive impairment. The associations between cardiac hemodynamics and brain outcomes were independent of socio-demographic and cardiovascular factors.

Increasing body of evidence indicates that patients with advanced heart failure carry a higher risk for manifestations of brain ageing including gray matter loss, white matter hyperintensities, infarcts, and cognitive impairment[9, 24-26]. Recently, it has been proposed that not only subjects with advanced heart failure but also those with suboptimal cardiac functioning might be at higher risk for accelerated brain ageing[8]. This hypothesis was tested in a limited number of studies based on community-dwelling older adults. In a cohort of individuals from the Framingham Offspring Study with a mean age of 61 years, Jefferson et al showed that higher cardiac index was associated with higher total brain volume but they could not demonstrate a clear association with most of the neuropsychological measures in the whole study population[12]. However, the

interpretation of those findings is hampered because brain outcomes were assessed about four years before cardiac MRI measurements. On average, this cohort is also younger than our study population. In an another study, middle aged to elderly subjects in the lowest and highest quintile of the LVEF showed worse performance in memory and executive functioning as well as visuoperceptual abilities compared to the reference groups[27]. In our cohort, with a mean age of 76 years, we showed that decreased cardiac hemodynamic parameters, in particular stroke volume and cardiac output, was strongly associated with structural brain changes including lower total brain volume and gray matter volume as well as functional brain abnormalities including worse performance in processing speed and executive functioning. Moreover, we showed that lower stroke volume and cardiac output associates with higher risk of mild cognitive impairment or dementia. Different mechanisms may explain a link between impaired cardiac functioning and brain ageing. One possibility is shared comorbidities and vascular risk factors[28]. That could, on the one hand, impair cardiac functioning and on the other hand, lead to brain structural and functional abnormalities[29]. Available evidence for the importance of early recognition and management of vascular risk factors such as hypertension in prevention of cardiovascular and cerebrovascular events support this notion[30]. However, we observed that correction of the analyses for established vascular risk factors did not essentially change our findings except for depressive symptoms. It is also possible the decline in cardiac functioning and brain ageing are an epiphenomenon and not causally related. While this possibility cannot be totally excluded, previous population-based cohort studies on older people free of dementia showed that subjects with heart failure are at a significantly higher risk for developing dementia[26]. As an alternative explanation, impaired cardiac functioning could result in a decreased cerebral blood flow and consequently long-standing cerebral hypoperfusion, via neuronal loss and injury, put the brain at higher risk for structural and functional abnormalities[31]. In line with this hypothesis, it has been shown that interventions to enhance left ventricular functioning improve cognitive performance in patients with heart failure[10]. In this study we observed that lower cardiac functioning was not related with markers of brain small vessel pathologies. This finding might indicate that long-term decreased cardiac functioning mainly influences neuronal energy homeostasis which ultimately results in neuronal cell death and brain tissue loss. Although a role for cerebral hypoperfusion as a mediator in the association between cardiac functioning and brain ageing seems plausible, further research is needed to confirm this hypothesis and we are not able to establish a causal relationship only based on our observational data.

This study has certain strengths and limitations. A relatively large cohort of community-dwelling older individuals with available data on cardiac function-

ing assessed by cardiac MRI, which is a reliable method and less operator dependent modality compared to conventional echocardiography[32], can be marked as the main strengths of this study. In addition, we had an extended set of data on structural and functional features of brain ageing. A possible limitation is that our study population consists of randomly selected individuals as well as a group of individuals with diabetes. However, the stratified analyses showed that the association between cardiac hemodynamics and brain outcomes was similar in both groups. As another limitation, due to the cross-sectional design of this study, it is not clear whether changes in cardiac hemodynamics preceded changes in brain outcomes. This highlights a need for future longitudinal studies to investigate whether disturbances in cardiac hemodynamics are also associated with progression of the structural and functional brain pathologies.

In conclusion, our findings suggest that suboptimal cardiac functioning, independent of conventional cardiovascular risk factors, is associated with structural and functional features of brain ageing in community-dwelling older subjects. The cross-sectional associations suggest that elderly patients presenting with cardiac or cognitive signs and symptoms may have both cardiac and cerebral disease and should be evaluated accordingly. Further, since there is a wider formulary for treating cardiac problems than there is for cognitive problems, clinicians treating cardiac problems should monitor a patient's cognition along with cardiac function. This is consistent with recent recognition and recommendations by the AHA that cardio-vascular risk factors lead to vascular cognitive impairment[33]. Future longitudinal and experimental studies will unravel the temporality and pathogenic mechanisms behind this association.

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6

Serum NT-proBNP, Blood Pressure and Cognitive Function

Manuscript based on this chapter has been submitted as:
van Vliet P, Sabayan B, Wijsman LW, Poortvliet R, Mooijaart SP, de Ruijter W, Gussekloo J, de Craen AJ, Westendorp RG. N-terminal pro-brain natriuretic peptide, blood pressure and cognitive function.

Summary

Hypertension in middle age is a risk factor for dementia, whereas in old age cognitive impairment is associated with lower blood pressures. Heart failure may play a role in this reversal of associations. In this chapter, we studied the relation between N-terminal pro-brain natriuretic peptide (NT-proBNP) levels, a marker of heart failure, blood pressure, and cognitive function over time in the oldest old. In the Leiden 85-plus Study, we measured NT-proBNP levels at baseline and annually global cognitive function (MMSE) and blood pressure (BP) in 560 subjects aged 85 years at baseline, who were followed for 5 years. Subjects in the highest tertile of NT-proBNP levels scored 1.8 points lower on the MMSE than subjects in the lowest tertile (p=0.004), and had a 0.7 point stronger decline in MMSE score per year (p=0.006). Subjects in the category highest tertile of NT-proBNP and the lowest tertile of systolic BP had a 3.7 point lower MMSE score at baseline ($p<0.001$) and a 0.6 point additional annual decline in MMSE score (p=0.040) compared to subjects in any other category. In the oldest old high NT-proBNP levels are associated with lower MMSE scores and with stronger declines in MMSE score. The combination of high NT-proBNP levels and low systolic BP is most detrimental for global cognitive function. Possibly, a failing pump function of the heart results in lower brain perfusion with resultant brain dysfunction.

Introduction

Cardiovascular diseases, such as myocardial infarction, generalized atherosclerosis, and stroke have been associated with increased risk of dementia and with steeper declines in cognitive function[1-4]. Hypertension on the contrary seems to be a risk factor for dementia only when present in middle age, whereas most studies show that dementia and cognitive decline in the oldest old associate with lower blood pressures[5]. In the oldest old blood pressures decrease over time[6,7], and stronger declines are related to the presence of dementia and cognitive decline[8,9]. One possible explanation for the decline in blood pressure in the oldest old might be the presence of (subclinical) congestive heart failure, with a failing pump function resulting in lower blood pressures[10].

Congestive heart failure has been associated with cognitive impairment in several studies. In two hospital-based studies, patients with heart failure had an increased risk of cognitive impairment, and with increasing severity of heart failure patients had worse cognitive performance[11,12]. Moreover, another study showed that lower left ventricular ejection fractions in elderly patients with heart failure associated with reduced verbal memory function[13]. A commonly used serum marker of heart failure is the N-terminal pro-brain natriuretic peptide (NT-proBNP), the inactive fragment of the proBNP hormone[14]. NT-proBNP has proven to be a quantitative marker of acute heart failure, with high sensitivity and reasonable specificity, and is also a useful tool to monitor (treatment of) heart failure[15]. Several cross-sectional studies have shown that higher NT-proBNP levels associate with lower cognitive function[16,17], and one follow-up study showed that higher NT-proBNP levels at baseline associated with worse cognitive function five to eight years later[18]. The relation between NT-proBNP levels, blood pressure, and cognitive function over time in the oldest old, has not been studied yet.

Here, we studied the association between NT-proBNP levels, blood pressure and cognitive function over time in the Leiden 85-plus Study, a population based follow-up study amongst subjects aged 85 years at baseline. We hypothesize that high NT-proBNP levels, and low systolic blood pressure associate with worse cognitive function.

Materials and Methods

Participants

Between September 1, 1997, and September 1, 1999, a total of 705 inhabitants of the community of Leiden, the Netherlands, reached the age of 85 years. Among these 85-year-old persons, we initiated a follow up study to investigate determinants of successful aging. There were no selection criteria on health or demographic characteristics. Fourteen inhabitants died before they could be enrolled. The response rate was 87%; a total of 599 subjects (397 women and 202 men) participated[19]. Of the 599 participants in the cohort, 38 refused to provide a blood sample, and NT-proBNP measurement failed in one participant, yielding a total number of 560 participants for the present study. Participants were visited within one month after their 85th birthday at their home for face-to-face interviews and neuropsychological testing. They were revisited annually until age 90 years. The Medical Ethical Committee of the Leiden University Medical Centre approved the study, and informed consent was obtained from all participants.

Plasma levels of NT-proBNP

Non-fasted blood samples were taken at the first visit early in the morning. Blood samples were kept frozen at -80ºC. In 2011 citrated plasma levels of NT-proBNP were measured in one batch using the NT-proBNP assay of Roche Diagnostics (Mannheim, Germany) on a Roche Modular E-170 automated immunoanalyser.

Blood pressure

Annually, blood pressure was measured, using a mercury sphygmomanometer, in seated position. During each home visit two blood pressure measurements were done, the first after at least five minutes of rest and no vigorous exercise in the preceding thirty minutes, the second after approximately 90 minutes at the end of the visit. The systolic value was measured at Korotkoff sound 1, and the diastolic value was measured at Korotkoff sound 5. For each year, the mean value of the two measurements was calculated and used in further analyses. Mean arterial pressure was calculated as 1/3(systolic blood pressure) + 2/3(diastolic blood pressure).

Global cognitive function

At age 85 years and then annually, global cognitive function was assessed in participants using the Mini-Mental State Examination (MMSE)[20]. The MMSE examines five areas of cognitive function: orientation, registration, attention and calculation, recall, and language. Possible scores on the MMSE range from 0 to

30 points, with lower scores indicating worse global cognitive functioning.

Other covariates

The level of education of each participant was determined by the number of years a subject went to school. Information was obtained at the first visit using a questionnaire. Low education was defined by six or less than six years of schooling, whereas high education was defined by seven or more years of schooling. The burden of vascular disease at baseline was determined by the number of cardiovascular and cerebrovascular pathologies (myocardial infarction, angina pectoris or myocardial ischemia, claudicatio intermittens, arterial surgery, stroke, and transient ischemic attack). Information on use of antihypertensive medication was obtained from pharmacist records, or from a questionnaire, filled out by the treating physician for institutionalized participants. Participants were classified as having diabetes when they met at least one of the following criteria: (a) history of type 2 diabetes obtained from the general practitioner or the subject's treating physician; (b) use of antidiabetic medication, based on information obtained from the subject's pharmacist; or (c) nonfasting glucose of 11.1 mmol/L or higher. Information on smoking was obtained from questionnaires, which were filled out by each participant at baseline. BMI was calculated by dividing the weight in kilograms by the square of length in meters (kg/m^2). Creatine clearance was estimated with the Cockroft-Gault formula. Prevalence of atrial fibrillation was determined by automated Minnesota Coding (Minnesota Code 8-3-1) of ECGs, which were recorded on a Siemens Sicard 440 (Erlangen, Germany), and were transmitted to the ECG Core Laboratory in Glasgow Royal Infirmary.

Statistical analyses

Characteristics of the study participants are reported as mean (standard deviation) for continuous variables and number (percentages) for categorical variables in tertiles of NT-proBNP levels. The associations of both NT-proBNP levels and systolic blood pressure with MMSE score at baseline and during follow up were analyzed using two different methods. First, linear mixed models were used for graphically representing the associations. Linear mixed models use all available data during follow-up and account for repeated measurements, thereby handling missing data more appropriately than traditional models[21]. For more accurate analyses using all available data, but without imputation methods for missing data, the associations of NT-proBNP levels and systolic blood pressure with MMSE score were analyzed using linear regression analysis. In these analy-

ses baseline associations were tested and for the longitudinal associations regression coefficients of change in MMSE score per year were calculated per subject, yielding new outcome variables used as the determinant. In the primary analyses categorical NT-proBNP levels and systolic blood pressure were the independent variable, whereby NT-proBNP levels and systolic blood pressure were divided in tertiles. For continuous analyses log-transformed NT-proBNP levels were used. In a multivariate model all analyses were corrected for level of education, use of antihypertensives, smoking status, prevalence of diabetes, body mass index, renal function, prevalence of atrial fibrillation, and, finally, the number of cardiovascular and cerebrovascular pathologies at baseline. In a final analysis a new variable consisting of four categories, using combinations of the lowest and highest tertile of NT-proBNP levels and the lowest and highest tertile of systolic blood pressure, was used as an independent variable with MMSE scores as the determinant. All calculations were performed using SPSS software (version 20.0.1, SPSS Inc, Chicago, Ill).

Results

Table 1 shows baseline characteristics of all 560 study participants in tertiles of NT-proBNP levels. Compared to subjects in the lowest tertiles, subjects in the highest tertile of NT-proBNP had a lower educational level, had lower blood pressures, had a lower body mass index, and a lower renal function, were more likely to use antihypertensives, and to have a history of smoking, and had a higher prevalence of cardiovascular pathologies and atrial fibrillation.

Table 1. Baseline characteristics of study population.

	Serum MT-proBNP			
Characteristics	**Low** (n=186)	**Middle** (n=187)	**High** (n=187)	p-value
Male (%)	62 (33%)	54 (29%)	71 (38%)	0.176[c]
Low education (%)[a]	115 (62%)	112 (60%)	134 (72%)	0.030[c]
SBP, mmHg (SD)[b]	156.8 (17.6)	155.9 (18.1)	152.9 (20.0)	0.045[d]
DBP, mmHg (SD)[b]	79.0 (9.0)	77.2 (9.4)	74.1 (9.6)	<0.001[d]
MAP, mmHg (SD)[b]	104.9 (10.5)	103.4 (11.0)	100.4 (11.4)	<0.001[d]
PP, mmHg (SD)[b]	77.8 (14.3)	78.7 (14.6)	78.8 (17.1)	0.553[d]
Body mass index, kg/m^2 (SD)[b]	28.1 (4.2)	27.4 (4.9)	26.1 (4.0)	< 0.001[d]
GFR, ml/min (SD)[b]	48.7 (11.0)	46.2 (10.6)	40.7 (11.5)	<0.001[d]
Use of antihypertensives (%)	66 (36%)	87 (45%)	96 (51%)	0.007[c]
Current smoking (%)	30 (16%)	24 (13%)	35 (19%)	0.307[c]
Former smoking (%)	90 (49%)	76 (42%)	99 (54%)	0.048[c]
Prevalence of DM type II (%)	30 (16%)	27 (14%)	35 (19%)	0.531[c]
Prevalence CVD (%)	49 (27%)	65 (36%)	111 (62%)	<0.001[c]
Prevalence of atrial fibrillation (%)	3 (2%)	4 (2%)	49 (27%)	<0.001[c]

[a] Level of education was dichotomized by six years of schooling

[b] Normally distributed continuous data are presented as means with standard deviations

[c] Statistical significance of the difference in number of subjects per tertile, using Chi^2 testing

[d] Statistical significance of the trend over the tertiles, using linear regression analysis

Abbreviations: SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, PP: pulse pressure

Figure 1a graphically shows the association between NT-proBNP levels and MMSE scores from age 85 to 90 years. At age 85 years, subjects in the highest tertile of NT-proBNP had a 1.8 point lower MMSE score than subjects in the lowest tertile (p=0.002). Moreover, during the five year follow-up, subjects in the highest tertile of NT-proBNP had a 0.24 point steeper decline in MMSE score per year compared to subjects in the lowest tertile (p=0.008). There was also an

association between systolic blood pressure and MMSE score (figure 1b). Subjects in the lowest tertile of systolic blood pressure had a 2.8 point lower MMSE score at age 85 years than subjects in the highest tertile ($p<0.001$). They also had a 0.39 point steeper decline in MMSE score per year compared to subjects in the highest tertile of systolic blood pressure ($p<0.001$).

When analyzing these associations with NT-proBNP levels and systolic blood pressure as continuous variables similar associations were found. An increase in log-transformed NT-proBNP levels associated both with lower MMSE scores as baseline (difference in MMSE score at age 85 years per 1 ln-NT-proBNP increase: -0.57, 95% CI, -0.98 to -0.16, p=0.007) and with stronger declines in MMSE score from age 85 to 90 years (additional annual change in MMSE score from age 85 to 90 years per 1 ln-NT-proBNP increase: -0.08, 95% CI, -0.15 to -0.02, p=0.012). An increase in systolic blood pressure associated with higher MMSE scores at baseline (difference in MMSE score at age 85 years per 10 mmHg increase in systolic blood pressure: +0.68, 95% CI, 0.43 to 0.93, $p<0.001$) and with weaker declines in MMSE score from age 85 to 90 years (additional annual change in MMSE score from age 85 to 90 years per 10 mmHg increase in systolic blood pressure: 0.08, 95% CI, 0.01 to 0.04, $p<0.001$).

When using linear regression analyses for analyzing the association between tertiles of NT-proBNP levels and MMSE scores at baseline and during follow-up, similar results were found as in the linear mixed models analyses (table 2). Subjects in the highest tertile of NT-proBNP had lower MMSE scores at age 85 years and had stronger declines in MMSE score from age 85 to 90 years. Also for the baseline association between systolic blood pressure and MMSE score similar results were found, with the lowest MMSE scores at age 85 years in subjects in the lowest tertile of systolic blood pressure. However, subjects in the lowest tertile of systolic blood pressure did not have a significant stronger decline in MMSE score from age 85 to 90 years compared to subjects in the highest tertile. In continuous multivariate analyses, with further correction for possible confounders, similar associations were found. After correction for sex, education, smoking, prevalence of diabetes mellitus and atrial fibrillation, use of antihypertensives, BMI, and renal function, higher ln-NT-proBNP levels associated with lower MMSE scores at age 85 years (difference in MMSE score at age 85 years per 1 ln-NT-proBNP increase: -0.49, 95% CI, -0.94 to -0.04, p=0.032), and with stronger declines in MMSE score from age 85 to 90 years (change in MMSE score from age 85 to 90 years: -0.19, 95% CI, -0.37 to -0.02, p=0.030). With further correction for the prevalence of cardiovascular pathologies the association was no longer significant for the baseline analysis (p=0.131), but stayed for the longitudinal analysis (p=0.043). In the multivariate analyses there was a strong association between systolic blood pressure and MMSE score at baseline (difference in MMSE score at age 85 years per 10 mmHg increase in systolic blood

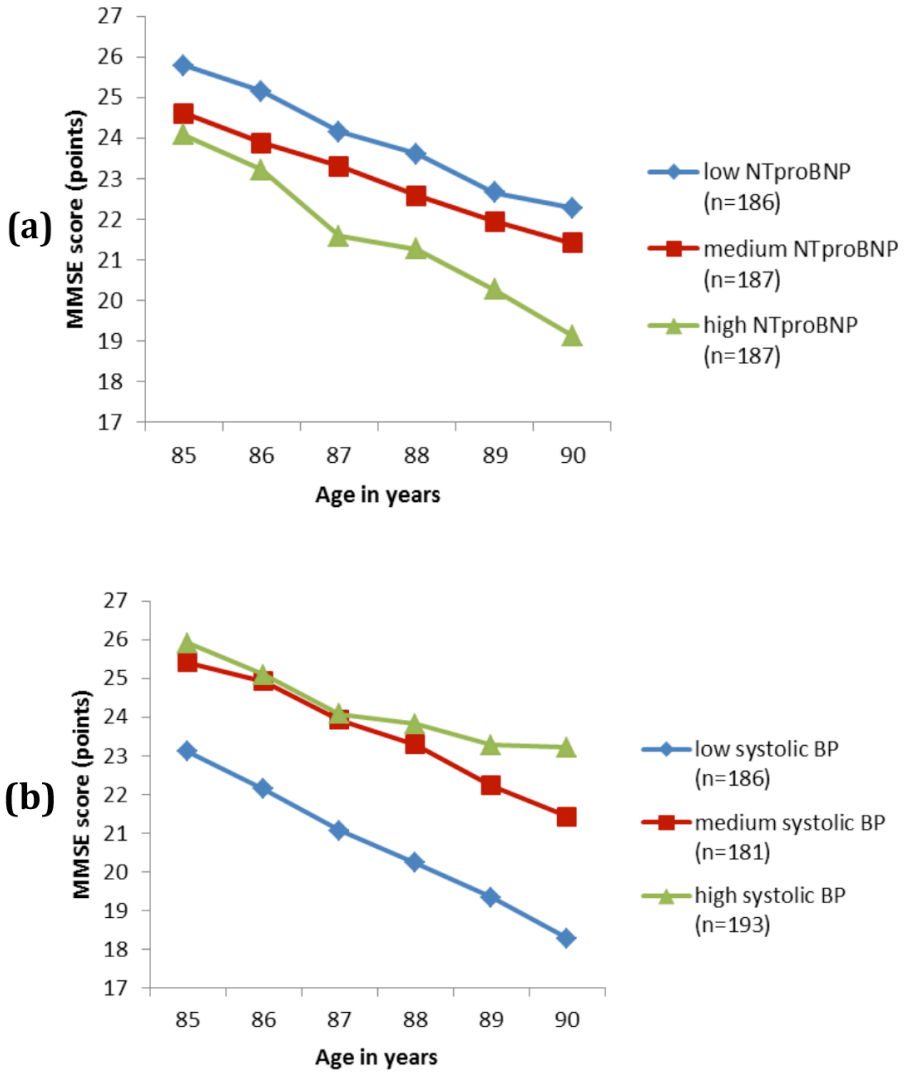

Figure 1. NT-proBNP and systolic blood pressure were divided in tertiles, based on the medians. Data points represent estimates and their standard errors, calculated using linear mixed models, with adjustment for sex and level of education. Number of subjects and range of values per tertile: low NT-proBNP, n=186, 20.8-200.9 pg/ml; high NT-proBNP, n=187, 552.9-28,362.7 pg/ml; low systolic blood pressure, n=186, 110.0-146.5 mmHg; high systolic blood pressure, n=193, 162.0-215.0 mmHg.

pressure: +0.49, 95% CI, 0.24 to 0.75, p<0.001), but not with MMSE score from age 85 to 90 years (change in MMSE score from age 85 to 90 years per 10 mmHg increase in systolic blood pressure: +0.08, 95% CI, -0.02 to 0.18, p=0.138).

Table 2. MMSE score at age 85 years and change in MMSE score from age 85 to 90 years dependent on tertiles of NT-proBNP and tertiles of systolic blood pressure.

	Tertiles of the determinant			
	Low	Middle	High	P-value
NT-proBNP				
Cross-sectional	25.8 (0.4)	24.6 (0.4)	24.0 (0.4)	0.004
Longitudinal	-0.9 (0.2)	-1.0 (0.2)	-1.6 (0.2)	0.006
SBP				
Cross-sectional	23.1 (0.4)	25.3 (0.4)	25.9 (0.4)	<0.001
Longitudinal	-1.4 (0.2)	-1.0 (0.2)	-1.1 (0.2)	0.139

Tertiles of NT-proBNP and tertiles of systolic blood pressure were created on the median. Estimates with their standard errors were calculated, with correction for sex and level of education. P-values represent the statistical significance of the trend over the tertiles of NT-proBNP and tertiles of systolic blood pressure (SBP), calculated using linear regression analysis, with adjustment for sex and level of education. Number of subjects per tertile NT-proBNP: lowest, n=186; medium, n=187; highest, n=187. Number of subjects per tertile systolic blood pressure: lowest, n=186; medium, n=181; highest, n=193.

Finally, to test for the combined effect of NT-proBNP levels and systolic blood pressure on MMSE scores, a new variable was created, consisting of combinations of the lowest and highest tertiles of NT-proBNP levels and systolic blood pressure. Figure 2 shows that subjects in the categor 'high NT-proBNP & low systolic blood pressure' had the lowest MMSE score at baseline and had the strongest decline in MMSE score from age 85 to 90 years. Compared to the other three categories, subjects in the category 'high NT-proBNP & low systolic blood pressure' had a 3.7 point lower MMSE score at age 85 years (95% CI, 2.1 to 5.3, p<0.001), and a 0.49 point annual additional decline in MMSE score from age 85 to 90 years (95% CI, 0.24 to 0.75, p<0.001). When using diastolic blood pressure, mean arterial pressure, and pulse pressure in the models, similar associations were found at baseline (all p≤0.001) and during follow up for pulse pressure (p<0.001), but not for diastolic blood pressure (p=0.580) and mean arterial pressure (p=0.089).

