Assessment of Vascular Contributions to Cerebral Small Vessel Disease: Data from the general population study "Good Aging in Skåne"

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LUND
UNIVERSITY

DOCTORAL DISSERTATION

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Title and subtitle: Assessment of vascular contributions to Cebral Small Vessel Disease: Data from the general population study “Good Aging in Skåne”

Abstract: Cerebral small vessel disease (CSVD) are highly age-related pathologies visualized by neuroimaging. CSVD can be silent lesions but are associated with cognitive decline, dementia, gait disorders, depression and stroke. A growing body of evidence links CSVD to systemic vascular processes, but the pathophysiology is not fully explained. The aim of this thesis is to investigate possible links between markers of vascular burden or hypertension-related organ dysfunction with CSVD or atrophy. The Swedish study Gott Åldrande i Skåne (Good Aging in Skåne, GÅS) is a longitudinal population study of older adults. In a sub-cohort aged 70-87 we performed carotid artery Doppler ultrasound and 3T magnetic resonance imaging (MRI). Markers of CSVD were assessed by visual rating scales and included: white matter hyperintensities (WMH), cerebral microbleeds (CMB), lacunar infarctions (LAC), and patterns of brain atrophy; medial temporal lobe (MTA), parietal (PAR), precuneal (PREC), central (CA) and global cortical (GCA).

In **paper I** (n=344) we cross sectionally investigated the association of hypertension and blood pressure variables, with CMB and spatial distribution of CMB. Deep CMB had a stronger association with hypertension and blood pressure measurements than lobar CMB.

In **paper II** (n=291) we cross sectionally investigated the association between common carotid artery (CCA) mean flow velocities and CSVD/atrophy. Peak systolic velocity (PSV) and end diastolic velocity (EDV) were assessed by Doppler ultrasound, and pulsatility index (PI) and resistivity index (RI) were calculated. Low EDV in CCA was associated with WMH, PAR, PREC and GCA. Low EDV and high PI and RI were associated with composite scores of CSVD burden.

In **paper III** (n=390) we cross sectionally investigated the association between chronic kidney disease (CKD) and CSVD, specifically considering hypertension status. An association between CKD and the presence of CMB and cortical atrophy was observed only in the hypertensive cohort.

In **paper IV** (n=401) we cross sectionally investigated the associations between 257 circulating plasma proteins investigated by proximity extension assay (PEA) by Olink Proteomics (CVD II, CVD III and inflammatory panels), and MRI manifestations. 18 proteins were significantly associated with markers of CSVD or atrophy.

In conclusion, CSVD is common in the aging population. CSVD can be linked to hypertension, carotid artery flow parameters, and CKD, which are all closely related to vascular aging and arterial stiffness. Identified circulating proteins are markers of vascular burden, immune regulation, blood-brain barrier, apoptosis and tissue maintenance. The results of these studies emphasize the importance of a holistic approach to age related organ dysfunction, and careful management of vascular risk factors in the aging individual.

Key words: cerebral small vessel disease, hypertension, blood pressure, carotid Dopler ultrasound, chronic kidney disease, proteomics, biomarker, risk factor, epidemiology, older adults, general population

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Populärvetenskaplig sammanfattning

Cerebral småkärlssjukdom (CSVD) är ett samlingsnamn för förändringar i hjärnan som är starkt kopplade till åldrande. De diagnosticeras främst genom hjärnavbildningar, såsom magnetkamera-undersökning (MR) eller datortomografi (DT), och syns exempelvis som vitsubstansförändringar (WMH), mikroblödningar (CMB), små djupa mikro-infarkter (lakunära infarkter, LAC) och atrofier (krympning av hjärnan) (1, 2). CSVD kan vara kliniskt asymtomatiskt under flera år, och hos en majoritet av en i övrigt frisk äldre befolkning ses CSVD-förändringar vid hjärnavbildning. Även om de är vanliga och ofta asymtomatiska är de förknippade med ökad risk för kognitiv svikt, demens, depression, rörelsemönsterstörningar och stroke (3). CSVD utgör därmed ett gränsland, eller ett kontinuum, mellan normalt åldrande och sjukliga förändringar som i förlängningen kan bidra till stor funktionsförlust. Även om mekanismerna inte är fullt klarlagda, är flera riskfaktorer kända – däribland hypertoni, diabetes och rökning.

Målsättning

Syftet med avhandlingen är att kartlägga förekomst av åldersförändringar i hjärnan samt att öka förståelsen kring riskfaktorer och bakomliggande mekanismer för utveckling av CSVD.

När vi åldras blir de större artärerna i kroppen styvare, vilket gör att blodtrycket stiger och flödesdynamiken i kärlen ändras. Ett högre blodtryck och kraftigare pulsationer i kärlen har potential att skada små känsliga artärer och mindre kärl i vävnaden. Vi vill undersöka om tecken till åldersförändringar i de större kärlen har koppling till förekomst av CSVD.

Njuren har likt hjärnan en hög genomblödning med mindre dämpning av det inkommande blodflödet än andra organ. Njursvikt har en stark koppling till hypertoni, både som bidragande orsak till, och konsekvens av, högre blodtryck. Njursvikt kan leda till högt blodtryck när förmågan att reglera salt- och vätskebalansen minskar, medan långvarig hypertoni kan skada njurarnas blodkärl och försämra dess funktion. Samvarians mellan njursvikt och CSVD är av intresse både av mekanistiska skäl och från ett kliniskt perspektiv.