When analyzing the associations between NT-proBNP levels, systolic blood pressure and MMSE scores with linear regression analysis instead of linear mixed models, similar results were found for baseline associations, and results

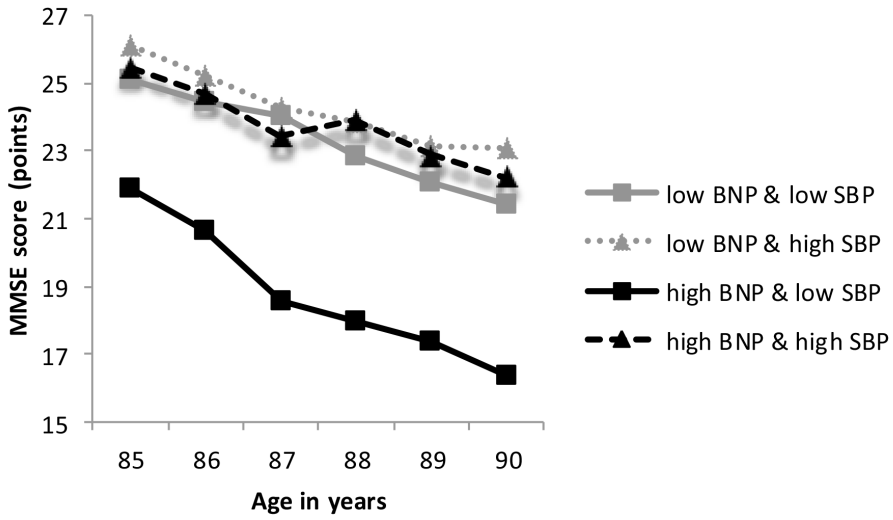

Figure 2. MMSE score from age 85 to 90 years dependent on categories of tertiles of NT-proBNP and tertiles of systolic blood pressure. Categories of NT-proBNP and systolic blood pressure were created by making tertiles, based on the medians, and using the highest and the lowest tertile of each parameter. Data points represent estimates, calculated using linear mixed models, with adjustment for sex and level of education.

were less outspoken for the longitudinal associations. When comparing the category 'high NT-proBNP & low systolic blood pressure' with the other three categories the difference in MMSE score at age 85 years was 3.7 points (95% CI, 2.1 to 5.3, $p<0.001$), and the additional annual decline in MMSE score from age 85 to 90 years was 0.6 points (95% CI, 0.0 to 1.3, $p=0.040$).

Discussion

The main finding of our study is that higher NT-proBNP levels are associated with worse global cognitive function at baseline and with stronger cognitive decline during follow-up in the oldest old. Moreover, lower systolic blood pressures associated with worse global cognitive function at baseline, but not clearly with stronger cognitive decline during follow-up. When combining NT-proBNP levels and systolic blood pressures in a new variable, subjects with both high NT-proBNP levels and low systolic blood pressures had the worst global cognitive

function and the strongest cognitive decline compared to all other subjects.

Our finding of higher NT-proBNP levels associating with worse cognitive function is in accordance with previous studies. In the Rancho Bernardo Study, a population-based study amongst 950 subjects with a mean age of 77 years, subjects with high NT-proBNP levels had worse cognitive test scores compared to subjects with low NT-proBNP levels[16]. A recently published study amongst elderly patients with type 2 diabetes also showed that higher NT-proBNP levels associated with lower cognitive function[17]. However, these studies reported on cross-sectional associations and lack follow up data. In the Hoorn Study, higher NT-proBNP levels at baseline associated with worse cognitive performance, which was measured after a follow up period of five to eight years[18]. There was no information on cognitive performance at baseline, and therefore conclusions on cognitive decline could not be drawn from these results. The only study reporting on prospective associations was performed in 464 subjects aged 75 years and over[22]. In this study, high NT-proBNP levels associated with both higher declines in MMSE score, measured at baseline and after five years of follow up, and with an increased risk of dementia. Information on blood pressure was not reported. Only one study has shown the absence of an association between NT-proBNP levels and cognitive function, despite the clear association between high NT-proBNP levels and vascular disease in the population of elderly patients with mental illness[23].

NT-proBNP has emerged as an important biomarker of cardiac function and is commonly used in clinical practice for the evaluation of congestive heart failure[24]. NT-proBNP is the biologically inactive N-terminal fragment of the prohormone proBNP, which is evenly split into the active BNP and NT-proBNP[25]. ProBNP is secreted predominantly by cardiac myocytes of the ventricles in response to stretching and distension of the cardiac wall and has a natriuretic and diuretic effect[26,27]. An experimental study amongst healthy male volunteers showed that infusion of low-dose BNP resulted in natriuresis and the inhibition of plasma renin activity[28]. Moreover, in animals, sustained infusion of natriuretic peptides resulted in a lower blood pressure[29]. Additionally, genetic variation in the natriuretic peptide precursor B gene was shown to associate with both lower NT-proBNP levels and higher blood pressures[30]. These findings might explain the observed association between high NT-proBNP levels and lower blood pressures in our study. However, this contradicts with results from previously published studies, in which high NT-proBNP levels associated with higher blood pressures, albeit in younger populations[31,32]. An explanation for these opposing results might be that initially, as a consequence of hypertension, higher filling pressures of the ventricles result in an increased release of NT-proBNP. However, with longer standing hypertension ventricular enlargement and dysfunction may develop. This leads to heart failure with lower car-

diac output and persisting NT-proBNP release with increased natriuresis and diuresis, subsequently resulting in lower blood pressures.

Since low NT-proBNP levels reliably exclude heart failure and high NT-proBNP levels are related to heart failure, our results may suggest that heart failure associates with worse cognitive function and with stronger cognitive decline in the oldest old. The relation between cognitive function and heart failure, irrespective of NT-proBNP levels, has been reviewed before[33,34]. The authors conclude that there is a strong cross-sectional association between heart failure and cognitive impairment, but that there are very few longitudinal data available. Possible pathophysiological mechanisms explaining for the association between heart failure and cognitive impairment may be found in direct and indirect pathways. An indirect pathway may be the presence of common risk factors that associate with both heart failure and cognitive impairment. Hypertension at midlife for instance is a risk factor for late-life cognitive impairment[5], but is also a well-known risk factor for heart failure up to old age[35]. Similar common risk factors are a history of smoking and prevalence of atherosclerotic disease, which all associate with both an increased risk of heart failure and an increased risk of cognitive impairment[36-38]. Specifically the latter one may explain the found associations for a substantial part, because correction for the prevalence of cardiovascular pathologies in the multivariate model diminished the cross-sectional association between NT-proBNP levels and MMSE scores at age 85 years. However, these indirect pathways may only explain part of the observed association between NT-proBNP levels and cognitive function in our study, since correction for all these risk factors in our analyses did not materially change the longitudinal results. Another likely pathophysiological explanation might be found in the direct effect of heart failure on cerebral perfusion. Heart failure is characterized by a decreased cardiac pump function, with resultant lower cardiac output. This eventually may result in cerebral hypoperfusion with concomitant decreased cerebral function. In an older population of patients with heart failure, ejection fractions below 30% were indeed associated with worse memory function compared to ejection fractions over 30% [39]. In further support of this possible pathophysiological mechanism we showed in a recently performed meta-analysis that both patients with Alzheimer's disease and patients with vascular dementia have lower cerebral blood flow velocities than age-matched controls[40]. Other explanatory factors that associate with both NT-proBNP levels and cognitive function, such as atrial fibrillation and renal function, are less likely to play a substantial role, since correction for these variables did not materially change the associations.

In cross-sectional analyses low systolic blood pressure associated with worse global cognitive function. However, results from the longitudinal analyses are less clear. When using linear mixed models low systolic blood pressure associ-

ated with stronger declines in MMSE score. This could however not be replicated when using linear regression analyses, using regression coefficients of change in MMSE scores over the years as outcome variable. A possible explanation may be found in the handling of missing values. Linear mixed models uses imputation techniques for missing values of MMSE scores, whereas the regression coefficients of change in MMSE score are created using available data points only, without imputation of missing values. Likely, the imputation of missing values in the linear mixed models gave a slight overestimation of the effect observed when using linear regression analysis. Worst global cognitive function and strongest cognitive decline was observed in subjects who had both high NT-proBNP levels and low systolic blood pressures, as opposed to all other subjects. This suggests that only the combination of the two is detrimental. A possible explanation for this finding might be that high NT-proBNP levels reflect a different pathomechanism in the group of subjects with low blood pressure compared to the group with high blood pressure. In subjects with low blood pressure high NT-proBNP levels may be the result of longer existing heart failure, which results in cardiac dysfunction, lower cardiac output and low blood pressure. This then eventually may result in cerebral hypoperfusion. In subjects with high blood pressure high NT-proBNP levels may be the result of cardiac stress caused by the hypertension, which does not necessarily have a direct negative impact on cerebral function. In other words, the latter group may reflect subjects with better cardiac function, and possibly better general health than the former group. This reasoning corresponds with previous findings, in which lower diastolic blood pressure in old age was shown to associate with increased mortality risk[41]. In the Leiden 85-plus Study we previously showed that in the oldest old declines in blood pressure during follow up associate with increased mortality risk[6,42]. Within the context of this reasoning low blood pressure and possibly high NT-proBNP levels in old age could also be a marker of underlying disease, such as general wasting, explaining for these findings.

A limitation of our study is that information on cardiac output, and cerebral perfusion is not available. Echocardiograhy was only performed in a convenience sample of 90 year olds, and unfortunately not at baseline. With additional information of these two parameters the possible pathophysiological mechanisms could have been further elucidated. Another limitation of our study is that these results cannot be extrapolated to younger populations, since the study was performed in the oldest old only.

In conclusion, high NT-proBNP levels in the oldest old associate with worse global cognitive function and with stronger cognitive decline. The combination of high NT-proBNP levels and low systolic blood pressure is specifically detrimental for cognitive function.

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Serum NT-proBNP and Risk of Cognitive Decline

Manuscript based on this chapter has been submitted as:
Wijsman L, Sabayan B, van Vliet P, Trompet S, de Ruijter W, Poortvliet R, van Peet P, Gussekloo J, Jukema W, Stott D, Sattar N, Ford I, Westendorp RG, de Craen AJ, Mooijaart S. NT-proBNP and cognitive decline in older adults at high cardiovascular risk

Summary

N-terminal pro-brain natriuretic peptide (NT-proBNP) has been related with cognitive impairment, which might be explained by clinical heart failure. Whether NT-proBNP associates with cognitive decline independent of clinical heart failure has not been studied. We investigated the association of NT-proBNP with cognitive decline in older subjects without advanced stages of clinical heart failure. We studied 5205 men and women (mean age 75 years) at high cardiovascular risk without prevalence or incidence of advanced stages of clinical heart failure (defined as New York Heart Association functional class III/IV at baseline or heart failure hospitalization during follow-up) of the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER). Four domains of cognitive function were tested at baseline and repeated during a follow-up period of 3.2 years. Participants with higher NT-proBNP levels ($\geq$ 450 ng/L) had a worse baseline cognitive function on all tests, including reaction time (mean difference high vs. low group (95% CI)) 2.84 seconds (0.60; 5.08); processing speed -0.94 digits coded (-1.57; -0.31); immediate memory -0.11 pictures remembered (-0.28; 0.06); and delayed memory -0.13 pictures remembered (-0.36; 0.11). Longitudinally, participants with higher NT-proBNP levels had a steeper cognitive decline during follow-up, including reaction time (mean annual change high vs. low group (95% CI)) 0.61 seconds (0.15; 1.07); processing speed -0.15 digits coded (-0.25; -0.05); immediate memory -0.05 pictures remembered (-0.09; 0.00); delayed memory -0.05 pictures remembered (-0.11; 0.01). Higher NT-proBNP levels associate with a steeper cognitive decline in older subjects without advanced stages of clinical heart failure. Although the exact underlying mechanism is unclear, NT-proBNP may serve as a biomarker of suboptimal left ventricular function, resulting in cognitive decline.

Introduction

Higher levels of N-terminal pro-brain natriuretic peptide (NT-proBNP), a hormone produced by cardiomyocytes in response to ventricular stretch, have been associated with cognitive impairment[1-4]. Evidence comes from several cross-sectional studies, which show that among community-dwelling older subjects, higher NT-proBNP levels were associated with worse cognitive function, in particular memory[1-4]. There are a limited number of longitudinal studies with relatively small sample sizes, which demonstrate that higher NT-BNP levels are also associated with steeper declines in Mini-Mental State Examination (MMSE) scores and higher incidence of dementia[5]. A potential mechanism behind the relationship between higher NT-proBNP levels and cognitive function is clinical heart failure, resulting in left ventricular dysfunction with subsequent reduced cardiac output. It is hypothesized that reduced cardiac output causes inadequate cerebral perfusion, leading to a higher risk of cognitive impairment[6-8]. Improvements in cognitive function in patients following cardiac transplantation suggests that impaired cardiac function might be a reversible risk factor for cognitive impairment[9,10].

Recent evidence demonstrates that higher NT-proBNP levels in older subjects without clinical heart failure are strongly associated with cardiovascular diseases and risk factors and predict an increased risk of atrial fibrillation, stroke, transient ischemic attack, myocardial infarction and mortality[11-14]. In addition, higher NT-proBNP levels have been related to left ventricular hypertrophy and systolic and diastolic dysfunction in subjects without clinical heart failure[15,16]. The relationship of cardiovascular diseases and risk factors with cognitive impairment is well-established[17,18]. Hence, cognitive impairment might already be present in asymptomatic subjects at early stages of reduced cardiac function. However, the association of higher NT-proBNP levels with cognitive impairment and decline in subjects without advanced stages of clinical heart failure has not been studied yet.

We hypothesized that elevated levels of NT-proBNP are associated with a steeper cognitive decline, even in subjects without the prevalence or incidence of advanced stages of clinical heart failure. Therefore, we studied the association of NT-proBNP with cognitive function cross-sectionally and longitudinally in a cohort of older men and women from the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER), in which participants with advanced heart failure, defined as New York Heart Association (NYHA) functional class III/IV at baseline or heart failure hospitalization during follow-up, were not included.

Methods

Study design

Data in this study were obtained from the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER), a randomized, double-blind, placebo-controlled trial designed to investigate the effect of pravastatin in prevention of vascular events in older men and women with pre-existing cardiovascular disease or risk factors thereof. This trial included 5,804 individuals aged 70-82 years old who were enrolled from three collaborating centers in Ireland, Scotland and the Netherlands. Approximately 50% of the participants showed evidence of cardiovascular disease including stable angina, intermittent claudication, stroke, transient ischemic attack, myocardial infarction and vascular surgery. The rest of participants had one or more cardiovascular risk factor, defined as hypertension, smoking or diabetes mellitus. Primary outcome of the trial was the combined endpoint of definite or suspect death from coronary heart disease, non-fatal myocardial infarction and fatal or non-fatal stroke during a mean follow-up period of 3.2 years. The institutional ethics committees of the three collaborating centers approved the study and all participants gave written informed consent[19,20].

Study participants

Participants with congestive heart failure, defined as New York Heart Association functional class III or IV, were excluded from PROSPER[19]. For the present study, we additionally excluded participants with heart failure hospitalization during follow-up (n=205).

Serum NT-proBNP

Blood samples were taken at 6 months after follow-up in EDTA tubes. NT-proBNP was determined using electrochemiluminescence immunoassay on a Roche Modulator E170. A number of 394 participants had missing NT-proBNP measurements. In keeping with existing literature on cutoff values in this age group, we defined three groups of NT-proBNP: low (<100 ng/L), middle (100-450 ng/L) and high NT-proBNP ($\geq$ 450 ng/L)[1].

Cognitive function

The MMSE was used to evaluate global cognitive function; participants with a baseline score below 24 points were excluded from PROSPER. Cognitive function was tested at baseline and at 9, 18, 30 months and at the end of the study. The time-point of the measurement at the end of the study varied between 36 and 48 months; therefore, we performed the analysis with their individually varying time point, but report the results for the mean of these time points (at 42 months). Four different neuropsychological tests were used to assess executive function, attention, immediate and delayed memory. The Stroop-Colour-Word-Test was used to test selective attention and reaction time of the participants. The participants were asked to read a color name which was displayed in a color different from the color it actually names. The outcome parameter was total number of seconds to complete the test; a higher score therefore indicates worse performance. General cognitive speed was tested by the Letter-Digit Coding Test. The participants had to match certain digits with letters according to a provided key. The outcome variable was the total number of correct entries in 60 seconds, and therefore higher scores represented better performance. The Picture-Word Learning Test was used to assess immediate and delayed memory performance. Fifteen pictures were presented at the participants, and they were asked to recall as many pictures as possible in three trials. After 20 minutes they were asked to repeat the test to measure their delayed recall. The outcome parameter is the accumulated number of correct recalled pictures, immediate and after 20 minutes. Higher scores thus indicate better performance. A detailed description of the cognitive tests and the procedures has been published previously[21]. Since treatment with pravastatin did not influence cognitive function during follow-up, we included participants from both pravastatin and placebo groups[22].

Statistical analysis

Baseline characteristics of the study participants are reported as mean (standard deviation) for continuous variables and number (percentage) for categorical variables for each group of NT-proBNP. Differences in continuous variables were tested with linear regression models, in which p-values were calculated using log-transformed NT-proBNP levels. Differences in categorical variables were tested by Chi-squared tests.

To investigate the cross-sectional association of NT-proBNP with cognitive function, we used linear regression models. Log transformed NT-proBNP levels were included as independent variable; outcome variable was the mean baseline score on each of the four cognitive function tests. Linear mixed models were used to examine the association between NT-proBNP and cognitive decline over

time. The models included log transformed NT-proBNP levels, time (in years) and the interaction term between time and log transformed NT-proBNP levels. We performed our analyses in three steps. In the first step, crude analyses were performed, in which we only adjusted for cognitive test version where appropriate. In the second step, we added the variables age, sex, education, country and apolipoprotein E genotype to the model to investigate the potential influence of these factors on the associations (minimally adjusted model). Furthermore, in a fully adjusted model we also added the following potential confounders: cardiovascular diseases and risk factors at baseline (history of cerebrovascular and cardiovascular disease, history of hypertension, history of diabetes mellitus, smoking status, HDL and LDL cholesterol levels, triglycerides, systolic and diastolic blood pressure, body mass index), statin treatment and serum creatinine level. Since the associations did not essentially change in different models, results of the minimally and fully adjusted models are presented in the manuscript. To further explore the influence of cardiovascular diseases and risk factors, additional analyses were performed in which we stratified for history of cardiovascular diseases and risk factors. To test whether the difference between participants with or without a history of cardiovascular disease or risk factor was significant, we calculated a p-value for interaction by using linear regression models. Furthermore, we performed additional sensitivity analyses in which we excluded 1) participants taking pravastatin treatment during follow-up; 2) participants with incident stroke and/or transient ischemic attack during follow-up; 3) participants with incident myocardial infarction during follow-up; 4) participants with incident atrial fibrillation during follow-up; 5) participants with vascular events, including coronary heart disease death, nonfatal myocardial infarction, nonfatal and fatal stroke and/or TIA during follow-up; and 6) participants taking loop diuretics, beta blockers or ACE-inhibitors at baseline.

Results

Table 1 shows baseline characteristics of study participants grouped by NT-proBNP levels. Participants with higher NT-proBNP levels were older and had a higher prevalence of hypertension, myocardial infarction, vascular disease and smoking (all p-values <0.001). Body mass index was lower in participants with higher NT-proBNP levels (p-value <0.001). Systolic blood pressure, pulse pressure and mean arterial blood pressure were higher among participants with higher NT-proBNP levels (p-values <0.001, $p<0.001$ and $p=0.001$ respectively). Furthermore, use of loop diuretics, beta blockers and ace-inhibitors was higher in participants with higher NT-proBNP levels (p-values <0.001, $p<0.001$ and

p=0.031 respectively). Participants with higher NT-proBNP levels had higher creatinine levels (p <0.001).

Table 2 shows the association of NT-proBNP levels with cognitive function at baseline. In the minimally adjusted model, participants with higher NT-proBNP levels had a worse performance on Stroop test (p=0.004) and Letter-Digit Coding test (p<0.001). The same trend was observed for immediate and delayed Picture-Word Learning tests, showing that participants with higher NT-proBNP levels had worse performance, albeit these associations were not significant (p-value=0.062 and p=0.065 respectively). When further adjusting for prevalent cardiovascular diseases or risk factors at baseline, we found similar differences in cognitive function between the groups. The association of NT-proBNP levels with Stroop test and Letter-Digit Coding test in the fully adjusted model remained significant (p-value=0.005 and p<0.001 respectively), whereas for immediate and delayed Picture-Word Learning tests the associations did not significantly differ (p-value=0.115 and p=0.083 respectively). Results from adjusted models did not materially differ from crude models.

Table 1. Baseline characteristics of study participants grouped by NT-proBNP

	NT-proBNP (ng/L)			
	Low N=1818 <100 ng/L	Middle N=2698 100-450	High N=689 ≥450 ng/L	P-value
Demographics				
Age, years	74.42 (3.04)	75.53 (3.37)	76.59 (3.40)	<0.001
Female, n (%)	850 (46.8)	1490 (55.2)	360 (52.2)	<0.001
Age left school, years	15.17 (2.06)	15.15 (2.06)	15.10 (2.08)	0.083
Vascular risk factors				
Hypertension, n (%)	1056 (58.1)	1736 (64.3)	444 (64.4)	<0.001
Diabetes mellitus, n (%)	245 (13.5)	245 (9.1)	57 (8.3)	<0.001
Stroke or TIA, n (%)	189 (10.4)	301 (11.2)	85 (12.3)	0.371
Myocardial infarction, n (%)	120 (6.6)	369 (13.7)	177 (25.7)	<0.001
Vascular disease, n (%)	630 (34.7)	1246 (46.2)	393 (57.0)	<0.001
Current smoker, n (%)	536 (29.5)	667 (24.7)	175 (25.4)	0.001
Body mass index, kg/m^2	27.22 (4.02)	26.69 (4.21)	26.17 (4.20)	<0.001
Total cholesterol, mmol/L	5.68 (0.90)	5.68 (0.91)	5.70 (0.93)	0.461
Systolic blood pressure, mmHg	152.60 (20.25)	155.11 (21.75)	158.75 (23.50)	<0.001
Diastolic blood pressure, mmHg	84.02 (10.95)	83.73 (11.33)	83.38 (12.01)	0.158
Pulse pressure, mmHg	68.58 (0.42)	71.38 (0.35)	75.37 (0.68)	<0.001
Mean Arterial Pressure, mmHg	106.88 (0.30)	107.53 (0.25)	108.51 (0.49)	0.001
Antihypertensive medications, n (%)				
Diuretics	650 (35.8)	1067 (39.5)	269 (39.0)	<0.001
Loop	153 (8.4)	327 (12.1)	107 (15.5)	< 0.001
Other	497 (27.3)	740 (27.4)	162 (23.5)	
Calcium channel blockers	459 (25.2)	692 (25.6)	151 (21.9)	0.125
Beta blockers	241 (13.3)	831 (30.8)	273 (39.6)	< 0.001
Ace-inhibitors	279 (15.3)	421 (15.6)	134 (19.4)	0.031
Creatinine level, Umol/L	97.92 (19.35)	100.76 (22.22)	108.58 (25.08)	< 0.001

Values presented as mean (standard deviation) except as noted.

Abbreviations: SD, standard deviation; n, number; TIA, transient ischemic attack. P-values were calculated using log-transformed NT-proBNP levels for continuous variables and Chi-squared tests for categorical variables.

Table 2. Association of NT-proBNP with baseline cognitive function

	NT-proBNP			
Cognitive tests (mean, SE)	Low N=1818 <100 ng/L	Middle N=2698 100-450	High N=689 ≥450 ng/L	P-value*
Stroop, seconds				
Minimally adjusted model	64.37 (1.46)	64.13 (1.42)	67.36 (1.63)	0.004
Fully adjusted model	66.28 (1.56)	66.12 (1.53)	69.26 (1.72)	0.005
LDCT, digits coded				
Minimally adjusted model	23.94 (0.41)	23.54 (0.40)	23.03 (0.46)	<0.001
Fully adjusted model	23.29 (0.44)	22.85 (0.43)	22.29 (0.48)	<0.001
PLTi, pictures remembered				
Minimally adjusted model	9.58 (0.11)	9.52 (0.11)	9.44 (0.12)	0.062
Fully adjusted model	9.49 (0.12)	9.44 (0.12)	9.37 (0.13)	0.115
PLTd, pictures remembered				
Minimally adjusted model	10.43 (0.16)	10.40 (0.15)	10.29 (0.17)	0.065
Fully adjusted model	10.21 (0.17)	10.18 (0.16)	10.08 (0.18)	0.083

Data represent mean (standard error) score of each cognitive function test.
∗ P-values were calculated using the continuous value of log-transformed NT-proBNP levels.
Abbreviations: SE, standard error; n, number; LDCT, Letter-Digit Coding test; PLTi, Picture-Word Learning test, immediate; PLTd, Picture-Word Learning test, delayed.
Minimally adjusted model: adjusted for age, sex, country, education, apoe genotype, test version for LDCT and PLT.
Fully adjusted model: Minimally adjusted model + adjustments for history of cerebrovascular and cardiovascular disease, history of hypertension, history of diabetes mellitus, current smoking, baseline systolic and diastolic blood pressure, HDL and LDL cholesterol, triglycerides, body mass index and serum creatinine.

Table 3 and Figure 1 show the association of NT-proBNP levels with changes in cognitive function during follow-up. Participants with higher NT-proBNP levels had a steeper cognitive decline on Stroop test, Letter-Digit Coding test and immediate and delayed Picture-Word Learning tests (all p-values < 0.001). Again, further adjustments for prevalent cardiovascular diseases or risk factors at baseline did not alter the observed associations (all p-values <0.001). The association of NT-proBNP levels with cognitive decline from crude models did not materially differ from adjusted models.

Table 3. Association of NT-proBNP with cognitive decline during follow-up

	NT-proBNP			
Cognitive tests (mean annual change, SE)	Low N=1818 <100 ng/L	Middle N=2698 100-450	High N=689 ≥450 ng/L	P-value*
Stroop, seconds				
Minimally adjusted model	0.46 (0.11)	0.71 (0.09)	1.04 (0.26)	0.001
Fully adjusted model	0.47 (0.11)	0.72 (0.09)	1.04 (0.26)	0.001
LDCT, digits coded				
Minimally adjusted model	-0.32 (0.02)	-0.36 (0.02)	-0.46 (0.04)	0.001
Fully adjusted model	-0.32 (0.02)	-0.35 (0.02)	-0.47 (0.04)	<0.001
PLTi, pictures remembered				
Minimally adjusted model	-0.00 (0.01)	-0.03 (0.01)	-0.05 (0.02)	<0.001
Fully adjusted model	0.00 (0.00)	-0.02 (0.01)	-0.04 (0.02)	<0.001
PLTd, pictures remembered				
Minimally adjusted model	-0.05 (0.01)	-0.06 (0.01)	-0.10 (0.03)	0.001
Fully adjusted model	-0.03 (0.01)	-0.05 (0.00)	-0.10 (0.03)	0.001

Data represent mean annual change (standard error) in each cognitive function test. *P-values were calculated using the interaction term of time x log transformed NT-proBNP levels.

Abbreviations: SE, standard error; n, number; LDCT, Letter-Digit Coding test; PLTi, Picture-Word Learning test, immediate; PLTd, Picture-Word Learning test, delayed.

Minimally adjusted model: adjusted for age, sex, country, education, apoe genotype, treatment group, test version for LDCT and PLT.Fully adjusted model: Minimally adjusted model + adjustments for history of cerebrovascular and cardiovascular disease, history of hypertension, history of diabetes mellitus, current smoking, baseline systolic and diastolic blood pressure, HDL and LDL cholesterol, triglycerides, body mass index and serum creatinine.

To further explore the influence of cardiovascular diseases and risk factors, we performed additional analyses in which we stratified for history of various cardiovascular diseases and risk factors, and tested for interaction. Figure 2 shows the association of NT-proBNP levels with cognitive decline, stratified by history of cardiovascular diseases and risk factors. There was no significant difference in change in cognitive function during follow-up between participants with and without cardiovascular diseases or risk factors, except for participants with a history of stroke and/or transient ischemic attack (TIA) and myocardial infarction. Participants with previous stroke and/or TIA had a less steep decline on Letter-Digit Coding test (p-value for interaction=0.003), while participants with previous myocardial infarction had a steeper decline on Letter-Digit Coding test (p-value for interaction=0.008). However, no such differences were observed for participants with previous stroke and/or TIA or myocardial infarction on

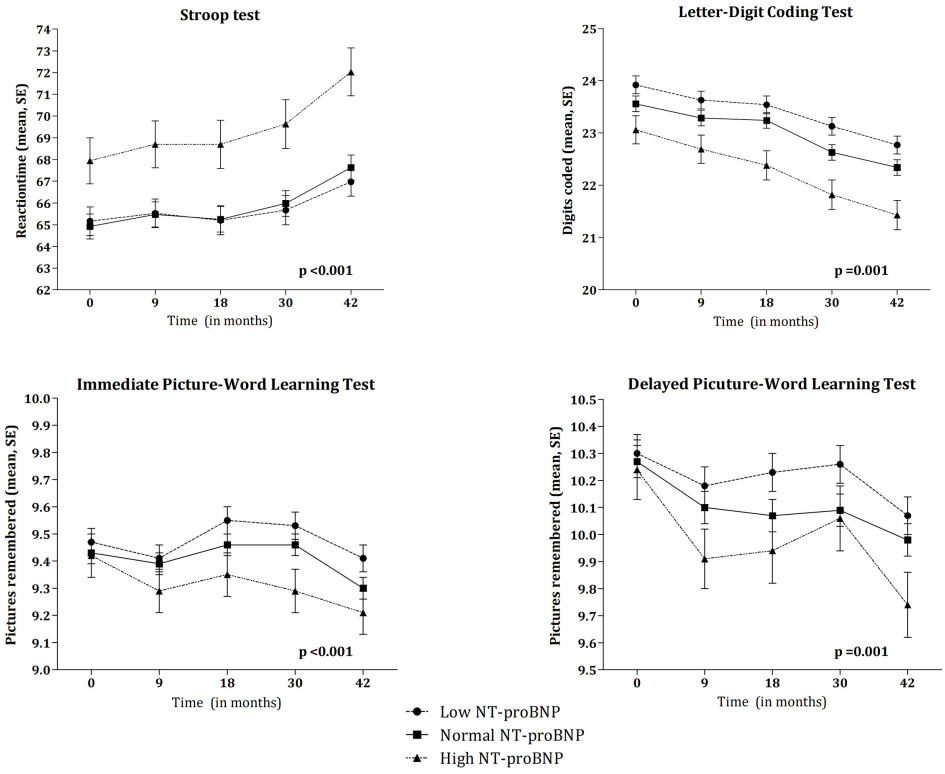

Figure 1. Association of NT-proBNP with cognitive decline during follow-up. Data represent mean score (95% confidence interval) of each cognitive test during follow-up, in each group of NT-proBNP. P-values were calculated using the interaction term of time x log transformed NT-proBNP levels. Adjustments were made for age, sex, country, education, apoE genotype and test version where appropriate.

any of the other cognitive tests.

Furthermore, we performed additional sensitivity analyses to investigate whether the association between NT-proBNP levels and cognitive function and decline could be affected by 1) participants taking pravastatin treatment during follow-up (n=2588); 2) participants with incident stroke and/or TIA during follow-up (n=355); 3) participants with incident myocardial infarction during follow-up (n=339); 4) participants with incident atrial fibrillation during follow-up (n=421); 5) participants with vascular events, including coronary heart disease death, nonfatal myocardial infarction, nonfatal and fatal stroke and/or TIA during follow-up (n=648); and 6) participants taking loop diuretics (n=588), beta blockers (n=1345) or ace-inhibitors (n=834) at baseline. Exclusion of these participants did not essentially change our results (data not shown).

Discussion

In this prospective cohort study including over 5000 men and women with mean age of 75 years, we showed that participants with higher NT-proBNP levels without advanced stages of clinical heart failure had worse cognitive function and steeper cognitive decline during a mean follow-up period of 3.2 years. These associations were independent of cardiovascular diseases and risk factors.

Our finding is in line with previous cross-sectional studies investigating the association of NT-proBNP and cognitive function[1-5]. The Rancho Bernardo Study investigated the association in 950 community-dwelling adults of 60 years and older and found that higher NT-proBNP levels were associated with poor global and executive function, but not with cognitive flexibility[1]. Another cross-sectional study including 1066 men and women aged 60-75 years with type 2 diabetes showed that higher NT-proBNP levels were weakly associated with lower general cognitive function[2]. Furthermore, The Hoorn study found that baseline BNP was associated with worse processing speed, memory, attention and executive function. Besides, they observed that an increase of BNP over time was associated with reduced attention and executive function[4]. So far only few other longitudinal studies investigated the association[5]. In a prospective study, we previously found that at age 85, subjects in the highest tertile of NT-proBNP had a lower baseline MMSE score and a steeper decline in MMSE during a 5-year follow-up period (unpublished results). This is in line with the study performed by Kerola et al, who showed that BNP was associated with worse baseline score on MMSE, a higher decline of MMSE, and a higher incidence of dementia during a mean follow-up period of 5 years[5]. To our knowledge, this is the first study

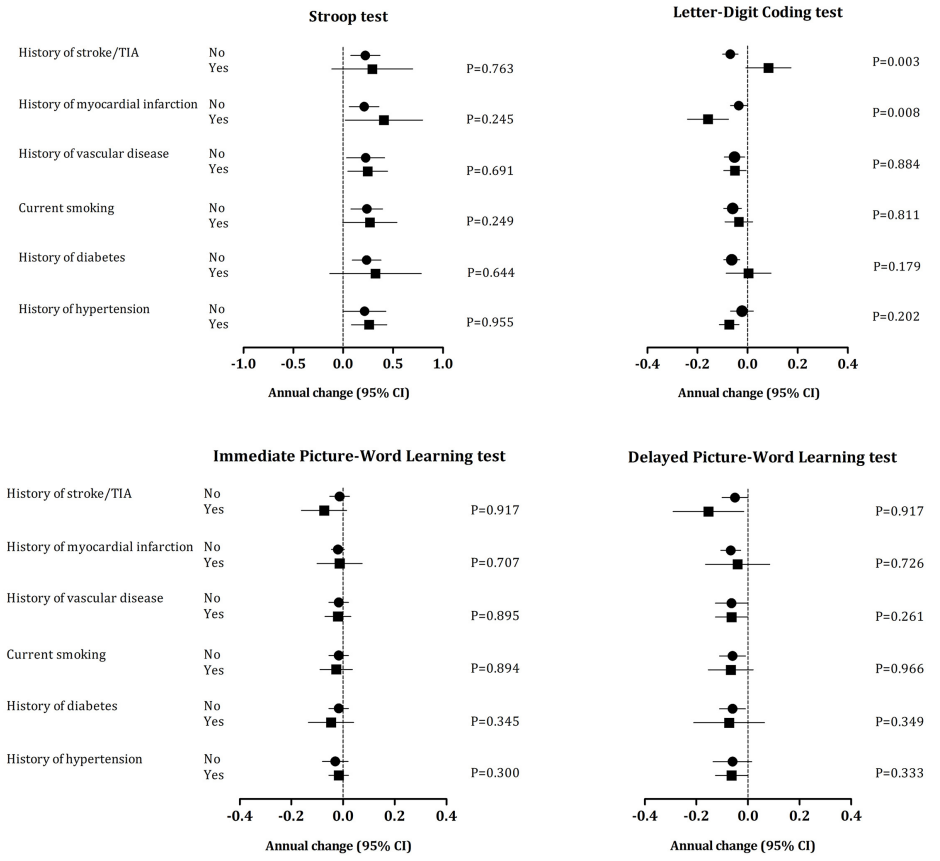

Figure 2. Association of NT-proBNP with cognitive decline during follow-up, stratified by cardiovascular diseases and risk factors. Data represent mean annual change (95% confidence interval) per 1 ng/L increase in log transformed NT-proBNP for each cognitive test, Stratified by cardiovascular diseases. Adjusted for age, sex, country, education, apoE genotype and test version where appropriate. P-values show p for interaction.

reporting on the association of NT-proBNP and cognitive function and decline in a large cohort of older participants without advanced stages of clinical heart failure at baseline and during follow-up.

Brain natriuretic peptide (BNP) and the biologically inactive N-terminal pro-brain natriuretic peptide are secreted by the ventricles of the heart in response to excessive stretching of cardiomyocytes[23]. BNP has favorable physiological properties, including increased natriuresis and diuresis, relaxation of vascular smooth muscle cells and inhibition of the renin-angiotensin-aldosterone-axis, eventually causing a reduction in blood pressure and ventricular preload[23]. Our results showed that higher NT-proBNP levels, and thus BNP as well, were associated with higher systolic blood pressure.

Different explanations can be proposed for the observed association of NT-proBNP with cognitive decline. First, NT-proBNP and cognitive decline may both reflect underlying cardiovascular damage and therefore stem from a common cause, rather than suggesting a causal relationship. Previous studies have shown that NT-proBNP levels have a prognostic value for the occurrence of cardiovascular events, such as myocardial infarction, atrial fibrillation, coronary heart disease, unstable angina, stroke and transient ischemic attack[11;14;24;25]. This has also been demonstrated in subjects with elevated NT-proBNP levels, but without clinical heart failure[14]. Furthermore, NT-proBNP levels provide predictive information for use of risk stratification in nonfatal cardiac events, stroke and mortality[25;26]. Cardiovascular diseases are closely linked to cognitive dysfunction and dementia[17;18]. This is in line with the finding that high NT-proBNP levels are associated with an increased prevalence of cardiovascular diseases and risk factors in the population under study. However, when adjusting and stratifying our analyses for cardiovascular diseases and risk factors, our results did not essentially change. Furthermore, excluding participants with incident myocardial infarction, stroke and/or TIA showed the same results. Nevertheless, we cannot totally rule out the possibility that unmeasured cardiovascular risk factors resulted in both increased NT-proBNP and cognitive decline. Second, impaired cardiac function may activate the renin-angiotensin system which in turn has been associated with cognitive decline[27]. Recent evidence suggests that the renin-angiotensin system is important in the regulation of cerebral blood flow: it impairs cerebrovascular regulation and promotes oxidative stress and amyloid protein deposition.[28] In line with this evidence, observational studies have shown that subjects receiving angiotensin receptor blockers have a lower decline in their cerebral perfusion and have a lower risk of developing dementia[29;30]. Therefore, activation of the renin-angiotensin system might be a possible explanation on the observed association between higher NT-proBNP levels and cognitive decline. Since only a small number of participants used angiotensin receptor blockers in the population under study ($n<100$), we could not further

investigate this issue. Third, since natriuretic peptides have first been identified in porcine brain extract, one could hypothesize that NT-proBNP could have a direct effect in the brain. However, filtered by the blood brain barrier, the concentration of NT-proBNP in the brain is very low, if not undetectable[31]. A fourth explanation might be that high NT-proBNP levels in subjects without advanced stages of heart failure indicate a suboptimal left ventricular functioning with subsequent decreased cardiac output and cerebral hypoperfusion[8;15]. Cerebral hypoperfusion, which impairs the delivery of oxygen and nutrients to the brain, has been associated with cognitive dysfunction and dementia[6-8]. In this scenario, high NT-proBNP levels could serve as a biomarker, reflecting suboptimal left ventricular function, which may result in cognitive decline. Although this explanation seems plausible, there is a need for interventional studies investigating the influence of improvement in cardiac function with its subsequent influence on cerebral perfusion, and eventually the prevention of cognitive decline in old age. Taken together, we favor the hypothesis that NT-proBNP could serve as a biomarker, reflecting suboptimal left ventricular function, which may result in cognitive decline. Major strengths of this study include the large sample size of over 5000 older participants and the repeated use of an extended standardized cognitive test battery to assess cognitive function over a mean follow-up period of 3.2 years. Furthermore, in contrast to previous studies, participants in our study had relatively preserved cardiac function, which gave us the opportunity to investigate the independent value of NT-proBNP in relation with cognitive function and decline. However, this study has several limitations. Our study population consisted of older participants at risk of cardiovascular diseases with relatively preserved cognitive function (MMSE $\geq$ 24 points), which might limit the extrapolation of our findings to a general population of older subjects. Furthermore, although participants with NYHA functional class III/IV were excluded from PROSPER, we might still have included participants with advanced stages of clinical heart failure but without ever being diagnosed with this condition. In addition, only information on heart failure hospitalization was available, which could have resulted in the inclusion of participants who developed clinical heart failure during follow-up, without being admitted to the hospital. However, excluding participants with NT-proBNP levels of $\geq$ 450 ng/L showed essentially the same results. In conclusion, higher NT-proBNP levels associate with worse cognitive function and steeper cognitive decline in older subjects without advanced stages of clinical heart failure. Although the exact underlying mechanism is unclear, NT-proBNP may serve as a biomarker of suboptimal left ventricular function, which through decreased cardiac output and cerebral hypoperfusion may result in cognitive decline.

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8

Cerebrovascular Hemodynamics in Alzheimer's disease and Vascular Dementia

Manuscript based on this chapter has been published as:
Sabayan B, Jansen S, Oleksik AM, van Osch MJ, van Buchem MA, van Vliet P, de Craen AJ, Westendorp RG. Cerebrovascular hemodynamics in Alzheimer's disease and vascular dementia: A meta-analysis of Transcranial Doppler studies
Ageing Res Rev. 2012;11(2):271-7.

Summary

Alteration in cerebrovascular hemodynamics has been reported in both ageing and dementia. However, it is still unclear whether this alteration follows similar pattern in ageing and in different dementia pathologies. The aim of this meta-analysis was to investigate changes in cerebral blood flow velocity and pulsatility index in two most common forms of dementia; Alzheimer's disease and vascular dementia, using transcranial doppler studies. A literature search was conducted in Pubmed, EMBASE and Web of Science. After initial screening of 304 articles and removing duplicates, a total of 53 articles, published between 1980 and 2010, were reviewed. Finally 12 articles were included in the meta-analysis. For each study, effect sizes (ES) indicating the standardized mean differences of the hemodynamic measures between two groups were calculated. Using random effect models, pooled estimates of ES were measured. Patients with Alzheimer's disease (ES=-1.09, 95% CI -1.77 - -0.44, p=0.004) and vascular dementia (ES=-1.62, 95% CI -2.26 - -0.98, $p<0.001$) had significantly lower cerebral blood flow velocity compared with healthy aged-matched controls. In addition, pulsatility index was significantly higher in both Alzheimer's disease (ES =0.5, 95% CI 0.28 0.72, $p<0.001$) and vascular dementia patients (ES=2.34, 95% CI 1.39 3.29, $p<0.001$). Patients with Alzheimer's disease had lower pulsatility index (ES= -1.22, 95% CI -1.98 0.46, P=0.002) compared to subjects with vascular type of dementia. Patients with Alzheimer's disease and vascular dementia have a pronounced disturbance in their cerebrovascular hemodynamics. The severity of disturbances in cerebral hemodynamics is significantly lower in Alzheimer's disease compared to vascular dementia.

Introduction

Over the decades, our knowledge regarding the pathogenic pathways behind dementia and its most common forms, Alzheimer's disease and vascular dementia, has changed considerably[1]. In contrast to previous thoughts about the pure neurodegenerative nature of Alzheimer's disease, it is now well established that vascular dysfunction and hemodynamic disturbances play a role in both Alzheimer's disease and vascular dementia[2, 3]. Several epidemiological studies have reported an association between vascular risk factors, such as hypertension, hyperlipidemia, diabetes mellitus type 2 and metabolic syndrome, and Alzheimer's disease[4]. Furthermore, pathologic investigations have shown that many autopsied brains of patients with Alzheimer's disease contain cerebrovascular pathologies[5]. In this setting, recently this pathologic condition was introduced as a vasocognopathy entity[6]. Although recognition of Alzheimer's disease as a vascular entity can have certain clinical, therapeutic and preventive implications, many issues regarding the mechanisms by which vascular risk factors initiate or promote cognitive impairment are still unknown[7].

While both ageing and Alzheimer's pathology have been shown to affect neurovascular and metabolic regulation of cerebral blood flow[8], there are inconsistent findings on differences in cerebrovascular hemodynamics in patients with Alzheimer's disease compared to healthy elderly subjects. Furthermore, it is not clear whether alterations in cerebrovascular hemodynamics follow similar patterns in different types of dementia. Transcranial doppler sonography is a non-invasive imaging technique widely used for the investigation of cerebrovascular hemodynamics in the major cerebral arteries[9]. This technique provides two major hemodynamic measures: mean cerebral blood flow velocity and pulsatility index. While the mean cerebral blood flow velocity shows a relative measure of the integrity in arterial perfusion, the pulsatility index reflects cerebrovascular resistance and intracranial compliance[10].

By the introduction of transcranial doppler sonography, the number of investigations on the changes of cerebral hemodynamic status among demented patients has increased[11-24]. However, this growing body of evidence on the cerebrovascular hemodynamic changes in Alzheimer's disease and vascular dementia, if not demonstrated in a systematic way, might lead to complexity of interpretations. We conducted a systematic review and meta-analysis to investigate changes in the cerebral hemodynamics in patients with Alzheimer's disease and vascular dementia in comparison with healthy aged match subjects. Furthermore, the differences in the cerebral hemodynamic measures were evaluated between Alzheimer's disease and vascular dementia. Since the middle cerebral artery is the most commonly investigated vessel by transcranial doppler sonog-

raphy and supplies the main cognitive areas in brain, we focused on changes of cerebral blood flow velocity and pulsatility index in the middle cerebral arteries of the included studies participants.

Methods

Search strategy

Pubmed, EMBASE and Web of Science were searched with the key words representing Alzheimer's disease, vascular dementia, transcranial doppler sonography, healthy ageing and cerebrovascular hemodynamics (see details in the appendix 1). The search was restricted to original articles published up to December of 2010 with English, German, French and Dutch languages (n=304). Two independent reviewers (BS and SJ) examined titles and abstracts to decide whether studies could be included. Then the full articles of included studies were checked for further determination. The reference lists of the key studies were reviewed for potential relevant articles. In order to avoid inclusion of repeated data, the whole final studies were checked for similar authors, patient characteristics and results. At the end, one study was excluded for this reason.

Study selection/Data extraction

Title and abstracts of 53 articles were screened initially and then a total of 23 studies were reviewed in detail. The following criteria were considered for eligibility of articles: (1) participants consisting of Alzheimer's disease patients and/or vascular dementia diagnosed by NINCDS-ADRDA [25], NINCDS-AIREN [26], DSM-III-R [27] or DSM-IV criteria [28] (in those studies that used the term multi-infarct dementia, criteria were assessed to be compatible with the mentioned criteria for vascular dementia); (2) transcranial doppler sonography investigation as the main tool for measurement of cerebral hemodynamics; (3) healthy and age-matched control subjects who had no history of neurological or psychiatric disorders, substance abuse and chronic medical conditions such as anemia; and (4) assessment of cerebrovascular hemodynamics including cerebral blood flow velocity (cm/second) or pulsatility index measured in the middle cerebral artery. In those studies that provided cerebral blood flow velocity and pulsatility index values in both left and right middle cerebral arteries (n=5), left side measures were considered for the final analysis. Key information on demographic and cognitive status of participants, transcranial doppler sonography examina-

tion procedure and hemodynamic outcomes of each study were extracted by one reviewer (SJ) and were then checked by another reviewer (BS). When hemodynamic data were not reported in mean and standard deviation but in mean and confidence interval, the standard deviation was calculated. In one study where hemodynamic measures were presented separately in male and female groups, the mean and standard deviation of the hemodynamic parameters in men and women were combined.

Data analysis

For each study effect sizes were calculated by the Hedges method which can be interpreted as standardized mean difference of the hemodynamic measures between two groups[29]. The larger the effect size, the greater the differences in the hemodynamic measures between the two groups. After calculation of the effect sizes for each study, the meta-analyses were performed by using a random effects model which computes the pooled effect size. The random effects model was applied since it takes into account the variability between studies. Furthermore, sensitivity analysis was performed to ensure that no single study biased the combined results by repeated excluding one study at a time and measuring of the combined effect size in the remaining studies. To assess heterogeneity, i.e., whether the differences between studies were greater than would be expected by chance alone, the Q method was used[30]. Possible sources of for heterogeneity were explored by evaluating the differences in the characteristics of the included studies. The limited number of studies did not allow us to investigate source of heterogeneity further by meta-regression analysis. Using weighted regression and rank correlation methods, we assessed publication bias i.e., the phenomenon in which small studies with negative results are not published[31]. All the statistical analyses were carried out using STATA version 10.

Results

Study population

Among 23 reviewed full-text articles, a total of 12 studies were included in the meta-analysis [1-12] (Table 1). The studies included a total of 795 participants: 268 with Alzheimer's disease, 200 with vascular dementia, and 327 controls. Females made 56%, 46% and 53% of the participants in Alzheimer's disease, vascular dementia and control groups respectively. Average MMSE scores ranged from 13

to 25 in Alzheimer's disease and from 12 to 23 in vascular dementia groups.

Hemodynamic measures

Hemodynamic measures from the individual studies are presented in the table 2. After pooling the data, in comparison with healthy aged-matched subjects, significantly lower cerebral blood flow velocity was observed in patients with Alzheimer's disease (effect size=-1.09, p=0.004, figure 1-A) and vascular dementia (effect size=-1.62, $p<0.001$, figure 1-B). Pulsatility index was significantly higher in both Alzheimer's disease (effect size=0.497, p=0.004, figure 2-A) and vascular dementia patients (effect size=2.34, $p<0.001$, figure 2-B). When we compared cerebral blood flow velocity between patients with Alzheimer's disease and vascular dementia, there was no significant difference (effect size=0.24, p=0.56, figure 3-A). However sensitivity analysis revealed that the outlier study [3] biased the pooled estimate. After excluding the outlier study, patients with Alzheimer's disease showed significant higher cerebral blood flow velocity (effect size=0.56, p=0.003). Compared to vascular dementia patients, patients with Alzheimer's disease had significantly lower pulsatility index (effect size= -1.22, P=0.002, figure 3-B).