Vidare hoppas vi att undersökning av cirkulerande proteiner (äggviteämnen) i blodet kan ge indikationer om vad som sker på cellnivå i samband med CSVD. I förlängningen även möjligheten att hitta blodprovsmarkörer för CSVD, eller proteiner som kan utgöra behandlingsmöjligheter.

Material och Metoder

Gott Åldrande i Skåne (GÅS) är en pågående longitudinell befolkningsstudie som startade 2001. Studien är en del av den nationella äldrestudien The Swedish National Study on Aging and Care, (SNAC) (4). Ett slumpmässigt urval av befolkningen från 60 års ålder och uppåt inbjuds att medverka, och återbesök erbjuds vart tredje år för deltagare 78 år och äldre samt vart sjätte år för deltagare under 78 år. Nya kohorter bjuds in vart sjätte år. Undersökningen omfattar läkarundersökning, fysisk funktionsförmåga, kognitiv förmåga med 14 olika test, EKG, lungfunktionsbedömning, ankel-arm-index, ortostatiskt test samt frågeformulär kring tidigare sjukdomar, läkemedel, symtom, sociodemografi, livsstilsfaktorer, och psykologiska faktorer. Prover för biobank sparas vid varje tillfälle.

En fördjupningsstudie har utförts på 647 deltagare i åldersintervallet 70–87 år, där kärlfysiologi, med bland annat ultraljudsundersökning av halspulsådern, har undersökts. På samma delurval har 408 deltagare genomgått MR-hjärna för kategorisering av småkärlssjukdom i hjärnan. Genom blodprovstagning har vi undersökt markörer för njurfunktion. Vidare har koncentrationen av ett stort antal proteiner i blodet undersökts genom Olink Proteomics i Uppsala, en teknik som bygger på antikroppsigenkänning.

Delarbete I

Tvärsnittsstudie publicerad juli 2019. Vi har tittat på om det finns samband ett mellan högt blodtryck och mikroblödningar i hjärnan. Det visar sig att personer med högt blodtryck eller hypertoni i större utsträckning hade framför allt djupare liggande mikroblödningar. Personer med välbehandlad hypertoni hade i lägre utsträckning mikroblödningar jämfört med de som trots diagnos och behandling ändå hade för högt blodtryck vid undersökning hos oss.

Delarbete II

Tvärsnittsstudie, publicerad januari 2023. Vi har utfört ultraljudsundersökning av halspulsådern, och mätt flödeshastigheten vid pulsationerna. Högsta hastigheten vid systole, och lägsta hastigheten vid diastole har registrerats, och baserat på dessa har vi räknat ut ett index som mäter resistens och pulsatilitet. Det visar sig att de som har lägre flödeshastighet vid diastole i större utsträckning har förekomst av vitsubstansförändringar och vissa av atrofierna i hjärnan. Det fanns även ett linjärt samband mellan graden av pulsatilitet och resistivitet, och mer uttalade CSVD förändringar.

Delarbete III

Tvärsnittsstudie publicerad mars 2024. Vi har tittat på om det finns ett samband mellan njursvikt och CSVD. Ett samband kan ses, men först efter att vi delat in materialet utefter förekomst av hypertoni. Njursvikt, likt CSVD, har en stark koppling till hypertoni och kärlstyvhet. Även om orsakssamband inte kan fastställas i denna tvärsnittsstudie, antyder resultaten att hypertoni, njursvikt och CSVD kan utvecklas parallellt eller vara ömsesidigt relaterade. Därför kan det vara av vikt att ha ett helhetsgrepp vid val av behandling och uppföljning av patienter.

Delarbete IV

Denna studie är ännu inte publicerad. Det är en s.k. explorativ studie, där vi letat efter eventuella samband mellan 257 olika proteiner i blodet och CSVD. Proteinerna är valda för att de tidigare kopplats till hjärt-kärlmanifestationer eller inflammation. Vi hittade samband mellan 18 olika proteiner och CSVD. Dessa speglar allmän metabol eller vaskulär börda, inflammationsmarkörer och proteiner kopplade till immunförsvaret, proteiner involverade i blod-hjärnbarriären, och markörer för apoptos (programmerad celldöd). Våra fynd behöver först och främst bekräftas genom ytterligare studier, men kan eventuellt hjälpa till att förklara bakomliggande mekanismer på en djupare nivå än vad övergripande riskfaktorer gör.

Sammanfattning

Det finns tecken på att småkärlssjukdom i hjärnan kan vara kopplat till storkärlssjukdom som hypertoni eller kärlstyvhet, där samband med andra organsjukdomar, såsom njursvikt, också kan ses. Avhandlingen öppnar dörren för att på molekylärnivå kunna undersöka och förstå mekanismer för utvecklingen av kognitiv svikt. Till dess vill vi poängtera vikten av ett gott förebyggande arbete med kärlriskfaktorer och andra kardiovaskulära riskfaktorer, samt god blodtryckskontroll. Tidigare forskning har visat att en av de viktigaste skyddsfaktorerna mot kognitivt åldrande är att hålla hjärnan aktiv och stimulerad genom hela livet.

Det verkar som att receptet för ett gott åldrande, förutom ett flexibelt sinne, även är spänstiga kärl.