Source of heterogeneity

Except for the set of studies on pulsatility index in patients with Alzheimer's disease when compared to control subjects, all the other comparisons showed a significant heterogeneity (all $p<0.001$). Interstudy differences in gender distribution [2, 11] and prevalence of cardiovascular risk factors[3, 6] in some of the included studies might be responsible for the observed heterogeneity. Furthermore differences in diagnostic criteria and presence of the mix pathologic states as well as differences in the applied methods to measure hemodynamic parameters might be considered as other potential sources for heterogeneity of findings among the studies.

Publication Bias

No indication of publication bias was found for the studies included in this meta-analysis except for the set of studies used for the comparison of cerebral blood flow velocity in Alzheimer's disease patients and healthy controls. In this set of studies the funnel plot was asymmetric and there was borderline evidence of bias using weighted regression method (p value for bias 0.05).

Table 1. Characteristics of the included studies.

Authors (Year)	Groups	No Participants	Female (%)	Mean age (SD)	Mean MMSE (SD)	Diagnostic criteria	Measured parameters	Blinded TCD examiner	MAP mmHg
Van Beek A. et al	pr.AD	21	57	72.9 (5.7)	21.3(4.6)	NINCDS-ADRDA	CBFV	n.s	93.8
(2010)	C	20	25	74.6 (2.7)	29.2 (1.3)				86.7
Claasen J. et al	pr.AD	9	66.7	67.9 (5.5)	25 (3.2)	NINCDS-ADRDA	CBFV, PI	n.s	96
(2009)	C	8	50	64.5 (5.0)	29 (0.5)				97
Vicenzini E. et al	pr.AD	60	51.7	70.7 (2.4)	19.9 (2.6)	NINCDS-ADRDA	CBFV, PI	+	n.s
(2007)	VaD	58	46.6	68.9 (2.9)	20.1 (2.3)	NINDS-AIREN			
	C	60	46.8	69.0 (3.1)	29.2 (0.8)				
Lee S et al	AD	17	58.8	67.1 (5.9)	22.1 (4.9)	NINDS-ADRDA	CBFV, PI	n.s	94.7
(2007)	C	17	58.8	67.1 (5.9)	28.7 (0.6)				96.3
Doepp F. et al	pr.AD	20	70	66 (13)	18 (17)	NINDS-ADRDA	CBFV, PI	n.s	97.6
(2006)	VaD	20	45	71 (11)	20.7	NINDS-AIREN			100
	C	12	50	65 (8)	-				102
Marcos A. et al	pr.AD	36	66.7	70.4	-	NINDS-ADRDA	CBFV, PI	+	n.s
(1997)	VaD	31	25.8	72	-	DSM-IV			
	C	32	59.4	67.2	-				
Biedert S. et al	pr.AD	23	43.5	60-69	14	NINDS-ADRDA	CBFV, PI	n.s	94.3
(1995)	VaD	19	42.1	60-69	13	DSM-III-R			98.7
	C	36	52.8	60-69	29.5				97
Ries F. et al	pr.AD	24	58.4	65.8 (9)	18.3	Stroke-ADRDA	CBFV	+	n.s
(1993)		17	58.5	69.1 (8.5)	20	DSM-III-R			
	C	64	57.8	61 (11.1)	-				
Caamaño J. et al	pr.AD	12	41.7	63.5 (6.6)	-	NINCDS-ADRDA	CBFV, PI	n.s	89.8
(1993)	VaD	12	75	72.8 (9.0)	-	DSM-III-R			95.9
	C	12	66.7	57.2 (7.5)	-				99.2
Provinciali L. et al	AD	20	-	67.8 (4.7)	-	DSM- III	CBFV, PI	n.s	n.s
(1990)	VaD	20	-	64.7 (7.3)	-	DSM- II			
	C	25	-	Age-matched	-				
Foerstl H. et al	pr.AD	9	44.4	60-69	13	NINCDS-ADRDA	CBFV, PI	n.s	94
(1989)	VaD	9	44.4	60-69	12	DSM-III-R			95
	C	14	64.3	60-69	>29				

Abbreviations: SD: standard deviation, TCD: transcranial Doppler, MAP: mean arterial pressure, AD: Alzheimer s disease, pr.AD: probable Alzheimer's disease, VaD: vascular dementia, C: controls, NINCDS-ADRDA: The national Institute of Neurologic and Communicative Disorders and Stroke-Alzheimer Disease and Related Disorder Association, NINCDS-AIREN: The National Institute of Neurological Disorder and Stroke-Association International pour la Recherche et L'Enseignement en Neurosciences, DSM-III-R: Diagnostic and statistical manual of mental disorders, CBFV: Cerebral blood flow velocity, PI: Pulsatility index, n.s.: not specified

Table 2. Hemodynamic measures in the individual studies included in this meta-analysis.

Authors (Years)	CBFV cm/sec (SD)			PI (SD)		
	Alzheimers Disease	Vascular dementia	Control	Alzheimers Disease	Vascular dementia	Control
Van Beek et al (2010)	34.4 (13)	-	39.5 (10.7)	-	-	-
Claasen et al (2010)	38 (7.1)	-	55 (19)	-	-	-
Lee et al (2007)	56.3 (12.2)	-	57.7 (15.4)	0.92 (0.18)	-	0.88 (0.12)
Vicenzini et al (2007)	38.7 (2.9)	46.3 (3.1)	54.9 (3)	1.08 (0.05)	1.1 (0.5)	0.91(0.5)
Doepp et al (2007)	43 (13)	36 (8)	59 (13)	1 (0.2)	1.1 (0.2)	0.9 (0.2)
Biedert et al (1995)	45.5 (8.8)	38.2 (9.5)	50.4 (1.2)	0.85 (12)	1.27 (0.15)	0.82 (0.13)
Caamano et al (1990)	42.7 (7.2)	38.6 (13.7)	57.5 (8.47)	0.93 (0.27)	1.5 (0.22)	0.78 (0.15)
Ries et al (1993)	46.7 (10.6)	41.9 (8)	53.1 (13.5)	-	-	-
Biedret et al (1990)	47.6 (9.8)	39.1 (10,2)	50.7 (1.3)	-	-	-
Proviciali et al (1990)	56.3 (11.6)	41.8 (13.9)	53.7 (5.8)	0.88(0.14)	1.06 (0.13)	0.73 (0.15)
Marcos et al (1997)	40.7 (7.5)	42.2 (9.7)	45.5 (8.13)	-	-	-
Forestel et al (1989)	47.6 (9.8)	39.1 (10.2)	50.7 (1.3)	0.95 (0.13)	1.27 (0.17)	0.86 (0.17)

Abbreviations: CBFV: cerebral blood flow velocity, PI: pulsatility index, SD: standard deviation.

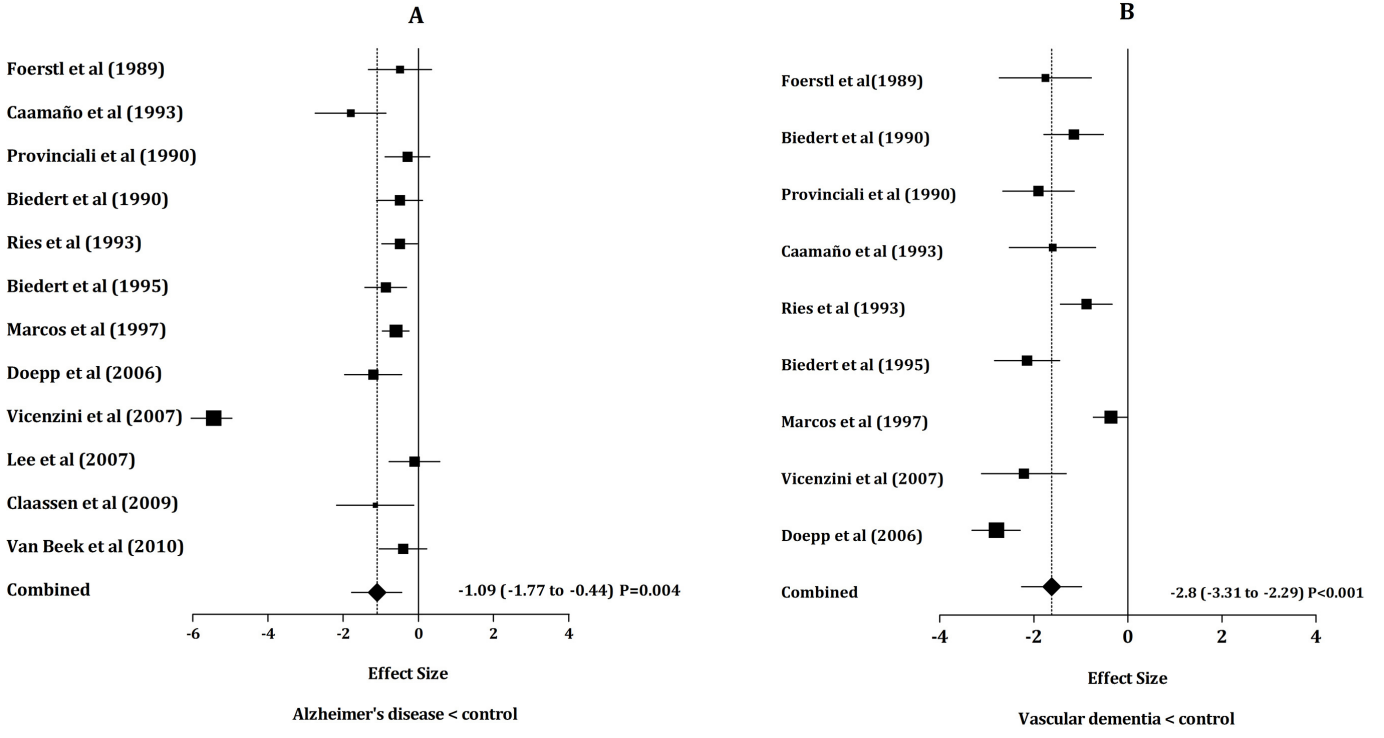

Figure 1. (A) Forest plot of cerebral blood flow velocity in patients with Alzheimer's disease compared to control subjects. (B) Forest plot of cerebral blood flow velocity in patients with vascular dementia compared to control subjects.

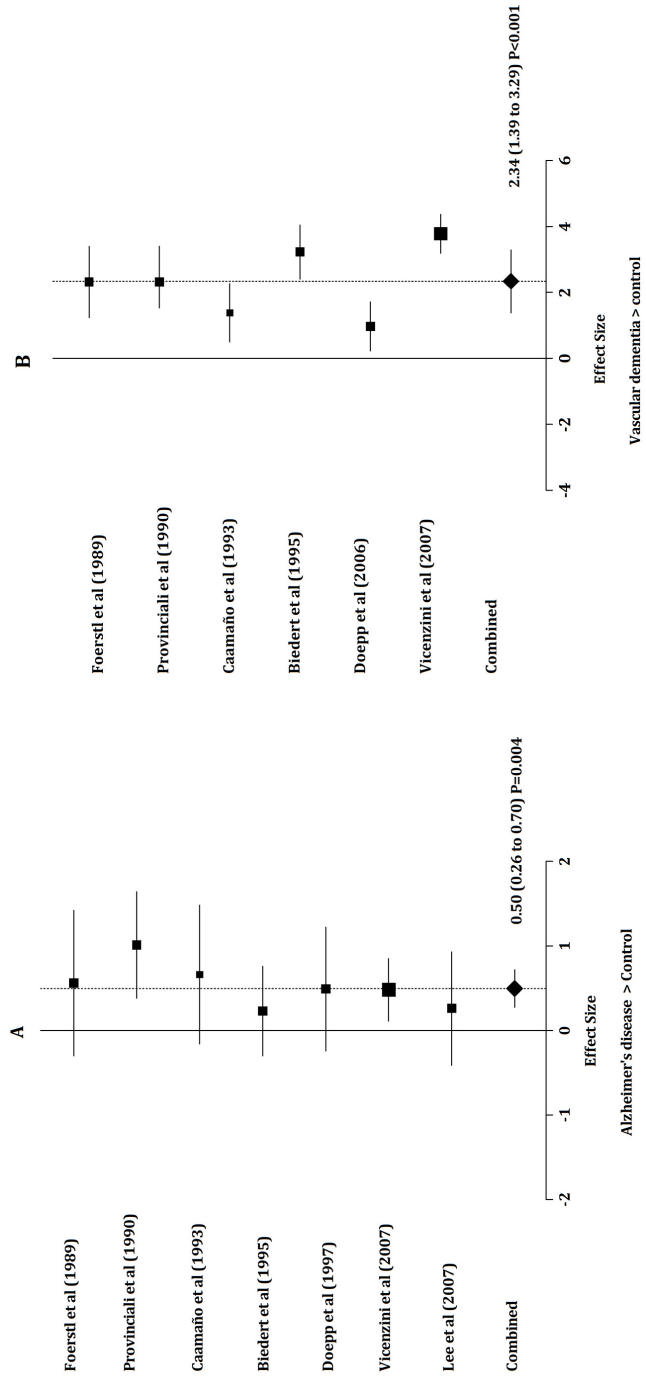

Figure 2. (A) Pulsatility index in patients with Alzheimers disease compared to control subjects. (B) Pulsatility index in patients with vascular dementia compared to control subjects.

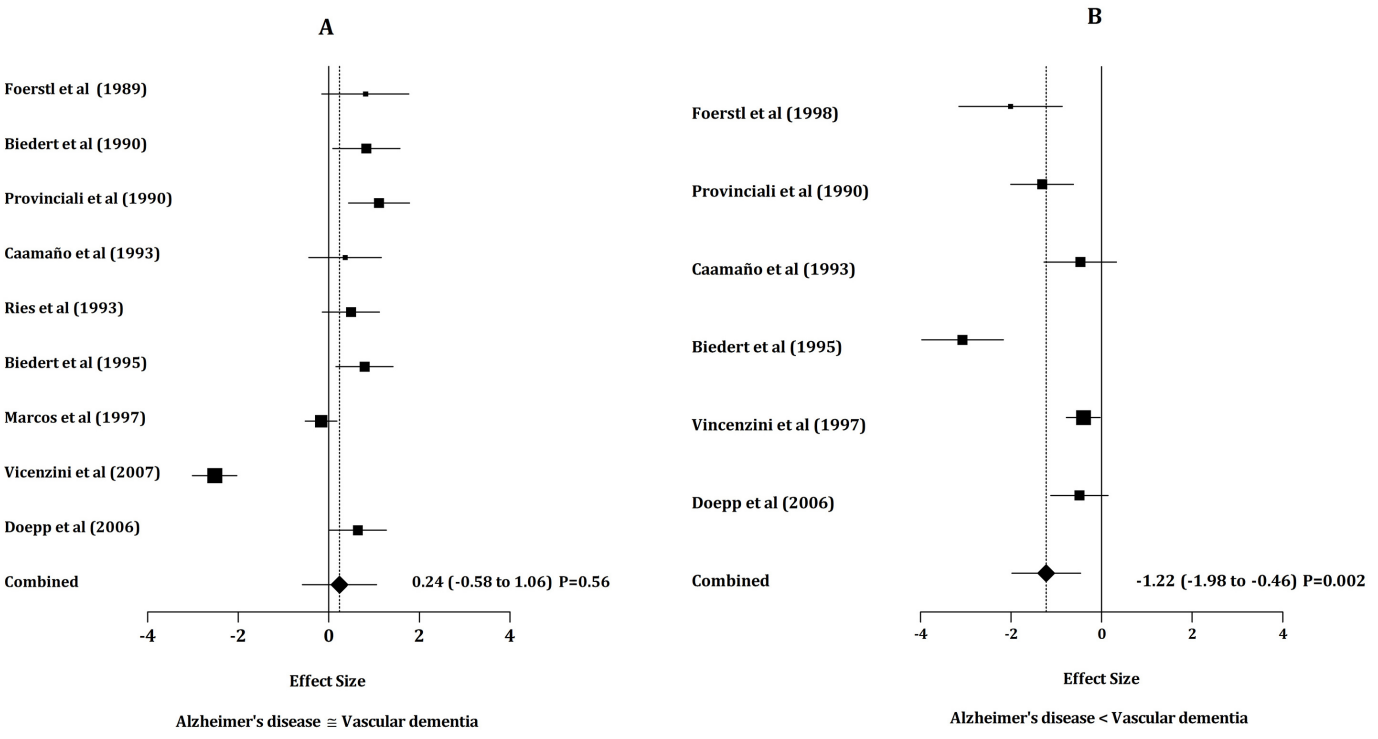

Figure 3. (A) Forest plot comparing cerebral blood flow velocity in patients with Alzheimer's disease and vascular dementia. (B) Forest plot comparing pulsatility index in patients with Alzheimer's disease and vascular dementia.

Discussion

This meta-analysis shows that patients with Alzheimer's disease and vascular dementia, in comparison with healthy aged matched subjects, have a pronounced disturbance in their cerebrovascular hemodynamics. The severity of disturbances in cerebral hemodynamics is significantly lower in Alzheimer's disease compared to vascular dementia.

Recently, the cerebrovascular hypothesis of dementia has received considerable attention[13]. This hypothesis implies that chronic cerebral hypoperfusion, in the presence of vascular risk factors leading to vascular and metabolic damages of the brain, is the main drive of neuronal dysfunction and cell death with consequent cognitive disability[14]. While the most common form of dementia, Alzheimer's disease, was first introduced as a primary neurodegenerative disorder, there is ongoing debate whether this entity should be recognized as a primary vascular disorder[15]. By the introduction of various neuroimaging techniques, a large number of studies have assessed whether Alzheimer's disease and vascular dementia have different patterns of vascular and hemodynamic characteristics[16].

Cerebral blood flow velocity, detected by ultrasound, has been used extensively as a proxy for cerebral blood flow[17]. Our results are in line with previous findings showing a state of cerebral hypoperfusion in dementia[18]. However, comparison of patients with Alzheimer's disease and vascular dementia revealed that Alzheimer's disease patients have significantly less impairment in their cerebral perfusion than vascular dementia patients, suggesting a potential spectrum of hemodynamic disturbances in different types of dementia. This difference between Alzheimer's disease and vascular dementia may be a reflection of differences in type, load or even location of vascular pathology. For instance, histological investigations have shown that amyloid angiopathy is the main vascular pathology in Alzheimer's disease, whereas atherosclerosis with consequent micro and macro vascular infarcts are the major pathologic features of vascular damage in vascular dementia[19]. In addition to the type of vascular damage, the severity of vascular changes might underlie this difference. For instance, although it was shown that patients with Alzheimer's disease have cerebral white matter lesions and microbleeds, as signs of small vessel disease, the severity of these pathologies are reported to be significantly higher in vascular dementia[20, 21]. Finally, different locations of vascular changes in the brains of patients with Alzheimer's disease and vascular dementia [22] might contribute to variation of cerebrovascular hemodynamics in these two types of dementia. In a large autopsy series of demented patients, evaluation of topographic patterns of brain vascular lesions showed that subjects with pure vascular dementia had

higher frequency of small subcortical lesions while cases with a definite diagnosis of Alzheimer's disease with vascular encephalopathy showed more lobar and cortical infarcts[23].

Significant increase in pulsatility index in both Alzheimer's disease and vascular dementia patients indicate a remarkable increase in cerebrovascular stiffness associated with decline in intracranial vascular compliance. A diffuse injury to micro-vascular structure imposed by atherosclerosis and amyloid angiopathy can be a potential explanation for this phenomenon[24]. However, most of the included studies in this meta-analysis have not provided detailed information on vascular pathologies proximal or distal to the middle cerebral artery. Furthermore, increased cerebrovascular resistance might highlight the role of chronic cerebral hypoperfusion not only as a consequence of neuronal loss and lower metabolic demand but also as the primary factor in development and promotion of dementia. The combination of high level of vascular resistance and low perfusion state suggests a global vascular pathology which could start from small vessel disease in both types of dementia and extend to larger vessels in the vascular type of dementia[23]. Collectively, it seems that hemodynamic disturbances and neurodegeneration act in concert in development and progression of dementia and no single mechanism can fully explain common mixed pathologic findings in the brains of patients with dementia.

Although dementia is a slowly progressing medical condition and pathogenic mechanisms may start working decades before apparent clinical symptoms[25], there is a limited data on hemodynamic changes in pre-clinical stages of dementia and mild cognitive impairment. Sun et al investigated the mean cerebral blood flow velocity in mild cognitive impairment patients and demonstrated that those patients had significantly lower cerebral blood flow velocity compared to age-matched controls[26]. While decrease in cerebral blood flow velocity and increase in pulsatility index are well-described phenomenon in ageing[27], the findings of our review highlight the intensified disturbances of cerebrovascular hemodynamics in aged people with dementia.

This meta-analysis has certain limitations. First, we could only include outcomes measured in the middle cerebral artery. Even though middle cerebral artery is the main vessel responsible for perfusion of parietotemporal areas in brain, there might be regional variations in hemodynamic disturbances through development and progression of dementia which we could not address. Nevertheless, recent studies among demented patients confirm that most of intracranial arteries follow similar hemodynamic pattern we presented for the middle cerebral artery[28]. Second, the studies were small and despite statistical analysis, there is concern of publication bias as small negative studies were unlikely to be published because of a lack of power compared to small positive studies. Finally,

transcranial doppler sonography cannot provide any estimation on the extent of brain atrophy. Therefore, neuronal loss and decrease in metabolic demand can be considered as an alternative explanation for reduction of cerebral blood flow in patients with dementia in comparison with healthy elderly. In this setting, interpretation of cerebral hemodynamic parameters before correction for the level of brain atrophy needs caution.

Regulations of cerebral blood flow and other hemodynamic measure follows complex mechanisms[29]. Though great achievement has been made in recognition of these mechanisms in physiologic states, our knowledge concerning the impairment of regulatory pathways in dementia is limited and needs further investigation. In future work, using pooled data of other hemodynamic indexes such as cerebral autoregulation and vasomotor reactivity measured at a regional level, more details on hemodynamic disturbances in different types of dementia might be revealed.

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9

Cognitive Impairment and Risk of Stroke

Manuscript based on this chapter has been published as:
Sabayan B, Gussekloo J, de Ruijter W, Westendorp RG, de Craen AJ. Framingham stroke risk score and cognitive impairment for predicting first-time stroke in the oldest old. Stroke. 2013 ;44(7):1866-71.

Summary

Predictive value of the conventional risk factors for stroke attenuates with age. Cognitive impairment has been implicated as a potential predictor for stroke in older subjects. Our aim was to compare the Framingham stroke risk score with cognitive functioning for predicting first-time stroke in a cohort of the oldest-old individuals. We included 480 subjects aged 85 years old from the Leiden 85-plus Study. At baseline, data on the Framingham stroke risk score and the Mini-Mental State Examination (MMSE) score were obtained. Risk of first-time stroke was estimated in tertiles of Framingham and MMSE scores. Receiver operating characteristic curves with corresponding areas under the curves (AUC) and 95% confidence intervals (CI) were constructed for both Framingham and MMSE scores. Subjects with high Framingham risk score compared to those with low Framingham risk score did not have a higher risk of stroke (Hazard Ratio (HR): 0.77, 95% CI: 0.39-1.54). Conversely, subjects with high level of cognitive impairment compared to those with low level of cognitive impairment had a higher risk of stroke (HR: 2.85, 95% CI: 1.48-5.51). In contrast to the Framingham risk score (AUC: 0.48, 95% CI: 0.40-0.56), MMSE score had discriminative power to predict stroke (AUC: 0.65, 95% CI: 0.57-0.72). There was a significant difference between AUC for Framingham risk score and MMSE score (p=0.006). In the oldest-old, the Framingham stroke risk score is not predictive for first-time stroke. In contrast, cognitive impairment, as assessed by MMSE score, identifies subjects at a higher risk of stroke.

Introduction

Oldest old subjects form a growing part of the stroke population in developed countries[1]. Since stroke in very old age puts a great burden on patients and health care systems, novel strategies for early identification of very old subjects at high risk of stroke is crucial[2].

With advancing age, conventional vascular risk factors such as hypertension and hypercholesterolemia lose their predictive value for stroke[3, 4]. This phenomenon has raised doubt whether well-established predictive models such as Framingham stroke risk score, which were constructed based on such factors in middle age and younger elderly, can accurately identify very old subjects at high risk of stroke[5]. Cognitive impairment is common in old age and is strongly associated with brain vascular pathologies and disturbances in cerebrovascular hemodynamics[6]. Some studies have shown that impaired cognitive function is a potential predictor for first-time stroke in middle aged and younger elderly people[7, 8]. Whether these findings can be extrapolated to very old subjects is unclear.

The Framingham stroke risk score has been validated in subjects younger than 85 years old[9] and there is a lack of evidence on the predictive value of cognitive impairment for first-time stroke in the oldest old. The aim of our study was to investigate the performance of Framingham stroke risk score and cognitive impairment in predicting five-year risk of first-time stroke in a population-based sample of very old subjects. We hypothesized that level of cognitive impairment better predicts risk of stroke in the oldest old.

Material and methods

Study design and participants

The Leiden 85-plus Study is an observational, prospective population-based cohort study of inhabitants of Leiden, the Netherlands. Between September 1997 and September 1999, all inhabitants of 1912-1914 birth cohort (n = 705) were contacted in the month of their 85th birthday. There were no selection criteria on demographic features or health status. Fourteen people died before enrollment; a total of 599 (397 women and 202 men) subjects agreed to participate (85%). As described previously, there was no significant difference between the demographic features and health status of those who participated and those who did not[10]. In this analysis we excluded 61 subjects who had a previous stroke. In

addition, 58 subjects were excluded because of missing data for the components of Framingham risk score or cognitive function, leading to a final sample size of 480 subjects. All participants were visited at their homes, where they underwent face to face interview, physical examination, blood sampling, electrocardiography and cognitive assessment. The Medical Ethical Committee of the Leiden University Medical Centre approved the study, and informed consent was obtained from all subjects.

Vascular risk factors included in the Framingham stroke risk score

Blood Pressure

Blood pressure was measured at baseline in the seating position, using a mercury sphygmomanometer. During the home visits, two blood pressure measurements were done two weeks apart and average of these two measurements were used in the analyses. Blood pressure measurements were recorded after at least 5 min of rest and no vigorous exercise in the preceding 30 min. The systolic blood pressure (SBP) was measured at Korotkoff sound 1, and the diastolic blood pressure (DBP) was measured at Korotkoff sound 5. Use of antihypertensive medication was extracted from pharmacy records.

Diabetes mellitus

Diabetes mellitus was considered present when present in the records of the primary care physician, when non-fasting glucose concentrations were greater than 11.0 mmol/l, or when a participant was taking antidiabetic medication according to their pharmacy records.

Smoking

All participants were interviewed about present and past smoking habits. Current and past smokers of cigarettes, cigars, and pipes were judged to have a history of smoking.

Cardiovascular diseases

Data on history of cardiovascular diseases including coronary artery disease,

heart failure, or peripheral vascular disease were obtained through interviewing individual's general practitioners or nursing home physicians.

Electrocardiogram based left ventricular hypertrophy and atrial fibrillation

Electrocardiograms were recorded on a Siemens Sicard 440 (Erlangen, Germany) and transmitted to the electrocardiograms core laboratory in the Glasgow Royal Infirmary for automated Minnesota coding [11]. All electrocardiograms were reviewed to exclude coding errors due to technical causes. Left ventricular hypertrophy was defined by Minnesota codes 3-1-0, 3-3-0, or 3-4-0. Atrial fibrillation was defined as the presence of Minnesota Code 8-3-1.

Cognitive assessment

At baseline, global cognitive function was assessed in all participants using the Mini-Mental State Examination (MMSE). The MMSE assesses five areas of cognitive function: orientation, registration, attention and calculation, recall and language. The MMSE has a maximum score of 30 in which higher scores indicate better cognitive function. We categorized participants based on the tertiles of MMSE score to low (MMSE $>$27), intermediate (MMSE =25-27) and high (MMSE $<$25) levels of cognitive impairment.

Stroke

In the Netherlands detailed information on health status, emergency events and patients' hospitalizations are all recorded with general practitioners. Occurrence of clinically recognized stroke during five years of follow up was assessed by annually interviewing general practitioners (for subjects living independently) or nursing home physicians (for subjects living in a nursing home). We used the World Health Organization definition of stroke of "rapidly developing clinical signs of focal (at times global) disturbance of cerebral functioning lasting $>$ 24 hours" to identify subjects with stroke events.[12] To obtain exact date of fatal stroke events, we retrieved specific data on causes of death from Statistics Netherlands, which assigns codes for all national death certificates according to the International Classification of Diseases and Related Disorders, 10th revision (ICD-10). Death due to stroke was classified as ICD-10 codes I60-I69.13

Statistical analysis

Characteristics of the study participants are reported as mean (standard deviation) for continuous variables and number (percentage) for categorical variables. Differences in values of continue parameters between participants who developed stroke and participants who did not were tested by independent samples t tests. Differences in frequency of categorical parameters between the two groups were evaluated using Chi-Square tests. To assess the performance of Framingham risk score and MMSE score in prediction of stroke we used following analyses. First, incidence rate of stroke with corresponding 95% confidence intervals, in each tertile of Framingham and MMSE score was estimated by dividing the number of events by the person-year at risk. In addition, we compared cumulative incidence of stroke in tertiles of both Framingham and MMSE scores. Kaplan-Meier method was used and strata were compared with log-rank test. In the next step, hazard ratios with corresponding 95% confidence intervals for stroke outcomes (second and third tertile of Framingham and MMSE score versus first tertile as reference) were calculated using Cox regression models. Finally, receiver operating characteristic (ROC) curves with corresponding areas under the curves (AUC, neutral value 0.50= risk prediction by pure chance) and 95% confidence intervals were constructed for the Framingham risk score and MMSE score. Significant difference between area under the curve for the Framingham and MMSE scores was tested using Chi-Square test. All analyses were carried out using SPSS software (version 20.0.0, SPSS Inc., Chicago, IL) except for the comparing of the AUC of ROC curves which was performed by STATA version 10.0 (Stata Corp., College Station, TX).

Results

Table 1 summarizes baseline characteristics of the participants. During five years of follow up 56 subjects developed a stroke (incidence rate: 30.3 per 1000 person-year). At age 85 years, prevalence of cardiovascular diseases, diabetes mellitus, smoking, atrial fibrillation, left ventricular hypertrophy and use of antihypertensive medication was not statistically different in participants with and without stroke events in subsequent years (all $P>0.05$). Moreover, systolic and diastolic blood pressure at age 85 years was also similar in participants with and without stroke events in subsequent years (both $P>0.05$). In contrast, subjects who developed stroke had significantly lower MMSE score at age 85 years ($P<0.001$).

Figure 1 shows cumulative incidence of stroke by tertiles of Framingham and

Table 1. Baseline characteristics of study participants

Characteristics	All	Occurrence of stroke during follow up		P-value*
	(n=480)	Yes (n=56)	No (n=424)	
Men, n (%)	163 (33.8)	16 (27.6)	147 (34.7)	0.28
History of CVD, n (%)	283 (58.7)	37 (66.1)	246 (58.0)	0.25
History of DM, n (%)	75 (15.6)	8 (14.3)	67 (15.8)	0.77
Ever smoking, n (%)	233 (48.3)	24 (42.1)	209 (49.3)	0.31
Atrial fibrilation, n (%) 42 (8.7)	6 (10.3)	36 (8.5)	0.64	
Left ventricular hypertrophy, n (%)	48 (10.0)	8 (13.8)	40 (9.4)	0.30
Use of antihypertensive medication, n (%)	213 (44.3)	22 (38.6)	191 (45)	0.36
Systolic blood pressure, mmHg mean (SD)	155 (18.3)	153.4 (20.1)	156 (18.0)	0.32
Diastolic blood pressure, mmHg mean (SD)	77 (9.3)	77.8 (9.8)	76.9 (9.3)	0.51
MMSE score, point, median [IQR]	26 [23-28]	24 [18-27]	27 [24-28]	<0.001

* P-value indicates significant difference between subjects with and without stroke occurrence

Abbreviations: CVD: cardiovascular diseases, DM: diabetes mellitus, BMI: body mass index, HDL: high density lipoprotein, LDL: Low density lipoprotein, SD: standard deviation, MMSE: mini mental state examination, IQR: inter quartile range

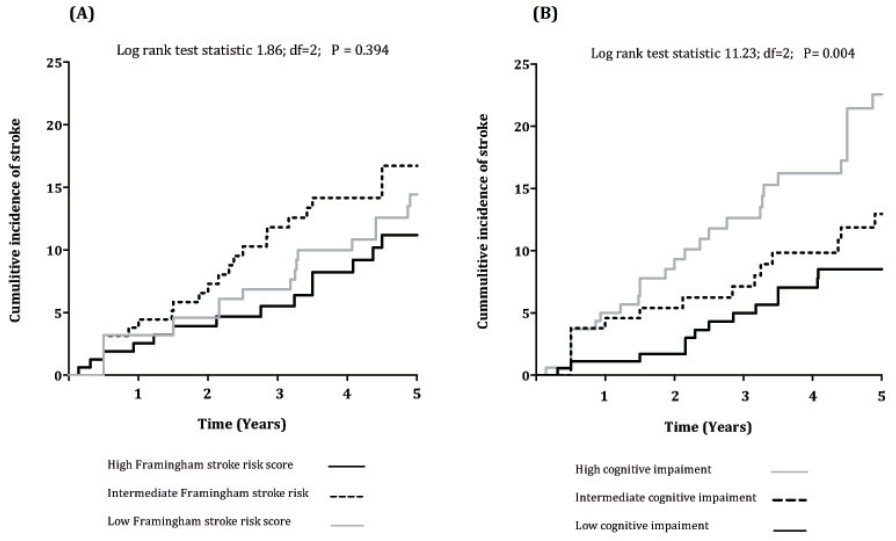

Figure 1. Cumulative incidence of fatal and nonfatal stroke during five years of follow-up according to level the Framingham risk score (1-A) and cognitive impairment (1-B)

MMSE scores. There was no significant difference in cumulative incidence of stroke among subjects with low, intermediate and high Framingham risk scores (log-rank p=0.39). In contrast, there was a significant difference in cumulative incidence of stroke among subjects with low, intermediate and high levels of cognitive impairment (log-rank p=0.004).

Number of stroke events and risk of stroke in tertiles of Framingham and MMSE scores are presented in table 2. Incidence rate of stroke was 23.3 (95% CI: 11.1-35.5) per 1000 person-year in subjects with high Framingham risk score, 37.1 (95% CI: 21.9-52.3) per 1000 person-year in subjects with intermediate Framingham risk score and 30.2 (95% CI: 16.6-43.8) per 1000 person-year in subjects with low Framingham risk score. Incidence rate of stroke was 49.5 (95% CI: 31.2-67.8) per 1000 person-year in subjects with high level of cognitive impairment, 27.8 (95% CI: 13.7-41.9) per 1000 person-year in subjects with intermediate level of cognitive impairment and 17.4 (95% CI: 8.1-26.8) per 1000 person-year in subjects with low level of cognitive impairment. Furthermore, subjects with high Framingham risk score did not have a higher risk of stroke compared to those with low Framingham risk score (HR: 0.77, 95 % CI: 0.39-1.54) whereas subjects with high level of cognitive impairment had a higher risk of stroke compared to those with low level of cognitive impairment (HR: 2.85, 95 % CI: 1.48-5.51). We further explored whether cognitive performance predicts risk of first-time

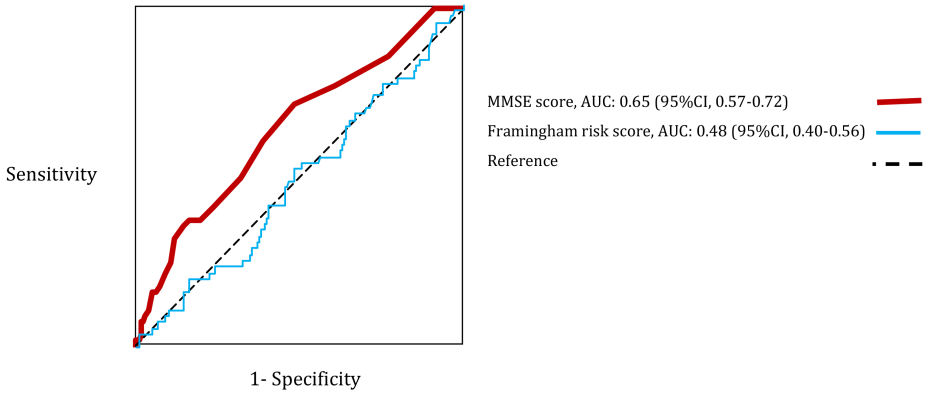

Figure 2. Receiver operating characteristic (ROC) curves showing area under the curve (AUC) of Framingham risk score and Mini Mental Status Examination (MMSE) for predicting five years risk of first-time stroke.

stroke independent of socio-demographic and cardiovascular factors and found similar associations between cognitive impairment and risk of stroke (table S-1 http://stroke.ahajournals.org/content/44/7/1866/suppl/DC1).

Figure 2 shows the ROC curve for Framingham risk score as well as MMSE score in prediction of stroke events. Framingham risk score did not predict stroke (AUC 0.48, 95%CI: 0.40-0.56). Conversely, MMSE score had discriminative power to predict stroke (AUC 0.65, 95%CI: 0.57-0.72). The AUC for MMSE score was significantly higher than the AUC for Framingham stroke risk score (p=0.006). In addition, to explore whether the association between cognitive impairment and risk of stroke was not only dependent on subjects with very low cognitive function, in a sensitivity analysis, we excluded subjects with MMSE score 16 or less (n= 42) and found similar outcomes (data not shown). In another sensitivity analysis, to test whether our findings are not due to short term stroke events, we excluded subjects who developed stroke in the first year of follow-up (n=14). This sensitivity analysis showed that the predictive value of MMSE score for stroke is not dependent on short term stroke events (table S-2 http://stroke.ahajournals.org/content/44/7/1866/suppl/DC1).

Discussion

In a cohort of very old individuals without a previous history of stroke, we observed that Framingham stroke risk score, composed of conventional vascular

Table 2. Risk of stroke in relation to Framingham risk score and level of cognitive impairment

	Number of participants	Number of stroke events	Incidence Rate* (95% CI)	Hazard ratio (95% CI)
Framingham five-year stroke risk				
Low (3.7%-13.2%)	160	19	30.2 (16.6-37.2)	1 (ref)
Intermediate (13.3% -22.2%)	161	23	37.1 (21.9-52.3)	1.22 (0.66-2.42)
High (22.3%-97.4%)	159	14	23.3 (11.1-35.4)	0.77 (0.39-1.54)
				P for trend: 0.501
Cognitive impairment				
Low (MMSE>27)	180	13	17.4 (8.0-26.9)	1 (ref)
Intermediate (MMSE 25-27)	136	15	27.8 (13.7-41.9)	1.59 (0.76-3.35)
High (MMSE<25)	164	28	49.5 (31.1-67.8)	2.85 (1.48-5.51)
				P for trend: 0.001

Incidence rates were estimated per 1000 person-year
Abbreviation: MMSE: mini mental state examination; HR: hazard ratio.

risk factors, did not predict risk of stroke. In contrast, impaired cognitive function assessed by low scores on the MMSE, identified subjects at higher risk of stroke.

A growing body of evidence indicates that the predictive value of conventional vascular risk factors for mortality and cardiovascular events attenuates with age.[3, 14-16] Previously, we reported that in very old people from the general population with no history of cardiovascular disease, risk factors such as hypertension, hypercholesterolemia, diabetes mellitus and left ventricular hypertrophy did not predict cardiovascular mortality.[5] Consistently, results from the current study suggest that conventional vascular risk factors included in the Framingham stroke risk score may not predict higher risk of stroke in the oldest old. Well-established prediction models for stroke are basically designed for middle aged or younger elderly people.[17] The Framingham stroke risk score is not an exception as it was constructed in a study population with an average age of 65 years and is validated for subjects younger than 85 years old.[9] This might explain why the Framingham risk score in our study population consisting of very old age subjects did not predict risk of stroke.

Cognitive impairment is common in old age and has a clear association with brain vascular pathologies and disturbances in cerebrovascular hemodynamics.[18-20] Thus, cognitive assessment has been proposed as a tool to identify younger elderly subjects at risk of stroke.[8, 21] In a population-based study including 9451 subjects aged 65 year and older, cognitive impairment was associated with a twofold increased risk for fatal incident stroke.[22] Similarly, a recent large study on 30959 individuals older than 55 years at increased cardiovascular risk showed that impaired cognitive function is associated with a graded increase in risk of stroke.[23] Findings of our study extend this evidence to the oldest old population and show that in very old age when the association between conventional vascular risk factors and cerebrovascular events is weak, cognitive assessment might be considered as a tool for identifying subjects at high risk of stroke. Given that the population of very old subjects is rapidly increasing worldwide and particularly in developed countries[24], our findings highlight a need for development of new prediction models for stroke in this age group and suggest that cognitive performance can be considered as a potential component in future prediction models.

We performed our analysis in the Leiden 85-plus study which is a population-based cohort with a relatively large number of participants, low attrition rate and a long follow up period. However, limitations of this study should be kept in mind when evaluating the results. Lack of neuro-imaging data to determine type of stroke can be marked as a limitation of this study although it is previously reported that the majority of strokes in very old age are of the ischemic type[25].

Moreover, silent strokes are frequently observed in older subjects[26]. Therefore, there is a possibility that subjects in the group with high cognitive impairment had more clinically unrecognized strokes which predisposed them to the subsequent stroke events. In addition, we assessed level of cognitive impairment only based on MMSE scores. Since there is no single criterion or definition for cognitive impairment, MMSE may not be the optimum tool to evaluate level of cognitive impairment. Despite this limitation, MMSE is commonly used in clinical settings and no expertise is involved in its application which makes it an appropriate candidate for identification of the oldest old subjects at high risk of stroke[27]. It is possible that cognitive tests such as Montreal Cognitive Assessment (MoCA) which are more sensitive to vascular cognitive impairment might better predict stroke events in old age[28].

In conclusion, we showed that the Framingham stroke risk score is not predictive for first-time stroke in a general population of the oldest-old individuals. In contrast, low cognitive function predicts higher risk of stroke in the oldest old. Assessment of cognitive function can be considered as an easily accessible tool to identify very old subjects at risk of stroke. Findings of this study need to be validated in the other cohorts of the oldest old people.

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10

Endothelial Dysfunction and Cerebral Blood Flow

Manuscript based on this chapter has been published as:
Sabayan B, Westendorp RG, Grond JV, Stott DJ, Sattar N, van Osch MJ, van Buchem MA, de Craen AJ. Markers of endothelial dysfunction and cerebral blood flow in older adults. Neurobiol Aging. 2013, S0197- 4580(13)00354-0.

Summary

We investigated the association of two markers of endothelial dysfunction, tissue plasminogen activator (t-PA) and von willebrand factor (VWF), with cerebral blood flow in 541 older participants at high risk for cardiovascular disease. Serum levels of t-PA and VWF were measured at baseline. Participants underwent two successive brain MRI scans, first at baseline and the second after a mean follow-up of 33 months. Total cerebral blood flow (CBF) was determined in each scan and also standardized for brain parenchymal volume. At baseline, higher t-PA was associated with lower CBF ($p = 0.034$). In the longitudinal analysis, higher levels of VWF were associated with a steeper decline in CBF ($p = 0.043$). There was no association between t-PA and decrease in CBF. These associations were independent of socio-demographic and cardiovascular factors. In conclusion, elevated markers of endothelial dysfunction are associated with lower cerebral blood flow in older adults at risk for cardiovascular disease.

Introduction

Endothelial cells play a crucial role in maintaining vascular homeostasis[1]. These cells contribute to regulation of vascular tone and the development of thrombosis and vessel wall inflammation[2]. Long-lasting exposure to cardiovascular risk factors results in activation of endothelial cells and consequently release of circulating markers reflecting endothelial cell injury[3].

Endothelial dysfunction is a systemic phenomenon involving vascular beds in different organs including the brain[4]. Elevated markers of endothelial dysfunction are related to impaired brain functioning[5]. However, the exact pathophysiologic mechanisms behind this association remain to be elucidated. Endothelial cells are actively involved in regulation of cerebral circulation[6]. In response to humoral, neuronal and metabolic stimuli, these cells produce and release relaxing and contracting factors that adjust vascular tone and change cerebrovascular hemodynamics[7]. Since regulation of cerebral blood flow (CBF) depends on proper functioning of vascular endothelial cells[8], endothelial dysfunction might lead to impaired brain functioning through changes in CBF.

Endothelial cells release t-PA in response to a range of factors mainly related to the activation of coagulation cascade[9]. Upon release, t-PA catalyzes the conversion of plasminogen to plasmin which facilitates thrombus dissolution. While acute release of t-PA contributes to prevent intravascular thrombosis, several studies have shown that elevated serum t-PA concentration is associated with higher risk of cardiovascular events[10, 11]. Hence, it has been suggested that high serum t-PA in people at high risk of vascular disease might reflect presence of long-lasting endothelial injury and subsequent endothelial dysfunction. On the other hand, VWF is a glycoprotein released from activated endothelial cells and plays a key role in thrombus formation[12]. Given this role, several studies have shown that higher levels of VWF predict vascular events[11, 13]. Different lines of evidence indicate that plasma VWF levels may also show a state of endothelial activation, which in itself may contribute to the pathogenesis of vascular events beyond the direct effects of released VWF[14]. Given this background and considering the pivotal role of endothelial cells in cerebrovascular regulation, we hypothesized that elevated levels of t-PA and VWF, as markers of endothelial cell injury and dysfunction, are associated with lower cerebral blood flow in older adults at high risk for cardiovascular disease.

Methods

Setting and participants

Participants were included from the nested MRI substudy of the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER), a large randomized controlled trial assessing the benefits of 40 mg pravastatin daily on vascular outcomes[15]. Study participants were men or women aged 70-82 years with either preexisting vascular diseases or with increased risk of vascular disease because of smoking, hypertension or diabetes mellitus. Participants with congestive heart failure and arrhythmia were not included in this study. Inclusion and exclusion criteria of the PROSPER study have been described in detail elsewhere[16]. A total of 553 Dutch participants in the PROSPER study underwent two successive MRI scans of the brain. In this report we included 541 participants for whom data on t-PA and VWF was available. Participants were included from both pravastatin and placebo groups since we previously showed that treatment with pravastatin does not influence level of CBF[17]. The Leiden University Medical Center institutional ethics review board approved the protocol for the MRI study and all participants gave written, informed consent.

Markers of endothelial dysfunction

Venous blood samples were obtained at baseline and stored at -80°C in the Haemostasis and Thrombosis Laboratory, Glasgow Royal Infirmary[18]. Level of tissue plasminogen activator (t-PA) antigen was measured by enzyme-linked immunosorbent assay (ELISA) from Hyphen BioMed (Paris, France). Level of von willebrand factor (VWF) antigen was measured by an in-house ELISA using antibody from DAKO (Copenhagen, Denmark). Measurements of t-PA and VWF were done before the start of statin treatment. All laboratory analyses were conducted by technicians blind to the identity of samples.

MRI Scanning

All imaging was performed on an MR system operating at field strength of 1.5 T (Philips Medical Systems, Best, The Netherlands). Dual fast spin echo [repetition time (TR) = 3,000 ms; echo time (TE) = 27/120 ms; flip angle = 90°; slice thickness = 3 mm; 48 slices; no interslice gap; field of view (FOV) = 220×220 mm; matrix = 256×204] were obtained from all participantss. The SIENAX technique was used to obtain estimates of total brain parenchymal volume. In summary,

SIENAX starts by extracting brain and skull images from the single whole-head input data. The brain image is then afne-registered to Montreal Neurological Institute (MNI) 152 space (by using the skull image to determine the registration scaling), done primarily to obtain the volumetric scaling factor to be used as normalization for head size[19]. In addition, single slice phase contrast MR angiography (TR/TE = 16/9 ms; flip angle = 7.5°; slice thickness = 5 mm; FOV = 250; RFOV = 75%; scan percentage = 80%; matrix = 256; 8 signal averages) with a velocity encoding of 100 cm/s was used for flow measurements[20]. The scans were performed in a plane perpendicular to the left and right internal carotid artery and the vertebral arteries, at the level of the vertical segment of the petrous portion of the internal carotid artery.

Total CBF

Level of CBF was analyzed by application of an in-house developed software package (FLOW; Division of Image Processing, Department of Radiology, Leiden University Medical Center) by using a workstation (UltraSparc 10; Sun Microsystems, Santa Clara, Calif)[21]. With this software, the blood vessel is identified manually, after which delineation of the vessel is performed automatically. For this method a region of interest (ROI) was manually drawn around the vessel in the magnitude image and copied into the phase images. For triggered measurements a vessel contour was drawn in one heart phase and the software copied this contour to the other phases. Visually, all phases were screened for correct positioning of the ROI. If required, the ROI was adjusted. Then flow volume is calculated by integrating the flow velocity values within this contour, multiplied with the area. Total CBF was calculated by adding flow from the left and right internal carotid arteries and the flow in both vertebral arteries. We standardized level of total CBF by brain parenchymal volume through dividing total CBF by each individual's brain volume (ml) and multiplying the obtained result by 100. Changes in levels of CBF were defined as the follow up measurement minus the baseline measurement.