Papers at a glance

Paper	I	II	III	IV
Aim	Investigate prevalence of CMB, and the association between blood pressure measurements and hypertension and CMB, with special regards to CMB localization	Investigate association between carotid artery duplex ultrasound flow parameters and MRI-markers of CSVD and brain atrophy	Investigate association between CKD and CSVD, with special regards to hypertension status	Exploratively investigate the associations between protein abundance of known cardiovascular or inflammatory proteins, and MRI-markers of CSVD or atrophy
Design	Cross-sectional	Cross-sectional	Cross-sectional	Cross-sectional
Population	Early finished participants of MRI-sub-study of Good Aging in Skåne Study n=344	MRI sub-study of Good Aging in Skåne Study. Subjects with peak flow velocity ≥ 120cm/s indicating stenosis >50% were excluded from analysis n=391	MRI sub-study of Good Aging in Skåne Study with available eGFR-values n=390	MRI sub-study of Good Aging in Skåne Study with available plasma from biobank n=401
Main statistical method	Multivariable logistic regression models	Multivariable linear and logistic regression models	Multivariable logistic regression models	Multivariable linear and logistic regression models. The Benjamini-Yekutieli method was used to keep false discovery rate at 5%.
Exposure variables	SBP, DBP, PP, ABI HT diagnosis, HT duration	Mean value from left and right CCA regarding: PSV, EDV, PI and RI	CKD (eGFR ≤ 60 ml/min/1,73m^2)	Normative plasma levels of circulating proteins identified by proximity extension immuno-assay, Olink panels CVDII, CVDIII and inflammation. Proteins with <75% accepted values in quality controll were excluded from analysis, leaving 257 proteins for analysis.
Co-variables	Age, sex	Age, sex	Age, sex	Age, sex
Main outcome	CMB, and CMB according to location	MRI markers of CSVD and specific patterns of atrophy,	MRI markers of CSVD and cortical atrophy,	MRI markers of CSVD and cortical atrophy, individually and combined

		and two different MRI-burden scores	individually and combined	
Main result	CMB occurred in 26% of subjects. 61,5% had a single lesion. Male gender, higher levels of measured blood pressure and HT diagnosis were associated with CMB. The effect of hypertension was stronger in the group with deeper CMB lesions compared to the group with strictly lobar distribution.	Low EDV was associated with WMC, GCA, PAR/KPA and PREC/PA, as well as the two measures of MRI-burden scores. PI and RI were linearly associated with two different CSVD-burden scores.	Among hypertensive subjects, CKD was associated with CMB and cortical atrophy.	11 plasma proteins (CTSL1, PGF, NTpBNP, TNFR2, GDF15, TNFR1, IL4RA, ADM, CXCL9, TFF3, BNP) were linearly associated with CSVD-score. 11 plasma proteins (CDH5, IL4RA, TNFR1, PGF, TF, TNFR2, CD93, CTSL1, LTBR, TNFRSF11A, TNFRSF10A) were positively associated with cortical atrophy. Increased levels of PI3 was associated with MTA. Increased levels of IL4RA was associated with Primary Atrophy.

CSVD: Cerebral small vessel disease, **MRI**: magnetic resonance imaging**, CMB**: cerebral microbleeds, **WMH**: White matter hyperintensities, **GCA**: Global Cortical Atrophy, **PAR/KPA**: Parietal Atrophy, **PREC/PA**: precuneus atrophy, **CCA**: common carotid artery, **PSV**: peak systolic velocity, **EDV**: end diastolic velocity, **PI**: Pulsatility index, **RI**: Resistivity index**, SBP:** systolic blood pressure, **DBP**: diastolic blood pressure, **PP**: pulse pressure, **ABI**: ankle-brachial index, **HT**: hypertension, **CVD**: cardio-vascular disease, **CKD**: chronic kidney disease, **eGFR**: estimated glomerular filtration rate, **FDR:** false discovery rate.

Abbreviations

ABI	Ankle-Brachial Index
AD	Alzheimer´s Disease
CA	Central Atrophy
CAA	Cerebral Amyloid Angiopathy
CCA	Common Carotid Artery
CKD	Chronic Kidney Disease
CMB	Cerebral Microbleeds
CSVD	Cerebral Small Vessel Disease
DBP	Diastolic Blood Pressure
EDV	End Diastolic Velocity
eGFR	Estimated Glomerular Filtration Rate
FDR	False Discovery Rate
FLAIR	Fluid-Attenuated Inversion Recovery
GCA	Global Cortical Atrophy
HT	Hypertension
LAC	Lacunar Infarctions
MCI	Mild Cognitive Impairment
MRI	Magnetic Resonance Imaging
MTA	Medial Temporal Lobe Atrophy
PAR	Parietal Atrophy
PI	Pulsatility Index
PP	Pulse Pressure
PREC	Precuneal Atrophy
PSV	Peak Systolic Velocity
PWV	Pulse Wave Velocity
RI	Resistivity Index
SBP	Systolic Blood Pressure
SWI	Susceptibility Weighted Imaging
WMH	White Matter Hyperintensities