Statistical analysis

Characteristics of the study participants are reported as mean (standard deviation) for continuous variables and number (percentage) for categorical variables. Participants were grouped based on t-PA and VWF tertiles. Baseline levels of total CBF were calculated for each tertile of t-PA and VWF. Similarly, we calculated changes in total CBF for each tertile of t-PA and VWF. The association between markers of endothelial dysfunction and CBF was tested using linear

regression models. We performed our analyses in two steps. In the first step, analyses were adjusted for age and sex. In the next step, further covariates were added to the statistical models based on their biological plausibility and their potential role as a confounder on the association between endothelial dysfunction and changes in CBF. The covariates included in the multivariate analyses were age, sex, body mass index, serum triglyceride, high density lipoprotein (HDL) cholesterol, history of diabetes, history of hypertension, smoking, alcohol intake, statin treatment and history of cardiovascular diseases (coronary, cerebral, or peripheral) at baseline. Associations between markers of endothelial dysfunction and changes in CBF were further adjusted for baseline CBF measures. All the probability values were estimated from normally transformed continuous data. We carried out all the analyses using SPSS software (version 20.0.0, SPSS Inc., Chicago, IL).

Results

Baseline characteristics of the participants are presented in table 1. Mean age was 75.0 years and 43.6% of participants were female. Mean values for t-PA and VWF were 11.3 ng/ml and 127.3 IU/dL respectively. At baseline, average of unstandardized CBF was 523.6 ml/min and average of standardized CBF for brain parenchymal volume was 49.3 ml/min/100 ml. The mean duration between the baseline and follow up MRI scans was 33$\pm$1.4 months.

Table 1. Baseline characteristics of the study participants (n=541)

Characteristics	Value
Socio-demographics	
Age, years	75.0 (3.1)
Women, n (%)	236 (43.6)
Current smoker, n (%)	110 (20.3)
Alcohol intake, unit per week	6.7 (8.3)
Cardiovascular factors	
SBP, mmHg	157.3 (21.6)
DBP, mmHg	85.8 (11.1)
Diabetes mellitus, n (%)	90 (16.6)
BMI, kg/m^2	26.6 (3.6)
Serum triglyceride, mmol/L	1.6 (0.7)
HDL cholesterol, mmol/L	1.3 (0.3)
History of vascular disease, n (%)	235 (43.4)
History of Stroke or TIA, n (%)	87 (16.1)
History of myocardial infarction, n (%)	67 (12.3)
Statin treatment, n (%)	269 (49)
Markers of endothelial dysfunction	
Tissue plasminogen activator, ng/mL	11.3 (3.8)
von Willebrand factor, IU/dL	127.3 (41.3)
Cerebral blood flow	
Unstandardized CBF, ml/min	523.6 (95.8)
Standardized CBF, ml/min/100ml	49.3 (8.9)

-All the values are presented as mean (standard deviation) except as noted. Abbreviations: SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, HDL: high density lipoprotein, TIA: transient ischemic attack, CBF: cerebral blood flow

Table 2 shows baseline and changes in CBF in relation to tertiles of t-PA. In the cross-sectional analysis adjusted for age and sex, baseline CBF was significantly lower in participants with higher t-PA circulating (p=0.008). Further adjustments for socio-demographic and cardiovascular factors did not materi-

ally change the results. In contrast, in the longitudinal analysis, there was no significant association between level of t-PA and changes in CBF. Associations between covariates and CBF from the multivariate analysis of t-PA with CBF are presented in the supplemental material (Table S-1 and Table S-2 http://www.sciencedirect.com/science/article/pii/S0197458013003540).

Table 3 shows baseline and changes in CBF in relation to tertiles of VWF. In the cross-sectional analysis, there was no association between level of VWF and baseline CBF. However, longitudinal analysis adjusted for age and sex showed that participants with higher VWF at baseline had a higher decline in CBF ($p = 0.014$). Further adjustments for sociodemographic and cardiovascular factors did not materially change the results. Associations between covariates and CBF from the multivariate analysis of VWF with CBF are presented in the supplemental material (Table S-3 and Table S-4 http://www.sciencedirect.com/science/article/pii/S0197458013003540).

Figure 1 shows percentages decline in CBF over a 33 months follow-up period dependent on tertiles of baseline t-PA and VWF at baseline. In the whole study population average decline in CBF was 1.6 ml/min/100ml. Between two MRI measurements, 26 participants developed stroke and 14 participants developed coronary artery events. To test whether the association between markers of endothelial dysfunction and CBF measures was independent of these events, we performed a sensitivity analysis excluding these participants and found similar outcomes (data not shown).

Table 2. Estimates of cerebral blood flow in relation to baseline serum level of tissue plasminogen activator

	Tissue Plasminogen Activator			
	Low (2.62-9.44 ng/mL) n=181	Middle (9.45-12.52 ng/mL) n=180	High (12.55-24.40 ng/mL) n=180	P-value
Baseline CBF, mean (SE)				
Age and sex adjusted model	50.2 (0.8)	49.8 (0.8)	48.1 (0.8)	0.008
Multivariate adjusted model*	50.0 (1.0)	50.0 (0.9)	48.6 (0.9)	0.034
Δ CBF, mean (SE)				
Age and sex adjusted model	-2.0 (0.8)	-1.1 (0.8)	-1.7 (0.8)	0.788
Multivariate adjusted model**	-2.0 (0.9)	-1.3 (0.9)	-2.6 (0.9)	0.236

Abbreviations: CBF: cerebral blood flow (ml/min/100ml), SE: standard error

Δ CBF: Follow up measure minus baseline measure

∗ Adjusted for age, sex, body mass index, triglyceride, HDL cholesterol, diabetes mellitus, hypertension, history of cardiovascular diseases, smoking, alcohol intake, statin treatment

∗∗ Further adjusted for baseline CBF

All probability values are calculated using tissue plasminogen activator as a continuous variable

Table 3. Estimates of cerebral blood flow in relation to baseline serum level of von Willebrand factor

	Von Willebrand factor			
	Low (39 -107 IU/dL) n=179	Middle (108-143 IU/dL) n=182	High (144-275 IU/dL) n=180	P-value
Baseline CBF, mean (SE)				
Age and sex adjusted model	49.2 (0.8)	50.0 (0.7)	49.3 (0.8)	0.120
Multivariate adjusted model*	49.1 (1.0)	50.0 (0.9)	49.3 (0.9)	0.077
Δ CBF, mean (SE)				
Age and sex adjusted model	0.1 (0.8)	-2.5 (0.8)	-2.1 (0.8)	0.014
Multivariate adjusted model**	-0.4 (0.9)	-2.4 (0.8)	-2.5 (0.9)	0.043

Abbreviations: CBF: cerebral blood flow (ml/min/100ml), SE: standard error

Δ CBF: Follow up measure minus baseline measure

∗ Adjusted for age, sex, body mass index, triglyceride, HDL cholesterol, diabetes mellitus, hypertension, history of cardiovascular diseases, smoking, alcohol intake, statin treatment

∗∗ Further adjusted for baseline CBF

All probability values are calculated using tissue plasminogen activator as a continuous variable

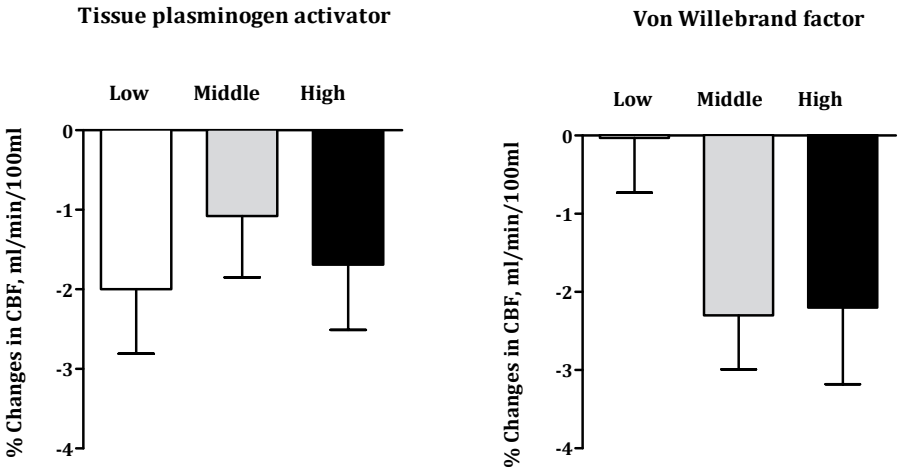

Figure 1. Longitudinal changes in cerebral blood flow in tertiles of tissue plasminogen acitivator and von willebrand factor. Each bar represents mean (standard error) for percentage decline in cerebral blood flow.

Discussion

This study shows that higher levels of t-PA and VWF, as circulating markers of endothelial dysfunction, are associated with lower cerebral blood flow in a cohort of older people at risk for cardiovascular events. We observed that high t-PA was cross-sectionally associated with lower cerebral blood flow, while higher levels of VWF were associated with a steeper decline in cerebral blood flow.

The capacity of endothelial cells to maintain vascular homeostasis decreases with advancing age[8]. Advanced age, on the other hand, is associated with a decrease in cerebral blood flow and increase in cerebral vascular resistance[22, 23]. Accordingly, it has been suggested that lower cerebral blood flow in older adults may be partly related to impaired endothelial cell functioning in the brain vasculature [24]. Exposure to cardiovascular risk factors activates endothelial cells which results in expression and release of inflammatory cytokines and coagulation factors into the circulation [25]. It has been demonstrated that t-PA and VWF are released from activated endothelial cells and they can serve as the markers of endothelial dysfunction [14, 26]. In this study, we found different associations of t-PA and VWF with cerebral blood flow measurements. While higher t-PA was associated with lower cerebral blood flow at baseline, VWF was associated with a steeper decline in cerebral blood flow. The reason for these differential associations is not clear. These findings might suggest that various circulating markers of endothelial dysfunction, probably because of their different biological properties, have different predictive values for CBF measurements. t-PA is a measure of activated endogenous fibrinolysis[9], a process that might carry counter-regulatory protection against reduction in CBF. VWF in contrast potentiates platelet activation [27], and elevated levels might then affect CBF change over time.

The observed association between circulating markers of endothelial dysfunction and lower cerebral blood flow can be explained in different ways. First, markers of endothelial dysfunction could represent higher loads of cardiovascular risk factors and disease [28] which may independently decrease level of CBF [29]. However, we showed that the association of the circulating markers of endothelial dysfunction with CBF measures did not essentially change after adjusting for established cardiovascular risk factors and diseases. A second explanation could be that higher degrees of endothelial dysfunction were associated with a loss of parenchymal brain volume due to previous neurovascular injuries [30]. Hence, those with higher t-PA and VWF might have required less CBF to meet the metabolic demand of their brains. Nevertheless, we found similar associations of the markers of endothelial dysfunction after standardization of cerebral blood flow for brain parenchymal volume. As a third possible explanation,

elevated t-PA and VWF could reflect presence of endothelial dysfunction not only in the systemic circulation but also in the brain vasculature which led to an increased cerebral vascular resistance and ultimately dysregulation of CBF. In line with this explanation, previous studies showed that medical conditions associated with endothelial dysfunction such as diabetes mellitus and cerebral microangiopathy are linked to disturbances in cerebrovascular hemodynamics [31, 32]. Further studies are needed to test whether elevated markers of endothelial dysfunction are associated with impaired functioning of cerebrovascular regulatory mechanisms such as cerebral autoregulation.

Increasing body of evidence shows that long-lasting exposure to cardiovascular risk factors with consequent cerebrovascular damage is associated with higher risk of cognitive impairment in older adults [33]. In line with this evidence, a recent systematic review and meta-analysis showed that individuals with elevated serum markers of endothelial cell injury including t-PA and VWF are at a higher risk for developing vascular dementia [5]. Although the exact mechanisms behind this association remain to be elucidated, it has been suggested that endothelial dysfunction might lead to cognitive impairment through changes in CBF [34]. Findings of our study support this hypothesis and might imply that strategies to prevent endothelial dysfunction can be considered as potential tools to reduce risk of cognitive impairment and dementia in older adults.

Our study has certain strengths and limitations. Major strengths of this study include a relatively large number of older participants and measurement of decline in CBF in two subsequent MRI sessions. As a limitation, our study population consisted of participants at high risk for cardiovascular disease which might reduce the generalizability of our findings to community-dwelling older adults. Nevertheless, this restriction provided us with an opportunity to investigate our research question in a homogeneous group of participants at high risk for development and progression of endothelial dysfunction [35]. As a second limitation, we assessed endothelial dysfunction indirectly using serum levels of t-PA and WVF antigens. Although different methods are available to assess endothelial dysfunction, no single test fulfills all the requirements for comprehensive assessment of impaired endothelial functioning [3]. Circulating markers of endothelial dysfunction including tPA and VWF are no exceptions and they cannot serve as the optimum tools to assess endothelial dysfunction. Therefore, our findings need to be replicated in future studies assessing endothelial dysfunction with other methods.

In conclusion, our findings suggest that endothelial dysfunction associates with lower levels of cerebral blood flow in older adults at risk for cardiovascular disease. Future interventional studies are needed to test whether strategies to maintain optimal function of endothelial cells can lead to preservation of CBF

and ultimately conservation of brain functioning in older adults.

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Cerebral Blood Flow and Survival in Old Age

Manuscript based on this chapter has been published as:
Sabayan B, Grond JV, Westendorp RG, Jukema W, Ford I, Buckly BM, Sattar N, van Osch MJ, van Buchem MA, de Craen AJ. Total cerebral blood flow and mortality in old age: A 12-year follow-up study
Neurology. 2013 26;81(22):1922-9.

Summary

The objective of this chapter is to examine the association of total CBF with all-cause, non-cardiovascular and cardiovascular mortality in older subjects at risk of cardiovascular disease. We included 411 subjects with a mean age of 74.5 years from the MRI substudy of the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER). Total CBF was measured at baseline and occurrence of death was recorded in an average follow-up period of 11.8 years. For each participant, total CBF was standardized for brain parenchymal volume. Cox regression models were used to estimate risk of all-cause, non-cardiovascular and cardiovascular mortality in relation to CBF. Mortality rates among participants in low, middle and high thirds of total CBF were 52.1, 41.5 and 28.7 per 1000 person-years respectively. Compared to participants in the high third of CBF, participants in the low third had 1.88-fold (95% confidence interval (CI): 1.30-2.72) higher risk of all-cause mortality, 1.66-fold (95% CI: 1.06-2.59) higher risk of non-cardiovascular mortality and 2.50-fold (95% CI: 1.28-4.91) higher risk of cardiovascular mortality. Likewise, compared to participants in the high third of CBF, participants in the middle third had 144-fold (95% CI: 0.98-2.11) higher risk of all-cause mortality, 1.29-fold (95% CI: 0.82-2.04) higher risk of non-cardiovascular mortality and 1.86-fold (95% CI: 0.93-3.74) higher risk cardiovascular mortality. These associations were independent of prevalent vascular status and risk factors. Low total CBF is linked with higher risk of all-cause, non-cardiovascular and cardiovascular mortality in older people independent of clinical cardiovascular status.

Introduction

Structural and functional integrity of the brain depends on adequate supply of nutrient and oxygen through blood flow.[1] The brain is a demanding organ and consumes about 20% of the oxygen inspired at rest while accounting for only 2% of the body weight.[2] This high metabolic demand renders the brain tissue vulnerable to low cerebral blood flow (CBF) as several animal studies have shown that long-standing cerebral hypoperfusion leads to neuronal loss, microvascular abnormalities and cognitive deficit.[3, 4]

Decreased cerebral perfusion in patients with acute traumatic brain injury and cerebrovascular accidents has been linked to adverse clinical outcomes and shorter survival.[5, 6] Likewise, clinical conditions with a state of chronic reduction in CBF, such as heart failure and carotid stenosis, have been associated with an increased risk of mortality.[7, 8] Despite this evidence, the independent role of CBF in the maintenance of health and survival in old age has not been thoroughly investigated.

In a cohort of older subjects at risk for cardiovascular disease, we investigated whether lower CBF is linked with a higher risk of all-cause, non-cardiovascular and cardiovascular mortality. We hypothesized that lower total CBF is independently associated with higher risk of mortality in old age.

Methods

Setting and participants

Participants were included from the nested MRI substudy of the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER), a large randomized controlled trial assessing the benefits of 40 mg pravastatin daily on vascular outcomes.[9] Study participants were men or women aged 70-82 years with either preexisting vascular diseases or with increased risk of vascular disease because of smoking, hypertension or diabetes mellitus. Subjects with congestive heart failure (NYHA class III/IV), arrhythmia and cognitive impairment (MMSE score $<$24) were not recruited in the PROSPER study. Inclusion and exclusion criteria of the PROSPER study have been described in detail elsewhere.[9] A total of 554 Dutch participants who completed the trial underwent MRI scans of the brain. In this study we included 411 participants for whom data on baseline total CBF, brain parenchymal volume and mortality was available. There was no significant difference between characteristics of the included participants and subjects

with missing values except for higher prevalence of coronary artery disease in subjects with missing values. Since we previously reported that treatment with pravastatin does not influence level of CBF, participants were included from both pravastatin and placebo groups.[10]

Standard Protocol Approvals, Registrations, and Patient Consents

The institutional ethics review boards of all participating centers approved the study protocol of the PROSPER study. The protocol was consistent with the Declaration of Helsinki. The institutional ethics review board of the Leiden University Medical Center approved the protocol for the MRI substudy. All participants gave written, informed consent.

MRI Scanning

All imaging was performed on an MR system operating at field strength of 1.5 T (Philips Medical Systems, Best, The Netherlands). Dual fast spin echo [repetition time (TR) = 3,000 ms; echo time (TE) = 27/120 ms; flip angle = 90°; slice thickness = 3 mm; 48 slices; no interslice gap; field of view (FOV) = 220×220 mm; matrix = 256×204], FLAIR (TR = 8,000 ms; TE = 100 ms; flip angle = 90°; slice thickness = 3 mm; 48 slices; no interslice gap; FOV = 220×176 mm; matrix = 256×153) and susceptibility weighted images (multislice gradient echo sequence; TR = 2593 ms; TE = 48 ms; flip angle = 60°; slice thickness = 6 mm; 22 slices; interslice gap = 6 mm; whole brain coverage; FOV = 220×198 mm; matrix = 256×176) were obtained from all subjects. The SIENAX technique was used to obtain estimates of total brain parenchymal volume, gray matter and white matter volume. In summary, SIENAX starts by extracting brain and skull images from the single whole-head input data. The brain image is then affine-registered to Montreal Neurological Institute (MNI) 152 space (by using the skull image to determine the registration scaling), done primarily to obtain the volumetric scaling factor to be used as normalization for head size.[11] Segmentation of white matter hyperintensities volume was performed automatically using software for Neuro-Image Processing in Experimental Research (SNIPER), an in-house developed program for image processing.[12] This segmentation was based on the T2-weighted and FLAIR images. Cerebral infarcts were defined as parenchymal defects seen on FLAIR images with the same signal intensity as CSF and a surrounding rim of high signal intensity following a vascular distribution. In addition, single slice phase contrast MR angiography (TR/TE = 16/9 ms; flip angle = 7.5°; slice thickness = 5 mm; FOV = 250; RFOV = 75%; scan percentage = 80%; matrix = 256; 8 signal averages) with a velocity encoding of 100 cm/s was used for flow

measurements.[13] The scans were performed in a plane perpendicular to the left and right internal carotid artery and the vertebral arteries, at the level of the vertical segment of the petrous portion of the internal carotid artery.

Total CBF

CBF was assessed using an in-house developed software package (FLOW; Division of Image Processing, Department of Radiology, Leiden University Medical Center) on a workstation (UltraSparc 10; Sun Microsystems, Santa Clara, Calif).[14] For this method a region of interest (ROI) was manually drawn around the vessel in the magnitude image and copied into the phase images. For triggered measurements a vessel contour was drawn in one heart phase and the software copied this contour to the other phases. Visually, all phases were screened for correct positioning of the ROI. If required, the ROI was adjusted. Flow volume is calculated by integrating the flow velocity values within these contours, multiplied with the areas within the vessel contours. Total CBF was calculated by adding flow from the left and right internal carotid arteries and the flow in both vertebral arteries. Total CBF was expressed in milliliters per minute (ml/min). We standardized level of total CBF by brain parenchymal volume (ml/min/100ml) by dividing total CBF (ml/min) by each individual's brain volume (ml) and multiplying the obtained result by 100.

Mortality

Participants were followed for occurrence of mortality until January 1, 2012 in an average follow-up period of 11.8 years. We obtained dates of deaths from the Dutch civic registry and specific data on causes of death from Statistics Netherlands, which assigns codes for all national death certificates according to the International Classification of Diseases and Related Disorders, 10th revision (ICD-10). Death due to cardiovascular mortality was classified as ICD-10 codes I00-I99 and death due to other reasons were recorded as non-cardiovascular mortality.

Other variables

At baseline a research nurse interviewed all the participants and data for their demographic characteristics and medication use were obtained. Information about history of vascular diseases was provided by each participant general practitioner. Diabetes mellitus was defined by self-reported history, a fasting blood glucose concentration of 7.0 mmol/L or self-reported use of antidiabetic

medication. Blood pressure was measured in sitting position using a fully automatic electronic sphygmomanometer (Omron M4®). Body mass index was measured using standard protocols. Global cognitive function was assessed using mini-mental state examination (MMSE).

Statistical analysis

Baseline characteristics of the study participants are reported as mean (standard deviation) or median (interquartile range) for continuous variables and number (percentage) for categorical variables. Differences in characteristics of study participants in thirds of CBF were tested by analysis of covariance or Kruskal-Wallis for continuous variables and pearson chi-square test for categorical variables. To compare cumulative incidence of all-cause, non-cardiovascular and cardiovascular mortality in thirds of total CBF, Kaplan-Meier graphs were made and strata were compared with log-rank test. We used Cox proportional hazard models to estimate risk of all-cause, non-cardiovascular and cardiovascular mortality associated with level of total CBF. Cox regression models were fit with time to death as the outcome variables and total CBF as the determinants. We performed our analyses in three steps. In the first step, analyses were performed unadjusted. In the second step analyses were adjusted for age and sex and finally analyses were adjusted for age, sex, cardiovascular risk factors and diseases including body mass index, smoking, serum cholesterol, mean arterial pressure, antihypertensive medication use, diabetes mellitus, statin treatment and history of vascular disease (coronary, cerebral, or peripheral) at baseline. In the multivariate analyses, body mass index, serum cholesterol and mean arterial pressure were entered as continuous variables and the other parameters were entered as dichotomous variables. In addition, we performed a series of sensitivity analyses to test whether our results were consistent in different subgroup of participants. In these sensitivity analyses, we separately excluded subjects with diabetes mellitus, hypertension, high body mass index (>25 kg/m^2), elevated serum levels of N-terminal pro-brain natriuretic peptide (NT-proBNP) (>400 ng/L)[15] as a marker of impaired cardiac function, coronary artery disease, cerebrovascular accidents (stroke or transient ischemic attack), brain infarcts, high load of white matter hyperintensities (>5 ml) and impaired cognitive function (MMSE score <28 points). All the analyses were carried out using SPSS software (version 20.0.0, SPSS Inc., Chicago, IL).

Results

Table 1 shows characteristics of the study participants in whole study population and in thirds of total CBF. Mean age of participants was 74.5 years and 55.5% of them were female. Mean values for total CBF and total CBF by parenchymal volume were 522.6 ml/min and 49.3 ml/min/100ml respectively. There was no significant difference in socio-demographic, cerebrovascular and cognitive characteristics of the participants in thirds of total CBF except that participants with lower total CBF were more frequently male (p=0.016) and had higher body mass index (p=0.041).

During follow-up period 195 subjects (47.4%) died, of whom 129 subjects (66.2%) died due to non-cardiovascular reasons and 66 (33.8%) died of cardiovascular causes. Numbers of deaths in low, middle and high thirds of CBF were 80 (41.0%), 66 (33.8%) and 49 (25.2%) respectively. All-cause mortality rates in subjects with low, middle and high total CBF were 52.1, 41.5 and 28.7 per 1000 person-years respectively. Non-cardiovascular mortality rates in subjects with low, middle and high total CBF were 32.6, 27.0 and 21.1 per 1000 person-years respectively and cardiovascular mortality rates in subjects with low, middle and high total CBF were 19.5, 14.4 and 7.6 per 1000 person-years respectively (Fig 1).

Table 2 shows the risks of all-cause, non-cardiovascular and cardiovascular mortality in relation to total CBF. In the multivariate adjusted model, compared to participants in the high third of CBF, participants in the low third had 1.88-fold (95% confidence interval (CI): 1.30-2.72) higher risk of all-cause mortality, 1.66-fold (95% CI: 1.06-2.59) higher risk of non-cardiovascular mortality and 2.50-fold (95% CI: 1.28-4.91) higher risk of cardiovascular mortality. Likewise, compared to participants in the high third of CBF, participants in the middle third had 144-fold (95% CI: 0.98-2.11) higher risk of all-cause mortality, 1.29-fold (95% CI: 0.82-2.04) higher risk of non-cardiovascular mortality and 1.86-fold (95% CI: 0.93-3.74) higher risk cardiovascular mortality. We found similar associations between lower CBF and higher risk of mortality when analyses were performed using CBF as a continuous variable (data not shown). The corresponding Kaplan-Meier survival curves showed that subjects with low total CBF had highest cumulative incidence of all-cause, non-cardiovascular and cardiovascular mortality (Fig 2).