Abstract

Cerebral small vessel disease (CSVD) are highly age-related pathologies visualized by neuroimaging. CSVD can be silent lesions but are associated with cognitive decline, dementia, gait disorders, depression and stroke. A growing body of evidence links CSVD to systemic vascular processes, but the pathophysiology is not fully explained. The **aim** of this thesis is to investigate possible links between markers of vascular burden or hypertension-related organ dysfunction with CSVD or atrophy. The Swedish study Gott Åldrande i Skåne (Good Aging in Skåne, GÅS) is a longitudinal population study of older adults. In a sub-cohort aged 70-87 we performed carotid artery Doppler ultrasound and 3T magnetic resonance imaging (MRI). Markers of CSVD were assessed by visual rating scales and included: white matter hyperintensities (WMH), cerebral microbleeds (CMB), lacunar infarctions (LAC), and patterns of brain atrophy; medial temporal lobe (MTA), parietal (PAR), precuneal (PREC), central (CA) and global cortical (GCA). In **paper I** (n=344) we cross sectionally investigated the association of hypertension and blood pressure variables, with CMB and spatial distribution of CMB. Deep CMB had a stronger association with hypertension and blood pressure measurements than lobar CMB. In **paper II** (n=291) we cross sectionally investigated the association between common carotid artery (CCA) mean flow velocities and CSVD/atrophy. Peak systolic velocity (PSV) and end diastolic velocity (EDV), pulsatility index (PI) and resistivity index (RI) were assessed. Low EDV in CCA was associated with WMH, PAR, PREC and GCA. Low EDV and high PI and RI were associated with composite scores of CSVD burden. In **paper III** (n=390) we cross sectionally investigated the association between chronic kidney disease (CKD) and CSVD, specifically considering hypertension status. An association between CKD and the presence of CMB and cortical atrophy was observed only in the hypertensive cohort. In **paper IV** (n=401) we cross sectionally investigated the associations between 257 circulating plasma proteins investigated by proximity extension assay (PEA) by Olink Proteomics (CVD II, CVD III and inflammatory panels), and MRI manifestations. 18 proteins were significantly associated with markers of CSVD or atrophy. In **conclusion**, CSVD is common in the aging population. CSVD can be linked to hypertension, carotid artery flow parameters, and CKD, which are all closely related to vascular aging and arterial stiffness. Identified circulating proteins are markers of vascular burden, immune regulation, blood-brain barrier, apoptosis and tissue maintenance. The results of these studies emphasize the importance of a holistic approach to age related organ dysfunction, and careful management of vascular risk factors in the aging individual.

Introduction

Rationale

Aging is a naturally occurring phenomenon in which tissue and organ function progressively decline due to altered cellular function and signalling. Rate of aging differs between individuals, reflecting both genetic and lifestyle effects on senescence. As we and others have shown, effects of cerebral aging can be visualized on imaging of the brain and are to be viewed as a natural occurrence in healthy older individuals. However, these same physiological changes can also be a prelude to accelerated or pathological aging, where loss of function leads to impairment and morbidity. Cerebral aging can result in a range of symptoms, such as cognitive impairment or dementia, executive disability, sleep-, mood-, and movement disorders, autonomic dysfunction, and increased risk of stroke (3). As the life expectancy of the world's population is expected to increase, so is the number of cognitively impaired individuals or individuals with dementia. Research has identified factors accelerating brain aging, such as dysmetabolism, hearing loss and sleep apnoea, as well as protective factors like physical activity and keeping a high cognitive activity (5).

During the recent decades, the vascular contribution to cerebral aging has been increasingly recognized (6). The vascular system in the brain is a complicated structure, crucial in maintaining cerebral integrity from the systemic blood flow meanwhile providing sufficient perfusion to meet tissue demands. Recent proteomic research has shed light on the dynamic interplay and signalling that occurs within and between the organ systems, including the vascular system and immune system. At the cellular level, aging appears not to be a simple progressive decline, but rather a complex balance of repair, degeneration, and regeneration in a delicate interplay. By understanding more about the progression of cerebral changes, from general risk factors to specific protein interactions, we hope to contribute to a cognitively healthier future older population. And as our understanding deepens, the postponement or even reversal of cognitive impairment may eventually become achievable.

Cognitive aging

Healthy cerebral aging results in structural and functional changes that lead to subtle declines in specific domains of cognition, while other domains often are spared or continue to excel. Normal changes in cognition are generally slow and predictable. Cerebral changes during aging can be compensated for by adaptive neuroplasticity (7) and relative spatial redistribution to cortical areas in brain activity (8).

Functionally, healthy cerebral aging can manifest as modest declines in certain cognitive domains, such as memory, conceptual reasoning, and processing speed. These abilities are often referred to as examples of fluid intelligence. Fluid intelligence is involved in problem solving, reasoning and learning new abilities. The so-called crystallized intelligence however, like skills, vocabulary and general knowledge, are often spared or may even continue to improve with age (9).

The implications of structural brain changes vary greatly between individuals (10). Cognitive reserve (11) describes the mismatch between structural changes and functional decline that can be observed in certain individuals. This was originally described among individuals with pronounced AD-pathologies in the brain but (relatively) spared cognition. Accounting for the histopathologic markers of the common causes of dementia (Alzheimer´s disease, vascular disease and Lewy Body), these collectively only explained 50% of the variation of decline in cognition (12). The resilience against cognitive decline in certain individuals reflects neuronal plasticity, increased reliance on frontal brain regions, and other compensatory mechanisms as well as genetics and lifestyle factors (10, 13). Several longitudinal studies have shown that lifestyle factors are associated with the rate of cognitive decline and even structural brain changes (14-16). Factors that have been associated with the rate of cognitive decline are educational level, and personal baseline level of cognitive performance like measure of literacy or vocabulary, as well as late life cognitive stimulation, physical activity, leisure activities and social engagement (9, 13, 16).