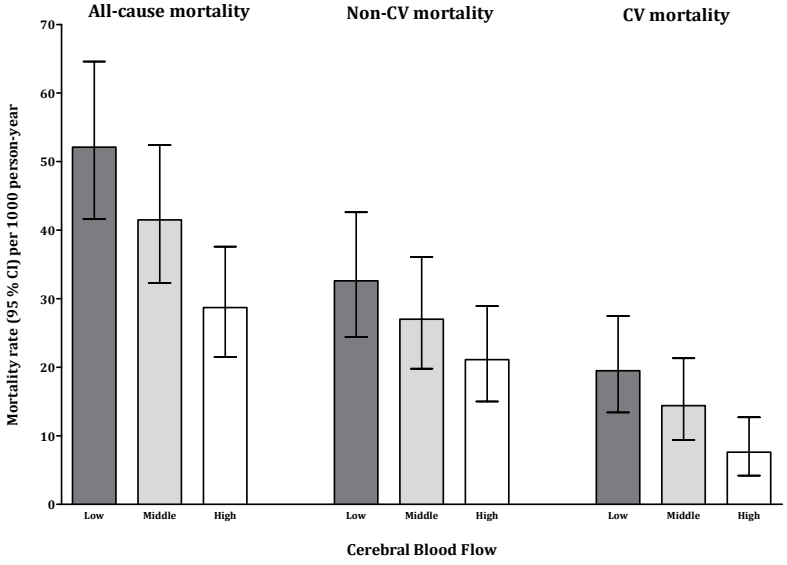

Figure 1. All-cause, non-cardiovascular (Non-CV) and cardiovascular (CV) mortality rates with 95% confidence interval (CI) per 1000 person-year in thirds of cerebral blood flow.

Table 1. Characteristics of study participants in whole study population and in thirds of total CBF

Characteristics	All	Thirds of total CBF, ml/min/100ml			P-value†
		Low (9.7- 45.7)	Middle (45.8- 52.2)	High (52.3- 84.8)	
	n= 411	n=137	n=137	n=137	
Socio-demographic factors					
Age, years	74.5 (3.2)	74.6 (3.3)	74.6 (3.2)	74.1 (3.1)	0.307
Men, n (%)	228 (55.5)	86 (62.8)	79 (57.7)	63 (46.0)	0.016
Age left school, years	15.6 (3.0)	15.7 (2.9)	15.5 (3.1)	15.5 (3.0)	0.153
Current smoker, n (%)	84 (20.4)	30 (21.9)	26 (19.0)	28 (20.4)	0.836
Cardiovascular factors					
History of CVD, n (%)	172 (41.8)	64 (46.7)	51 (37.2)	57 (41.6)	0.281
History of Stroke or TIA, n (%)	66 (16.1)	21 (15.3)	20 (14.6)	25 (18.2)	0.685
History of CAD, n (%)	48 (11.7)	20 (14.6)	14 (10.2)	14 (10.2)	0.428
NT-proBNP*, ng/L, Median (IQR)	105 (55.1-211.5)	104 (46.4-199.0)	107 (64.9-235.4)	106 (58.1-197.2)	0.651
Serum cholesterol, mmol/L	5.7 (0.8)	5.6 (0.9)	5.8 (0.8)	5.7 (0.8)	0.153
Body mass index, kg/m^2	26.6 (3.4)	27.2 (3.6)	26.7 (3.3)	26.1 (3.3)	0.041
Diabetes mellitus, n (%)	74 (18.0)	26 (19.0)	24 (17.5)	24 (17.5)	0.936
Antihypertensive therapy, n (%)	254 (61.8)	80 (58.4)	86 (62.8)	88 (64.2)	0.585
SBP, mmHg	157 (21)	159 (22)	159 (21)	154 (21)	0.089
DBP, mmHg	86 (11)	87 (10)	86 (10)	84 (11)	0.102
MAP, mmHg	110 (13)	111 (13)	110 (13)	108 (13)	0.057
MRI findings and cognition					
WMH volume, ml	5.3 (9.9)	5.8 (8.9)	5.9 (12.1)	4.3 (8.4)	0.311
Presence of infarcts, n (%)	135 (32.9)	52 (38)	44 (32.4)	39 (28.5)	0.244
MMSE score, Median (IQR)	28 (27-29)	28 (28-29)	29 (27-30)	29 (27-30)	0.441

Data are presented as mean (standard deviation) except as noted.

Abbreviations: CBF: cerebral blood flow, CVD: cardiovascular disease, TIA: transient ischemic attack, CAD: coronary artery disease, IQR: inter quartile range, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure

† Probability values indicate significant difference in thirds of total CBF

∗ Serum NT-proBNP was measured six months after inclusion time

Table 2. Risk of mortality in relation to level of total cerebral blood flow

	Total CBF, ml/min/100ml			
	Low 9.7- 45.7 HR (95%CI)	Middle 45.8- 52.2 HR (95%CI)	High 52.3- 84.8 HR (95%CI)	P for trend
All-cause mortality				
Unadjusted model	1.98 (1.39-2.83)	1.51 (1.05-2.19)	1 (ref)	<0.001
Age and sex adjusted model	1.82 (1.27-2.61)	1.41 (0.98-2.06)	1 (ref)	0.001
Multivariate adjusted model*	1.88 (1.30-2.72)	1.44 (0.98-2.11)	1 (ref)	0.001
Non-cardiovascular mortality				
Unadjusted model	1.71 (1.11-2.62)	1.35 (0.87-2.11)	1 (ref)	0.014
Age and sex adjusted model	1.57 (1.02-2.42)	1.27 (0.82-1.99)	1 (ref)	0.040
Multivariate adjusted model*	1.66 (1.06-2.59)	1.29 (0.82-2.04)	1 (ref)	0.026
Cardiovascular mortality				
Unadjusted model	2.74 (1.43-5.25)	1.96 (0.99-3.87)	1 (ref)	0.002
Age and sex adjusted model	2.52 (1.31-4.85)	1.76 (0.89-3.51)	1 (ref)	0.005
Multivariate adjusted model*	2.50 (1.28-4.91)	1.86 (0.93-3.74)	1 (ref)	0.007

Abbreviations: HR: hazard ratio, CBF: cerebral blood flow

∗ Adjusted for age, sex, body mass index, smoking, serum cholesterol, mean arterial pressure, antihypertensive medication, diabetes mellitus, Statin treatment and history of vascular diseases (coronary, cerebral or peripheral)

- Number of participants in each third is 137

We performed a series of sensitivity analyses on the associations of total CBF with all-cause, non-cardiovascular and cardiovascular mortality separately excluding participants who had diabetes mellitus, hypertension, high body mass index, impaired cardiac functioning, coronary artery disease, cerebrovascular accidents, brain infarcts, high load of white matter hyperintensities and impaired cognitive function (Fig 3). We found that exclusion of subjects with cardiovascular risk factors, cerebrovascular diseases and impaired cognitive function did not essentially change the results. In another sensitivity analysis, to test whether our findings are not due to short term death events, we excluded subjects who died in the first two years of follow-up (n=12). This sensitivity analysis showed that the associations were not dependent on short term death events (data not shown).

Discussion

In this study we showed that lower total CBF is associated with higher risk of all-cause, non-cardiovascular and cardiovascular mortality in older subjects at high risk of cardiovascular diseases. These associations were independent of

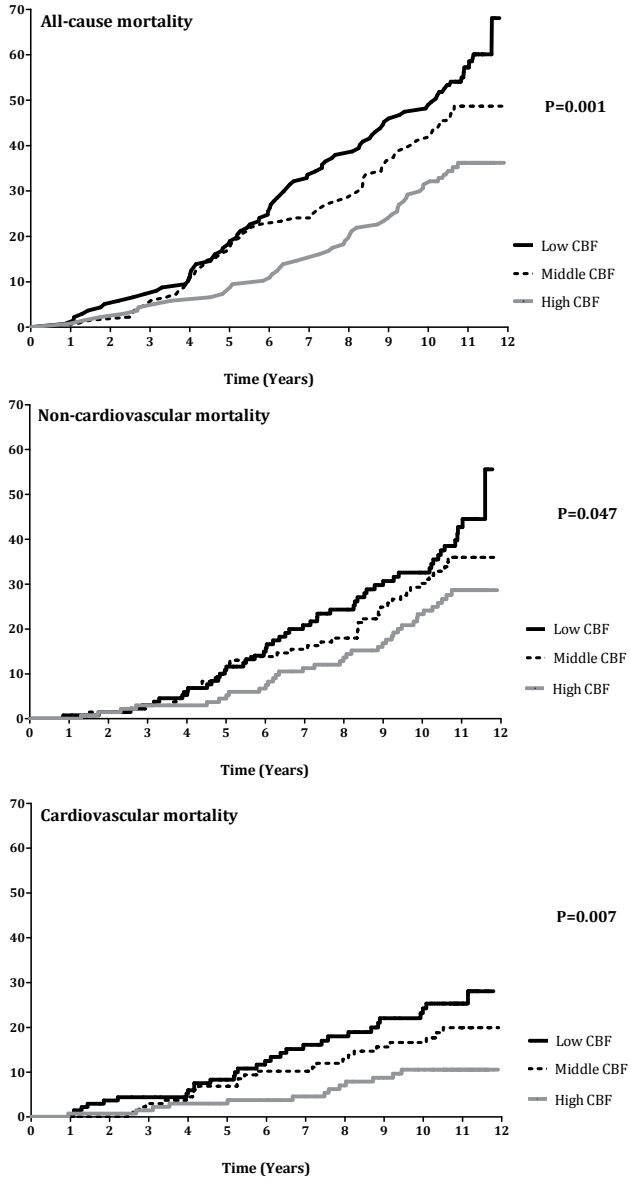

Figure 2. Kaplan meier curves for all cause, non-cardiovascular and cardiovascular mortality in thirds of total cerebral blood flow (ml/min/100ml).

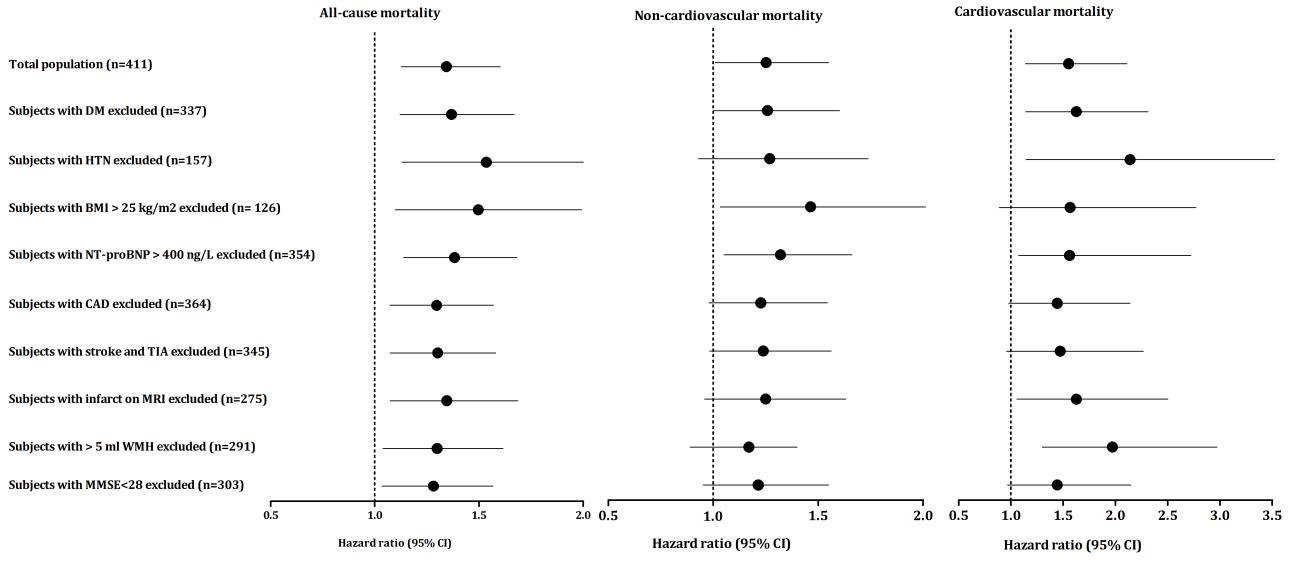

Figure 3. Hazard ratios for all-cause, non-cardiovascular and cardiovascular mortality for each third decrease in level of total cerebral blood flow in the whole study population and in subgroups of participants without diabetes mellitus (DM), hypertension (HTN), high body mass index (BMI) >25 kg/m2, serum levels of N-terminal pro-brain natriuretic peptide (NT-proBNP) >400 ng/L, history of coronary artery disease (CAD), stroke or transient ischemic attack (TIA), brain infarcts, high load of white matter hyperintensities (WMH) (> 5 ml) and mini-mental state examination (MMSE) score of less than 28 points. All the analyses were adjusted for age and sex.

prevalent vascular diseases (coronary, cerebral, or peripheral) and cardiovascular risk factors such as hypertension, diabetes mellitus, obesity, smoking and hypercholesterolemia.

Despite the fact that multiple systemic and cerebrovascular mechanisms act in concert to maintain optimal CBF, total CBF decreases with increasing age.[16] Decrease in CBF has been implicated in the pathogenesis of neurodegenerative disorders such as dementia and certain cerebrovascular events such as low flow infarcts.[17, 18] A recent study showed that lower total CBF is an independent risk factor for mortality and adverse clinical outcomes in patients with heart failure.[19] However, little is known about the independent role of CBF in the maintenance of health and survival in older people. In the current study, we observed that lower total CBF in older subjects at risk of cardiovascular disease is associated with higher risk of mortality.

Different explanations can be proposed for the association of lower CBF with higher risk of all-cause, non-cardiovascular and cardiovascular mortality. First explanation could be that lower total CBF in older subject is related to lower metabolic demand of the brain due to parenchymal atrophy which independently puts subjects at higher risk of mortality.[20] Since in each participant we normalized level of total CBF for brain parenchymal volume, this explanation seems unlikely. In addition, we observed that exclusion of participants with impaired cognition, which is closely related to a decreased in number and activity of neurons in the brain[20], did not materially change the associations between CBF and mortality outcomes.

A second explanation might be that lower total CBF reflects the presence of vascular risk factors and pre-existing vascular pathologies in the brain and heart[21-23] and that themselves rather than decreased CBF are responsible for increasing risk of mortality in older subjects. In line with this explanation, we found that subjects with lower CBF tended to have higher blood pressure and body mass index. Previous studies also showed that vascular risk factors such as hypertension can lead to a decline in CBF.[24] We observed that the adjustments of the analyses for cardiovascular risk factors and diseases did not essentially change the estimates and the sensitivity analyses showed that the associations between total CBF and mortality outcomes were not dependent of presence of impaired cardiac functioning, history of coronary artery events, cerebrovascular accidents, cerebral infarcts and white matter hyperintensties. Nevertheless, the possibility of residual confounding from unmeasured cardiovascular risk factors cannot be excluded. We observed that correction of all analyses for various well-established cardiovascular risk factors only slightly influenced the associations. Therefore, we expect that if we could account for potential unmeasured confounders, this would result in minor changes of the measures of association

between lower CBF and higher risk of mortality.

A third possible explanation involves the critical role of adequate CBF in maintenance of brain structure and function.[1] There are different lines of evidence indicating that lower CBF is associated with structural and functional abnormalities in the brain.[17] Since the brain is a vital organ in regulation of homeostasis[25], it is likely that suboptimal CBF, via neuronal injury and cell death, independently alters normal function of the brain leading to an increased risk for mortality.[26] It has been shown that neuronal damage is not only associated with structural and functional changes in the brain but also with disturbances in the immune system, energy homeostasis, autonomic stress response and endocrine regulation.[27-30] As it is not possible to make a causal inference solely based on our observation, this hypothesis needs to be tested in future studies investigating the long-term consequences of suboptimal CBF on major homeostatic mechanisms.

The main strengths of this study include a long follow-up time of 12 years and availability of extensive data on various socio-demographic and cardiovascular factors. A possible limitation is the inclusion of older subjects with or at high risk for cardiovascular disease which limits the generalizability of our findings to a general population of older subjects. However, the study population represents a substantial part of the aging subjects given the high prevalence of cardiovascular diseases and risk factors in the elderly. As another limitation, we only assessed total CBF and were not able to investigate the association between regional CBF and mortality outcomes. Future studies, using arterial spin-labeling MRI perfusion techniques, might unravel whether there is any association between regional CBF and cause-specific mortality in old age. In addition, we assessed total CBF with an in-house developed software package which needs further validation in the future studies.

Our findings show that lower total CBF standardized for brain parenchymal volume, independent of cardiovascular diseases and risk factors, is linked with higher risk of all-cause, non-cardiovascular and cardiovascular mortality in elderly subjects at high risk of cardiovascular diseases. These findings need to be replicated in future population-based studies with larger number of participants.

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12

Key Findings and General Discussion

Increasing life span and decreasing birth rate have given rise to a rapid increase of the aged population in many countries, both in absolute and relative terms[1]. This demographic transition has resulted in increasing number of older people with disorders linked to the accelerated brain aging such as dementia[2]. Different lines of evidence from epidemiological, pathological and neuroimaging studies indicate that exposure to cardiovascular risk factors are closely related to structural and functional changes in the brain[3]. This cumulative evidence on the role of vascular factors in acceleration of brain aging has been reflected in the statements by the American Heart Association / American Stroke Association (AHA/ASA) and the National Institute of Health (NIH) emphasizing that cardiac diseases and vascular pathologies should be considered as potentially modifiable risk factors for cognitive impairment and dementia[4]. Despite achievements in the understanding of the brain structure and function, the mechanisms by which cardiovascular factors contribute in brain aging are not fully understood.

The aims of this chapter are to (1) review the key findings of this thesis in the context of current knowledge and evidence (2) present pathophysiological models on potential contribution of cardiovascular and hemodynamic factors in development and progression of brain aging, (3) address the methodological issues and (4) provide directions for future research.

Lowering blood pressure: a double-edged sword for the aging brain

Higher midlife blood pressure increases risk of cognitive impairment and stroke, whereas studies on late-life hypertension have shown mixed findings[5, 6]. There is a complex interaction between blood pressure and the brain. Long-lasting hypertension damages cerebral vessels through hemodynamic stress and puts subjects at high risk for cerebral small vessel disease and stroke[7]. Once vascular pathologies are established and the brain tissue is extensively damaged, the brain fails to play a central role in regulation of blood pressure[8]. If this situation coincides with a decrease in cardiac function, a drop in blood pressure may further decrease brain perfusion and accelerate brain aging[9]. In line with this notion, life course studies have shown that men who develop dementia experience a steeper increase in systolic blood pressure from midlife to late life, followed by a steeper decrease in late life[10]. The dynamic connection between the brain and blood pressure has made it difficult to give a single answer to the clinical question whether lowering blood pressure can protect the brain in late life. A limited number of clinical trials investigated the effect of antihypertensive medications in prevention of dementia and stroke in the oldest old. Most of these studies showed some benefits[11, 12]. However, such trials generally recruited healthy participants with low levels of co-morbidities and frailty. This has raised a concern whether findings of these studies can be generalized to general populations of very old people in which functional disability and multi-morbidity are relatively common[13]. In the second and third chapters of this thesis the association of high blood pressure with risk of cognitive decline and stroke in very old subjects is examined. The findings indicate that higher blood pressure is not associated with adverse brain outcomes. In addition, in subjects with disability, high blood pressure might be even beneficial. These results possibly imply that the biological age rather chronological age should be a basis for treatment of high blood pressure in older people. In very old people with higher degrees of frailty and morbidities, higher blood pressure might be needed to ensure perfusion of different organs including the brain[14, 15].

Limitations of the hypertension hypothesis in old age: significance of variability

Although hypertension has been recognized as a strong risk factor for cerebrovascular damage, current evidence shows that the predictive value of high blood pressure for stroke attenuates with age[6]. Based on the common hypoth-

esis of hypertension, the main determinant of blood pressure-related end organ damage is higher levels of blood pressure. However, recent data suggest that other parameters such as blood pressure variability also contribute in increasing risk of vascular events[16]. Blood pressure fluctuates around average values over short and long period of times. Exaggerated minute-to-minute and diurnal blood pressure variability has been mentioned as a cardiovascular risk indicator[17]. Apart from short-term blood pressure variability, a substantial variation in blood pressure exists when a subject is observed over months with repeated clinical visits[18]. Recent studies have shown that visit-to-visit blood pressure variability, independent of average blood pressure, is associated with higher risk of stroke, carotid artery atherosclerosis and cerebral small vessel disease in older subjects[19]. In the fourth chapter of this book, it is demonstrated that higher visit-to-visit blood pressure variability has a strong relationship with cognitive impairment, manifestations of cerebral small vessel disease and lower hippocampus volume. Higher blood pressure variability might reflect a long-term hemodynamic instability that puts stress on the vascular endothelium and leads to alterations in brain structure and function. Furthermore, increased fluctuations in systemic blood pressure might result in repeated episodes of cerebral hypoperfusion causing neuronal injury and accelerated brain aging[19]. Increase in age is associated with a decrease in cardiac function, alternations in the mechanical and the structural properties of the vessels and diminished activities of blood pressure regulatory mechanisms[20]. Therefore, it seems that standard blood pressure measurements are not adequate for the evaluation of cardiovascular risk in older people[18]. Most of the current guidelines put emphasis on reduction of blood pressure in older subjects and less attention has been paid to management of blood pressure variability[16]. Further studies are needed to evaluate whether strategies to lower blood pressure variability can contribute in preservation of the brain structure and function in older subjects.

Blood pressure dysregulationn, cerebrovascular dysfunction and brain aging: A pathophysiological model

Normal regulation of blood pressure is necessary for adequate organ perfusion and prevention of vascular damage. In the presented pathophysiological model, hypertension, hypotension and blood pressure variation contribute in cerebrovascular damage and promote cerebral hypoperfusion (figure 1). Hemodynamic stress imposed by hypertension damages large and small cerebral vessels and results in endothelial dysfunction. Impaired function of the vascular endothelium leads to efflux of abnormal proteins and waste metabolic products.

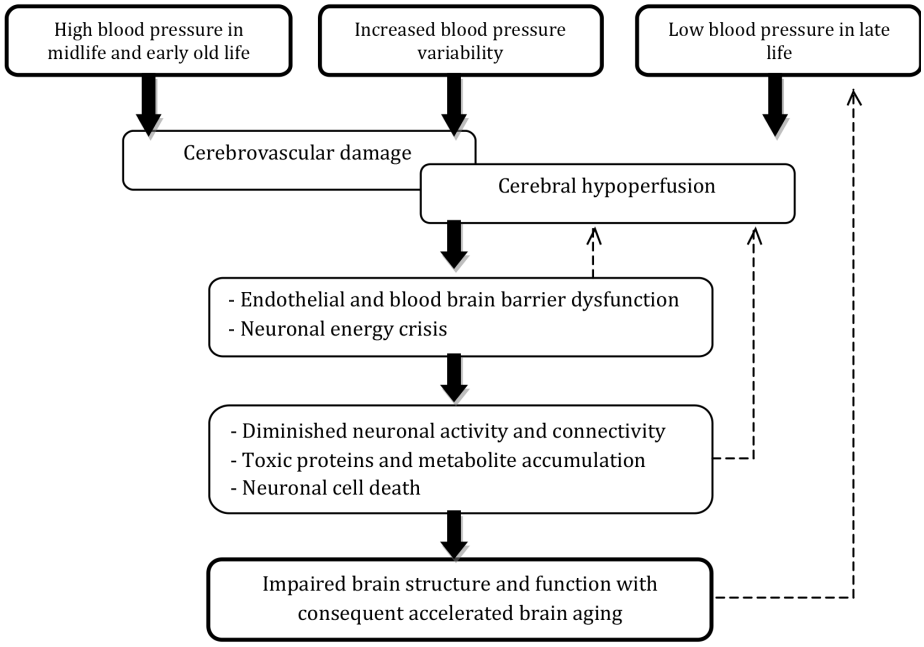

Figure 1. Pathophysiological model on the role of blood pressure dysregulation in brain aging

In addition, neurovascular unit loses its capacity to adjust the regional perfusion according to the metabolic demands of neurons. Long-lasting cerebral hypoperfusion results in neuronal enrgy crisis which ultimately lead to neuronal cell death. Extensive brain tissue damage may perpetuate the situation by further dysregulation in blood pressure and cerebral blood flow.

Impaired cardiac function: an emerging risk factor for brain aging?