Cerebral small vessel disease

Cerebral Small Vessel Disease (CSVD) are common, highly age-associated structural changes occurring in and around the small (10-200 micrometer diameter) vessels of the brain (17, 18). Historically identified at autopsy, it is now primarily diagnosed and researched by neuroimaging. Classical manifestations of CSVD are recent small subcortical infarcts, lacunes of presumed vascular origin, white matter hyperintensities of presumed vascular origin, cerebral microbleeds, perivascular spaces, cortical superficial siderosis, cortical microinfarcts, and brain atrophy (1, 19). CSVD can be silent for a long time in the aging individual, and the clinical

impact varies greatly. The most common clinical manifestations are cognitive impairment and lacunar syndrome (20). CSVD is the leading cause of vascular dementia and can exacerbate the symptoms of other dementias such as Alzheimer's dementia (AD) (21), while contributing to functional decline (22). Other common manifestations of CSVD are effects on movement, personality changes, apathy, depression and incontinence, as well as increasing the risk of ischemic and hemorrhagic stroke (3, 20). In a large-scale population cohort from the UK Biobank, WMH volume was associated with several clinical phenotypes, including falls, respiratory problems, sleep disturbances and eye- and hearing problems; representing impairment across several different systems of the body (23). This emphasizes that underlying mechanisms to CSVD are not singular processes limited to the brain but rather connected to simultaneous occurrences in multiple systems of the body. Leading causes of CSVD are arteriosclerosis (caused by aging, hypertension and other vascular risk factors), and cerebral amyloid arteriopathy (CAA), (the buildup of amyloid-beta in the vessel walls (2, 24)). This thesis focuses on the former.

Historical perspectives

The concept of CSVD was clinically reported in the late 1890s, as arising predominately in males in their 50s. Post-mortem examinations showed white matter atrophy and cortical thinning, and the condition was described by Otto Binswanger (1852-1929) as "encephalitis subcorticalis chronica progressiva" (25). Around that time, the term "lacune" was used to describe hollowed infarctions in small subcortical vessels (26). In the 1960s the term "lipohyalinosis" was used by C.M. Fisher to describe the histological changes that could be seen in arterioles and other smaller vessels involved in small deep brain infarcts that were seemingly without an embolic source (27). With the introduction of computed tomography, CT, white matter changes, referred to as "leukoaraiosis", were visualized as areas of reduced attenuation, and could be found in about 50% of older adults (28). The first magnetic resonance (MR) machine was available for a clinical setting already during the early 1980s, but it was not until the 1990s that the MRI became readily available (28). In 1987 Fazekas et al published a paper where they quantified the burden of white matter signal abnormalities, later known as Fazekas scale for WMH. By 1.5 T MRI they examined 12 AD-patients, 4 patients that were categorized as multi-infarct dementia by a high score on the Rosen-Haschinski scale, and 9 healthy controls (29). With the generalization of MRI, the interest for age-associated cerebral lesions grew, and these lesions were also more frequently detected by MRI than by CT. During the late 1990s, susceptibility sensitive sequences revealed tiny iron deposits, i.e. cerebral microbleeds, previously barely detectable and most often overlooked by earlier imagery techniques (30, 31) .

During the 1990s and 2000s the nomenclature varied and was used interchangeably. By example, WMH was referred to as vascular leukoencephalopathy, leukoaraiosis, Binswanger encephalopathy, subcortical arteriosclerotic encephalopathy, ischemic microangiopathy, microvascular ischemic lesions and more (28). Standards for classification of CSVD were first proposed by the US National Institute of Neurological Disorders and Stroke and the Canadian Stroke Network in 2006 (32). Definitions and terminology were further enhanced in the Standards for Reporting Vascular Changes on Neuroimaging (STRIVE)-criteria published in 2013 (19). The STRIVE-criteria have greatly influenced the research community with the goal of unifying the nomenclature, meanwhile proposing a nomenclature that avoids presumption of mechanisms of pathogenesis, many of which originated from before neuroimaging. The STRIVE-criteria defined the following manifestations: recent small subcortical infarcts, lacunes of presumed vascular origin, white matter hyperintensities of presumed vascular origin, perivascular spaces, cerebral microbleeds, superficial siderosis and brain atrophy. Ten years later an update on the criteria was published, including updates on STRIVE features and the inclusion of novel imaging features like cortical cerebral microinfarcts and incidental DWI-positive lesions (1).

MRI manifestations of CSVD

White matter hyperintensities

The white matter of the brain lies in the deeper structures, beneath the cortex. It is responsible for the communication between cerebral areas, and between cortex and the rest of the CNS. White matter consists of supportive cells surrounding axons; the long sprouts emanating from neurons. Axons carry information between neurons in the form of action potentials (chemical currents). Like electrical wiring, the conduction of the axon is increased by insulation, in this case fat in the form of living cells, oligodendrocytes, that uses their cell processes as wrapping around the nerve fibers (33).

White matter lesions are visible as hyperintensities on T2-weighted MRI and hypointense or isointense on T1-weighted sequences. The Fluid-Attenuated Inversion Recovery (FLAIR)-sequence is often applied to investigate WMH. The FLAIR-sequence suppresses the signal from fluids, such as cerebrospinal fluid (CSF), making the signal from lesions more visible. White matter hyperintensities are often bilaterally symmetrical and can be found in the deep white matter or around the ventricles. WMH can also occur in the brainstem or in subcortical grey matter structures, such as the basal ganglia (the latter two often referred to as subcortical hyperintensities). In the STRIVE-criteria, the proposed term is white matter hyperintensity of presumed vascular origin, in order to differentiate from

white matter lesions of other origins, like multiple sclerosis or leukodystrophies (19).

The pathophysiological explanation to WMH is still not clearly understood. The increased signal intensity of MRI represents a greater water concentration in the tissue, which is thought to represent both increased interstitual fluid, demyelination and areas of gliosis (repair by glial cells) (34). White matter hyperintensities on neuroimaging correlate with histological signs of oedema and myelin pallor (35) and advanced lesions show signs of matrix disentanglement, myelin- and axonal loss and reactive gliosis (36). Also, normal appearing white matter of individuals with CSVD has been found to have a higher degree of free water by diffusion tensor imaging, which has been hypothesized to represent a general increase in extracellular fluid, and vessel permeability (2, 37).

WMH are the most common CSVD markers, detectable in some individuals already in the early 40s (38). WMH burden is typically doubled for every decade (39) and present in more than 90% of individuals in advancing age. Furthermore, representation is heterogenic and in a pooled large-scale study of 15 population cohorts, also the *variance* in WMH volume increased exponentially with age (39). Because it is common, it is often regarded as a benign or incidental finding on brain imaging (40). However, WMH has repeatedly shown to be predictive of stroke (41), cognitive impairment (41), dementia (42), and death (42), as well as outcome after adverse events (43, 44). Furthermore, WMH are associated with gait disturbances, impaired balance and an increased risk of late onset depression (34, 45).

WMH are associated with vascular risk factors including hypertension, markers of arterial stiffness, diabetes and hyperlipidemia (3, 34, 46). Longitudinal studies have shown that manifestations of CSVD are dynamic and can be affected by risk factor management such as lowering of blood pressure (47, 48).

Taken together, WMH can be viewed as general markers of brain health. Although common and studied since at least the middle of the last century, white matter hyperintensities are still quite poorly understood. The cause and effect of WMH differ greatly between individuals, and known risk factors explain very little of the variance in severity of WMH and clinical outcome between individuals.

Cerebral microbleeds

Cerebral microbleeds are small perivascular hemosiderin deposits corresponding most probably to small foci of past micro hemorrhages. CMBs are visible on MRI images in sequence T2*-GRE and susceptibility-weighted imaging (SWI) (19) and were first discovered in the 1990s when these sequences were developed. Histologically the deposits are made up of hemosiderin-laden macrophages, and the paramagnetic properties of the iron in the hemosiderin causes a reduced signal due to susceptibility (signal loss due to faster T2* relaxation) (49).

Prevalence in studies is highly depending on cohort age and neuroimaging parameters. In the Rotterdam Scan Study (I,5T 3D T2*-GRE), prevalence increased from 6,5% in subjects aged 45-50, to 35.7% in individuals over 80. (50) CMBs, like other markers of CSVD, can occur as silent lesions, but are also associated with severe clinical outcomes and symptoms. Stronger associations are observed with increasing number of CMBs. Presence of CMB is associated with increased risk of hemorrhagic and ischemic stroke, dementia, both vascular and AD, depression, gait disorders and cognitive decline (20, 49).

A major contributor to CMB is cerebral amyloid arteriopathy (CCA), the buildup of amyloid-beta protein in the vessel walls. CCA-associated microbleeds are more common in a lobar distribution in the brain, while deeper CMBs have a stronger association with vascular risk factors (49, 51). CCA is believed to be responsible for the majority of sporadic lobar hemorrhages.

A higher presence of CMBs is found in patients with first-ever and recurrent hemorrhagic or ischemic stroke, dementia, hypertension or cerebral amyloid angiopathy (CAA) (52, 53), but conditions other than CSVD, like head injury, coagulopathies, infections and neuroinflammatory conditions can also present with a higher prevalence of CMBs (49). Some classical cerebrovascular risk factors show consistent association with CMBs, such as male gender, age and smoking, but others, such as diabetes or hyperlipidemia show inconsistent results across studies (30, 54). Other risk factors for CMB that also are associated with increased risk of intracerebral hemorrhage are anticoagulant use and excessive alcohol consumption (53). Several observational studies have reported greater prevalence of intracerebral hemorrhage in antithrombotic users with CMB compared to those without (53), but, to my knowledge, RCTs have not shown an unbeneficial effect of antithrombotic treatment when there is an indication for it, if incorporating the risk of future ischemic events (53, 55).

Lacunar infarctions

"Lacunes" represent small fluid filled cavities mainly emanating from small infarctions, and were described already in the 1800s. Autopsy studies by CM Fischer in the 1960s gave rise to the term "lipohyalinosis", describing structural changes in the affected micro-vessels (27). In the STRIVE-criteria (19) "lacunes", small iso-intense fluid filled cavities 3-15mm, are differentiated from "recent small subcortical infarction", which can be larger (≤20mm), appear hyperintense on DWI, and represent a recent (weeks) infarction -although still small enough to emanate from a single penetrating end arteriole. Authors emphasized that nomenclature of "recent small.." should not include the term "lacune", as not all small infarctions cavitate into "lacunes", and it has not been shown that all "lacunes" have formed from previous infarctions.