Experimental studies in animal models have shown that chronic cerebral hypoperfusion results in neuronal energy crisis, glial cell activation, accelerated amyloid beta accumulation and blood brain barrier disruption which all contribute to development of neuronal dysfunction and injury[21]. Likewise, human studies have revealed that lower cerebral blood flow is associated with higher risk of developing white matter lesions and brain atrophy[22, 23]. It is known that the heart

plays a pivotal role in generation and regulation of cerebral blood flow[24, 25]. Despite activities of multiple regulatory mechanisms, it has been shown that patients with New York Heart Association (NYHA) class III/IV heart failure have about 30% lower total cerebral blood flow compared to healthy controls[26]. On the other hand, interventions to improve cardiac functioning such as cardiac transplantation have been shown to restore cerebral hemodynamics to the normal levels[24]. Patients with congestive heart failure frequently present with cognitive impairment[27]. Decline in cerebral blood flow and hemodynamic abnormalities have been suggested to mediate the association between congestive heart failure and cognitive impairment[25]. A large prospective study showed that congestive heart failure in older people independent of cardiovascular comorbidities associates with higher risk of dementia[28]. Recent reports suggest that not only presence of congestive heart failure but also a graded decrease in cardiac functioning might be a risk factor for brain aging[29]. Findings of the chapters five, six and seven of this thesis show that the association between cardiac function and impaired cognition is not limited to patients with heart failure. These data suggest that a graded decrease in cardiac function as reflected in cardiac hemodynamics and serum NT-proBNP level, a marker of ventricular dysfunction, is associated with cognitive decline in general populations of old and very old subjects as well as in older subjects at risk of cardiovascular disease without heart failure. In line with these results, role of the heart-brain axis in brain aging has been suggested[30]. Long-lasting decline in cardiac functioning in interaction with pathologies in the systemic and cerebral circulations results in chronic brain hypoperfusion (figure 2). Chronic brain hypoperfusion gives rise to neuronal energy crisis and this ultimately leads to neuronal cell death. This cascade of events may put subjects at risk of accelerated brain aging.

Cognitive impairment in old age: a red flag for future stroke

Postmortem and neuroimaging studies have confirmed that not only patients with vascular dementia but also patients with Alzheimer's disease carry a high load of brain vascular pathologies[31]. In this thesis, it is demonstrated that patients with Alzheimer's disease and vascular dementia have a pronounced disturbance in their cerebrovascular hemodynamics (chapter eight). Accordingly, it has been suggested that cognitive impairment might reflect covert brain vascular pathologies, predisposing subjects to higher risk of stroke[32]. A number of longitudinal studies have investigated whether cognitive impairment is associated with increased risk of stroke and whether cognitive assessment can be considered as a tool for identification of subjects at high risk of stroke[33, 34].

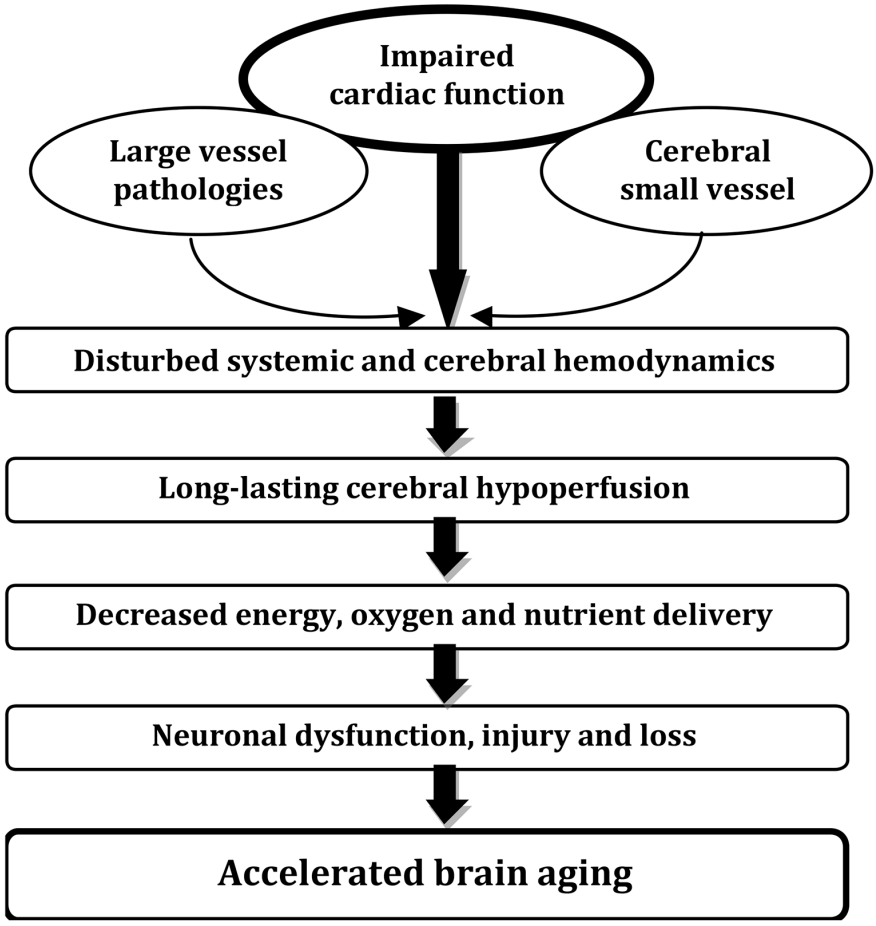

Figure 2. Proposed pathophysiological model on the link between abnormalities in the heart-brain axis and accelerated brain aging. Long-lasting decline in cardiac functioning in interaction with pathologies in the systemic and cerebral circulations results in chronic brain hypoperfusion. Chronic brain hypoperfusion gives rise to neuronal energy crisis and this ultimately leads to neuronal injury and cell death. This cascade of events renders subjects vulnerable for development of accelerated brain aging.

Most of these studies reported that middle aged or younger elderly subjects with lower cognitive performance were at a higher risk for developing stroke. In the ninth chapter of this thesis, we showed that lower cognitive function measured by mini-mental-state examination (MMSE) was associated with risk of first-time stroke in the oldest old. Furthermore, MMSE score appeared to be a better predictor for stroke when compared to the well-established Framingham stroke risk score. Collectively, available data suggest that older subjects with cognitive impairment should be considered among high-risk groups for development of future stroke.

Vascular endothelium; roles in regulation of cerebral blood flow

Cerebral autoregulation is the inherent ability of the brain to maintain a relatively constant level of cerebral blood flow despite fluctuations in perfusion pressure[35]. Changes in cerebral vascular tone play a key role in regulation of cerebral blood flow. Vascular tone in the cerebral circulation is regulated by several mechanisms[36]. One major mechanism involves endothelial factors[37]. Endothelium produces and releases potent relaxing and contracting factors that regulate tone of underlying vascular muscle and may also influence vascular growth[38]. Cardiovascular risk factors such as hypertension and diabetes have been linked to a decline in cerebral perfusion and it has been postulated that endothelial dysfunction due to chronic exposure to these risk factors is responsible for a decline in cerebral blood flow[39]. In line with this hypothesis, data presented in the tenth chapter of this thesis demonstrate that two serum markers of endothelial activation (von willebrand factor and tissue plasminogen activator) are associated with lower levels of cerebral blood flow. Given the critical role of cerebral perfusion in the maintenance of brain structure and function, preservation of the vascular endothelium can be a potential approach for delaying brain aging.

Cerebral blood flow; a determinant of survival in old age

The brain has an extraordinary metabolic demand[40]. This high metabolic demand is met by adequate and constant levels of cerebral blood flow that deliver energy, nutrient and oxygen to the brain[41]. Normal function of the brain is crucial for maintenance of health and homeostasis[42]. The brain is involved in regulation of stress response, circadian rhythm, endocrine system, fluid and elec-

trolyte balance, body energy expenditure and several other key pathways that act in concert to keep our body in a steady state[43, 44]. It has been shown that animal models with a state of hypoperfusion live shorter and develop weight loss[45]. In human, development of stroke is associated with disturbances in autonomic control, impaired sleep cycles and immune response[46, 47]. In the eleventh chapter of this thesis, the association between cerebral blood flow and 12-year survival in older people at risk for cardiovascular disease is investigated. The observed associations of cerebral blood flow with all-cause; cardiovascular and non-cardiovascular mortality might highlight the importance of cerebral perfusion in maintenance of health and survival in older people. Interestingly, all these associations were independent of socio-demographic and cardiovascular risk factors. There are a limited number of studies investigating the link between decreased brain blood flow and mortality. For instance, a recent study showed that lower cerebral blood flow is a strong predictor for mortality in patients with heart failure[48]. This finding might further highlight the importance of sufficient cerebral blood flow in maintenance of health and survival. Strategies to slow down decline of cerebral blood flow might decelerate rate of brain aging and improves survival in older people.

Methodological considerations

Scientific background, accurate observation, experimentation and applicability have been regarded as the fundamental elements of clinical research[49]. In this thesis, we build up our hypotheses based on the current evidence on the link between cardiovascular factors and age-related disorders of the brain. While findings of this thesis are in line with growing evidence exist supporting a role for vascular factors in brain aging, cardiovascular aging can be a cause or an epiphenomenon for brain aging[50, 51]. Hence, further life-course studies are needed to show the temporal relationship between dysregulations in cardiovascular system and changes in the brain structure and function. Such studies might unravel how early life events influence the susceptibility of the brain to cardiovascular and hemodynamic abnormalities. Findings presented in this thesis come from three well-establsihed cohorts of older subjects. Despite strengths in the design, inclusion of participants and availability of a wide range of variables, observational nature of these studies limits causal inference from the results. Therefore, interventional studies are needed to test whether correction of cardiovascular and hemodynamic abnormalities can decelerate process of brain aging.

It is important to keep in mind that the translation of findings from clinical studies to clinical practice has been always a challenge[52]. While clinical studies

usually look at outcomes in a population under study, in real life clinicians need to make decision for individual subjects. In this thesis we emphasized on the associations independent of sociodemographic and cardiovascular factors which might serve as potential confounders. Nontheless, large proportions of older subjects have multiple cardiovascular risk factors and co-morbidities[53]. These cardiovascular factors might individually or in interaction with each other contribute to brain aging. Further sophisticated analyses are waranted to account for cumulative contribution of multiple factors in development and progression of brain aging.

Future directions

Despite major efforts have been made, our understanding about etiology of abnormal brain aging is still limited. Current evidence indicates that the brain aging is a complex and multi-facet phenomenon[54]. This complexity has been reflected in the etiology of age-related disorders of the brain like dementia. It is well-described that a combination of factors act in concert in the pathogenesis of cognitive impairment and no single mechanism can explain the pathologic features observed in the brain of patients with dementia[55]. In this setting, studies which targeted specific brain pathologies, such as amyloid β deposition, have failed to show any improvement in cognitive function[56]. Future studies might be needed to focus on strategies which cover multiple aspects of brain aging. It has been shown that long-lasting cerebral hypoperfusion promotes oxidative stress, activation of inflammatory pathways, endothelial dysfunction, and amyloid β deposition[21]. Therefore, optimization of brain perfusion by prevention of pathologies in the heart-brain axis and neurovascular unit might modulate pace of aging in the brain[30]. In addition, findings of this thesis suggest that the association between cardiovascular factors and features of brain aging, like cognitive performance, might be not only age dependent but also functional status dependent. This calls for future studies investigating individualized approach in treatment of cardiovascular risk factors in order to preserve brain structure and function.

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13

Summary/ Samenvatting

Summary in English

The structural and functional integrity of the brain depends on the adequate and constant supply of energy and oxygen through cerebral blood flow. Hence, factors playing role in generation, delivery and regulation of brain blood flow may contribute in the pace of aging in the brain. Accelerated brain aging presents with pathological changes which impair cognitive function. This thesis aimed to show that cardiovascular and hemodynamic factors are related to the structural and functional features of brain aging. Findings of this thesis might open new avenues in prevention and progression of the accelerated brain aging.

Chapter 1 provides a background on the demographic, biologic and cardiovascular aspects of brain aging. In **Chapter 2**, we showed that higher blood pressure is associated with lower cognitive decline in very old age. This association was more prominent in older people with higher disability in their acticities of daily living. This has brought us to the next step to test whether levels of cognitive and functional disability moderate the association between high blood pressure and stroke in very old age. The findings presented in the **Chapter 3** indicate that higher blood pressure is associated with lower risk of stroke in very old subjects with higher degrees of disability.

Chapter 4 expands our current knowledge on the association of higher visit-to-visit blood pressure variability with impaired brain structure and function. Previously it has been shown that increased blood pressure variability is related to higher risk of stroke. Consistently, we showed that visit-to-visit blood pressure variability might put subjects at a higher risk for accelerated brain aging. In **Chapters 5, 6 and 7**, we focused on the association between level of cardiac function and brain aging. We observed that a strong association exists between a graded decrease in cardiac function and lower brain volumes and cognitive performance. Furthermore, we observed that in very old age, subjects who have both low blood pressure and left ventricular dysfunction have a higher risk for cognitive decline. These findings support the hypothesis that strategies to preserve cardiac function might also prevent abnormal brain aging in older subjects.

Disturbances in cerebrovascular hemodynamics have been implicated in the pathogenesis of cognitive impairment. Several studies have investigated whether patients with dementia have a lower cerebral blood flow and higher cerebrovascular resistance. In a meta-analysis (**Chapter 8**) we demonstrated that in patients with two most common forms of dementia; Alzheimer's disease and vascular dementia have profound disturbances in their cerebrovascular hemodynamics. However, the severity of disturbances was higher in patints with vascular dementia.

Chapter 9 deals with the identification of very old subject at risk for stroke using cognitive assessment. This chapter shows that in late life, conventional cardiovascular risk factors loss their predictive value while impaired cognitive function better predicts risk of stroke. This finding highlights that the assessment of cognitive function might be an easily accessible tool to recognize older subjects at risk of stroke.

In **Chapter 10**, we showed that increased serum markers of endothelial dysfunction are associated with lower levels of cerebral blood flow in older subjects at risk for cardiovascular disease. This is in line with previous lab findings indicating that endothelial cells play an important role in regulation of cerebral blood flow. Furthermore, it supports the hypothesis that cardiovascular risk factors accelerate process of brain aging, through ptomotion of the endothelial dysfunction and decrease in cerebral blood flow.

The brain is a key regulator of homeostasis. Previous studies have shown that subjects who carry a higher load of cerebrovascular damages have a shorter survival indepenet of cardiovascular risk factors and diseases. Given the significance of cerebral blood flow in the maintenance of brain structure and function, in **Chapter 11**, we showed that older subjects with lower cerebral blood flow have a shorter survival. This observation merits future studies investigating how preservation of cerebral blood flow influence health and survival in old age.

Chapter 12 reviews the key findings of this thesis and discusses them in the context of current knowledge and evidence. Based on the findings of this thesis, we suggested pathophysiological models on the contribution of cardiovascular and hemodynamic factors in development and progression of brain aging.

Samenvatting

De structurele en functionele integriteit van de hersenen is afhankelijk van een adequate en constante aanvoer van energie en zuurstof via de cerebrale bloedtoevoer. Factoren die een rol spelen in de productie, afgifte en regulatie van cerebrale bloedtoevoer kunnen derhalve bijdragen aan de snelheid van hersenveroudering. Versnelde hersenveroudering presenteert zich met pathologische veranderingen die de cognitieve functie beperken. Dit proefschrift heeft als doel om aan te tonen dat cardiovasculaire en hemodynamische factoren gerelateerd zijn aan structurele en functionele kenmerken van hersenveroudering. De bevindingen in dit proefschrift kunnen mogelijk nieuwe paden openen in de preventie van versnelde hersenveroudering.

Hoofdstuk 1 verschaft een achtergrond van de demografische, biologische en cardiovasculaire aspecten van hersenveroudering. In **Hoofdstuk 2** laten we zien dat een hogere bloeddruk geassocieerd is met een lagere cognitieve achteruitgang op zeer hoge leeftijd. Deze associatie was meer uitgesproken in oudere mensen met ernstigere beperkingen in hun dagelijkse activiteiten. Dit bracht ons tot de volgende stap, waarbij we testten of het niveau van cognitieve en functionele beperkingen de associatie tussen hoge bloeddruk en beroerte op zeer hoge leeftijd beïnvloedt. De bevindingen, zoals gepresenteerd in **Hoofdstuk 3**, tonen aan dat een hogere bloeddruk geassocieerd is met een lager risico op beroerte in zeer oude personen.

Hoofdstuk 4 verbreedt onze huidige kennis over de associatie tussen een hogere variabiliteit in bloeddruk, gemeten op meerdere momenten, en beschadigde hersenstructuren en functie. Eerder was reeds aangetoond dat een toegenomen bloeddrukvariabiliteit gerelateerd is aan een hoger risico op beroerte. Overeenkomstig deze bevindingen hebben wij aangetoond dat bloeddrukvariabiliteit mogelijk een groter risico geeft op versnelde hersenveroudering.

In **Hoofdstuk 5, 6 en 7** hebben we de aandacht gevestigd op de associatie tussen het niveau van cardiale functie en hersenveroudering. Onze observatie was dat er een sterke associatie bestaat tussen een graduele achteruitgang in cardiale functie en lagere hersenvolumes en slechtere cognitieve prestaties. Daarnaast vonden we dat op zeer hoge leeftijd personen met zowel een lage bloeddruk als linker ventrikel dysfunctie een hoger risico hebben op cognitieve achteruitgang. Deze bevindingen ondersteunen de hypothese dat strategieën die gericht zijn op het behoud van cardiale functie mogelijk ook preventief werken tegen abnormale hersenveroudering in oudere personen.

Verstoringen in cerebrovasculaire hemodynamiek zijn betrokken bij de pathogenese van cognitieve beperkingen. Meerdere studies hebben onderzocht of

patiënten met dementie een lagere cerebrale bloedtoevoer en hogere cerebrovasculaire weerstand hebben. In een meta-analyse (**hoofdstuk 8**) laten we zien dat patiënten met de twee meest voorkomende vormen van dementie, de ziekte van Alzheimer en vasculaire dementie, uitgesproken verstoringen hebben in cerebrovasculaire hemodynamiek. Echter, de ernst van de verstoringen was meer uitgesproken in patiënten met vasculaire dementie.

Hoofdstuk 9 behandelt de identificatie van zeer oude personen met een verhoogd risico op een beroerte, door gebruik te maken van cognitieve beoordelingen. Dit hoofdstuk laat zien dat, op hogere leeftijd, conventionele cardiovasculaire risicofactoren hun voorspellende waarde verliezen, terwijl een beperkte cognitieve functie juist het risico op beroerte beter voorspelt. Deze bevinding onderstreept dat de beoordeling van cognitieve functie mogelijk een eenvoudig beschikbaar middel kan zijn om oudere personen met een verhoogd risico op beroerte te identificeren.

In **Hoofdstuk 10** laten we zien dat verhoogde serum markers van endotheel dysfunctie geassocieerd zijn met lagere niveaus van cerebrale bloedtoevoer in oudere personen met een verhoogd risico hebben op cardiovasculaire ziekte. Dit komt overeen met eerdere bevindingen uit laboratoriumonderzoek, wat aangeeft dat endotheelcellen een belangrijke rol spelen in de regulatie van cerebrale bloedtoevoer. Daarnaast ondersteunt het de hypothese dat cardiovasculaire risicofactoren het proces van hersenveroudering versnellen door het bevorderen van endotheel dyfunctie en een afname in cerebrale bloedtoevoer.

De hersenen spelen een sleutelrol in de regulatie van homeostase. Voorgaande studies hebben aangetoond dat personen met een hogere accumulatie van cerebrovasculaire schade een kortere overleving hebben, onafhankelijk van cardiovasculaire risicofactoren en ziekten. Uitgaande van de importantie van de cerebrale bloedtoevoer in het onderhoud van hersenstuctuur en functie, laten we in **Hoofdstuk 11** zien dat oudere personen met een lagere cerebrale bloedtoevoer een kortere overleving hebben. Deze observatie vraagt om toekomstige studies, waarin onderzocht wordt hoe het behoud van cerebrale bloedtoevoer van invloed kan zijn op gezondheid en overleving op hoge leeftijd.

Hoofstuk 12 vat de belangrijkste bevindingen van dit proefschrift samen en bediscussieert deze bevindingen in de context van de huidige kennis en bewijzen. Gebaseerd op de bevindingen in dit proefschrift hebben we suggesties gedaan voor pathofysiologische modellen, waarin de bijdrage van cardiovasculaire en hemodynamische factoren aan de ontwikkeling en progressie van hersenveroudering wordt geëxpliciteerd.

List of Publications

1. **Sabayan B**, van der Grond J, Westendorp RG, Jukema JW, Ford I, Buckley BM, Sattar N, van Osch MJ, van Buchem MA, de Craen AJ. Total cerebral blood flow and mortality in old age: A 12-year follow-up study. Neurology. 2013 26;81(22):1922-9.

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About the author

Behnam Sabayan was born on June 13, 1984 in Shiraz, Iran. From 1998 to 2002, he attended in the Shahid Dastgheib high school affiliated to the National Organization for Development of Exceptional Talents.

In 2002, he entered the school of medicine at Shiraz University of Medical sciences. Studying medicine, he was honored as the distinguished student by the Ministry of Health and Medical Education in 2008. He also received the national award as a young researcher in the 14th Razi Festival. Between 2003 and 2009, he was as an active member and head of the student research committee at Shiraz University of Medical Sciences. Because of his interest in extracurricular activities, he volunteered in the activities of UNICEF, UNESCO and Student Organization of Iran.

In 2009, he obtained his medical degree and became researcher at the Health Policy Research Center. In August 2010 he started his PhD under supervision of Prof.Westendorp and Prof.van Buchem at Leiden University Medical Center, Leiden, the Netherlands. At the same time, he studied Master of Science in Ageing and Vitality and graduated from the Leyden Academy on Ageing and Vitality in 2011. Since 2011, he is the member of executive committee of the International Society of Vascular Behavioural and Cognitive Disorders.

Acknowledgements

I would like to express my deepest gratitude to all those who have supported me to complete this stage of my life and my scientific career. Doing PhD for me was not just about getting a better knowledge in a scientific field; it was also about being in touch with people who inspired me to explore my curiosities and challenge my intellectual boundaries.

Foremost, my sincere gratitude to my caring, loving, and supportive wife, Sanaz without her supports this achievement could never be possible. Sanaz, you are the meaning of a true friendship and you are a real friend who walks in when the rest of the world walks out. My lovely parents, I am truly grateful for your encouragements and supports. You helped me to follow my dreams. I would also like to take the opportunity to thank my grandmother "Azizjan", my brother and sister for their best wishes.

I had a privilege to study and work with great scientists who provided me with an excellent atmosphere for doing research. My sincere appreciation goes to Dr. A J de Craen. Dear Ton, I've learned many new things from you about clinical research and critical thinking. I really appreciate your honesty, understanding and sense of humor. I would like to thank Dr. M.J.P. van Osch, my Co-Promoter. Dear Thijs, I really enjoyed working with you not only as a supervisor but also as a friend. Your smart comments and remarks have always inspired me to think about new aspects of my research projects.

Being far from family in the last few years, now I better understand the person who said "friends are the family you choose". Ramin, Azita, Abbas, Raha, Maryam and Payam, you are wonderful friends who made joyful moments for me. Thank you so much for your friendship, support and laughs. Frouke and Peter, thanks for being my paranymphs! Frouke, it is about four years that we met for the first time in a student conference in Tehran. You are a great friend and I've always enjoyed chatting and sharing my opinions with you. Peter, I remember our first talk about doing PhD in Leiden. You have always provided me with your smart and realistic points of view and I very much enjoyed talking with you about different sort of things from scientific excellence to football matches! Besides, I appreciate you being always keen to make me more familiar with cultural, political and social aspects of life in the Netherlands.

Getting settled down in a new country can be a big challenge. Silvia, you and your family with your kind support, sympathy and understanding helped us to experience a smooth start.

Studying in the Netherlands, gave me the opportunity to become acquainted with some great friends; Moein, Akbar, Eidrees, Otto, Llywela, Kazem, Nahid, Anna, Sam, Aafke, Geranne, Koen, Amin, Pim, Maarten, David, Sylvie, Astrid, Ulrika, Shiva, Ali, Behrooz, Shima and Mahsan. Moein and Amin, I would like to express my gratitude to you for your kind assistance in preparation of this dissertation and arrangements before my PhD defense.

I would like to thank my great Shirazi friends. Abdolali (Zoli), Amin, Kasra, Nima, Hossein, Faraz, Alireza, Afshin and Omid, I still think fondly of wonderful moments we spent together.

My sincere thanks go to my friends and colleagues at the Leiden University Medical Center and Leyden Academy on Vitality and Ageing; Ania, Liselotte, Simon, Wouter, Sophie, Jasper, Xingxing, Jeroen, Diana, Stella, Iris, Karel, Steffy, Abi, Justin, Jessica, Christine, Elmi, Simin, Somayeh, Ineke, David, Jolanda, Marieke, Herbert and Julia.

My appreciation extends to all my co-authors for their great inputs and collaborations. Prof. J Gussekloo, Dr. W de Ruijter, Prof. A Maier, Prof. DJ Stott, Prof. I Ford, Prof. BM Buckley, Prof. N Sattar, Prof. JW Jukema, Dr. L Launer and Dr. A Arai, it was a great pleasure for me working with you.

Finally, as I stressed earlier in this dissertation, I am deeply grateful to all the patients and participants without whom it was not possible for me to study medicine and complete this PhD project.