It is estimated that roughly 25% of all ischemic strokes are lacunar, meaning that they are located in the deeper brain structures, and small enough to be caused by the occlusion of a single small penetrating artery (19, 26). These small, penetrating arteries are end arterioles without collateral blood supply, and occlusion typically produce a small infarcted area. Lacunar infarctions can be clinically silent or overt. It is not uncommon that small infarctions in this area are silent – and noticed in the later stages as fluid filled lacunes upon brain imaging. However, lacunar infarctions can also present with symptoms, collectively referred to as lacunar syndromes, and vary depending on location. CM Fischer described 21 syndromes, the most classical being "pure motor stroke", "pure sensory stroke" and "sensory-motor stroke". Most importantly, cortical symptoms such as aphasia, neglect or visual field defects are not present. Many of the silent lacunar infarctions, however, might have given rise to more diffuse symptoms such as cognitive impairment or movement disturbances, apathy and incontinence (26, 28).

Histologically, a few different etiologies to lacunar infarctions have been identified. Microatheromas are believed to be the most common cause, emanating from sources of atherosclerosis in more proximal vessels (but oftentimes not as proximal as the carotid artery, which has a stronger association with cortical ischemic strokes). Atheromatous deposits in the walls of the greater vessels are not uncommon, but in hypertension the atherosclerosis is more pronounced, and reaches further into the brain, to smaller arteries. Lacunar infarctions can also present without histological finds of an occluded vessel, associated with more distant changes including segmentally occurring lipohyalinosis and fibrinoid necrosis, both associated with hypertensive arteriopathy. Lipohyalinosis describes the accumulation of hyaline material in the vessel walls. Vessel walls are thickened, often resulting in narrowed lumen, but they can also become dilated. There is a loss of smooth muscle cells, and a reorganisation with fibrous tissue (2, 26).

Other causes of lacunar infarctions include genetic disorders such as CADASIL, infections, vasospasm, hypoperfusion and others (17, 26). Lacunar infarctions share many risk factors with other types of strokes, where hypertension and diabetes mellitus are the most important. Compared to cortical ischemic strokes, lacunar infarctions have a weaker connection to embolisms from the heart, carotid stenosis, or ischemic heart disease (24, 56).

Cerebral Atrophies

Brain atrophy in the concept of CSVD is defined by focal or general loss of brain volume that does not relate spatially to a specific macroscopic injury, such as trauma or infarctions (19). Atrophy can be visualized on brain imaging as sulcal dilation, representing cortical/grey matter atrophy, and central atrophy, representing loss of white matter. On a single image, atrophy is the assumed cause of sulcal dilation or ventricular enlargement, but in a strict sense it can only be confirmed by longitudinal imaging. In recent years advanced software has aided in volumetric assessment of

brain volume, either compared to previous images of the individuals, or normalized to intracranial cavity volume representing original brain size of the individual (57).

Causes of atrophy are heterogenic and occur in many disorders, vascular disease and neurodegenerative dementia diseases being a leading cause in the older population. Atrophy is also regarded a natural phenomenon following aging, beginning around the fourth decade of life. Atrophy rate ranges from 0.2% per year in middle aged adults, to below 1% per year in healthy older adults, and around 2% yearly in accelerated atrophy rates such as late-stage AD (57). In a longitudinal assessment, the Rotterdam Scan Study investigated the trajectories of cerebral aging in the general public. Mean age at the first scan was 64.4 years (45.7 – 97.9), and mean intracranial volume (proxy for original brain size) was 1138.2 mL (813.6 – 1699.4 mL) White matter volume and hippocampal volume changed more rapidly with increasing age, whereas grey matter showed a more linear and smaller decrease with advancing age (58). Since this was a general population, prevalence of other CSVD manifestations also increased more rapidly with advancing age.

Atrophy can represent neuronal loss, but this is not the only explanation to visible atrophy. Loss of glial cells and extracellular matrix also contribute to brain volume loss. Patterns of brain atrophy seem to follow a distinct pattern. By example, atrophy occurs early in the hippocampal formation and seems to follow a rostro-caudal order on a general population level; first affecting the frontal lobe (the lobe that matured last during adulthood) and affecting occipital lobe last. Moreover, secondary atrophy can be seen following brain lesions. By example, cortical thinning can be seen in areas distal to severe white matter hyperintensities (2). Lesions such as acute ischemic infarctions induce white matter degeneration of connecting fiber tracts as well as distal cortical thinning (59). Furthermore, accumulation of smaller lesions such as cortical microinfarcts might contribute to volume loss (1).

Risk factors for brain atrophy include age, hypertension, cardiac disease, diabetes mellitus, kidney disease, smoking and alcohol, as well as neuroimaging features of CSVD (19).

Neurodegenerative disorders are associated with increased rates of atrophy. Hippocampal atrophy occurs in the healthy aging individual but is accelerated in MCI and AD, and a validated and established marker of AD (60). It can also be accelerated in other dementias, such as vascular dementia or frontotemporal dementia, in which parietal lobe atrophy often also is a feature (61). As late as 2019, a new form of dementia has been recognized as a common co-pathology with Alzheimer´s disease, a proteinopathy presenting predominately with hippocampal atrophy. The degree of hippocampal atrophy is typically out of proportion to global burden or clinical representation. Limbic predominant age-related TDP-43 encephalopathy neuropathologic change (LATE-NC) is seen in approximately one third of individuals 85 years or older, and is associated with amnestic cognitive

disorder, especially if the condition is combined with AD pathology in the same individual (62).

The focal patterns of cortical atrophy, such as parietal lobe atrophy or precuneal atrophy, are also highly prevalent, although not exclusive, in MCI and AD patients (63, 64).

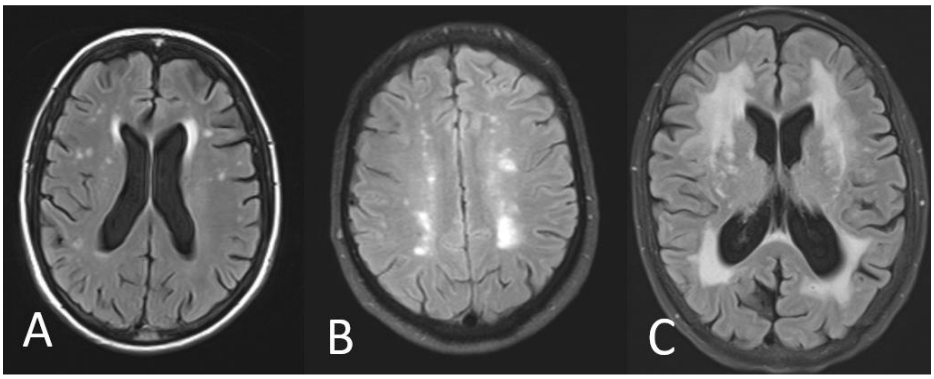


Figure 1. MRI exams of three diffrent patients: Axial FLAIR-images show three different degrees of white matter hyperintensities, classified according to Fazekas grade 1, mild (A), grade 2, moderate (B), and grade 3, confluent (C).

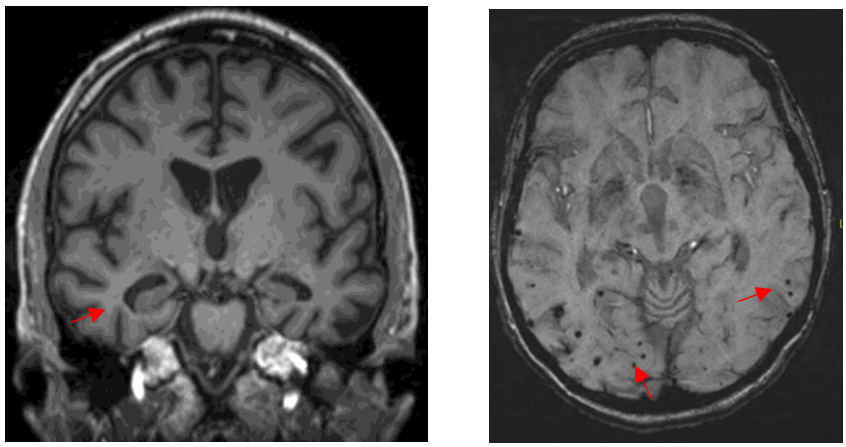


Figure 2: MRI exam of a 62 year old man with Alzheimer´s disease. 3D T1-weighted image showing dilated temporal horn and slight volume reduction of hippocampus (mild medial temporal atrophy) MTA scale 2 according to Sheltens´ scale. **Figure 3:** MRI exam of an 85 year old man with mild cognitive impairment shows multiple microbleeds in the occipital and temporal lobes

Implications of CSVD

Disruption of white matter networks and grey matter atrophy eventually leads to functional impairment in the individual. Associations with cognitive impairment and dementia can be found across the CSVD spectra (2).

With the exception of the acute onset of some lacunar strokes, CSVD is associated with progressive decline in function rather than the abrupt onset of symptoms. Cognitive impairment is a key feature of CSVD, that ranges from subtle changes in executive function and slowing of processing speed, to vascular worsening of neurodegenerative symptoms associated with e.g. Alzheimer's dementia. In CSVD episodic memory is often spared, while effects on working memory are common even early in disease progression (24). WMH volume has been found as the most important predictor for processing speed and executive functions among the CSVD markers (65), while hippocampal volume was the most important predictor for memory. The former might reflect the global effect of disruption of white matter networks that follows white matter impairment.

Depressive symptoms and apathy (loss of motivation and goal-oriented behaviour compared to previous levels) have been associated with strokes but increasingly recognized also in CSVD. Movement disorders, with a typical slowing of walking speed as a result of shortened stride length, as well as impaired balance are other typical symptoms of CSVD. Urinary incontinence is another feature of CSVD (24).

Normal function of supportive structures in the brain

The brain is critically dependent on a constant delivery of oxygen and metabolites for proper function. The metabolic demands of the brain constitutes around 20% of the blood flow when the body is at rest (17). The brain autoregulation keeps blood flow in the brain nearly constant across a wide range of blood pressures. This by a number of metabolic and myogenic controls, mainly on the arteriolar level (17, 33). Local blood flow is determined by the demands of the tissue by *neurovascular coupling*, which is interactions between neurons, astrocytes, pericytes, endothelial cells and smooth muscle cells (24). Potassium (K+) and NO are important neurovascular coupling mediators, but there are others. Some capillaries have pre capillary sphincters of elastin and specific smooth muscle cells (24) that contribute to regulate local blood flow. In addition, pericytes can regulate contractility of capillaries (18).

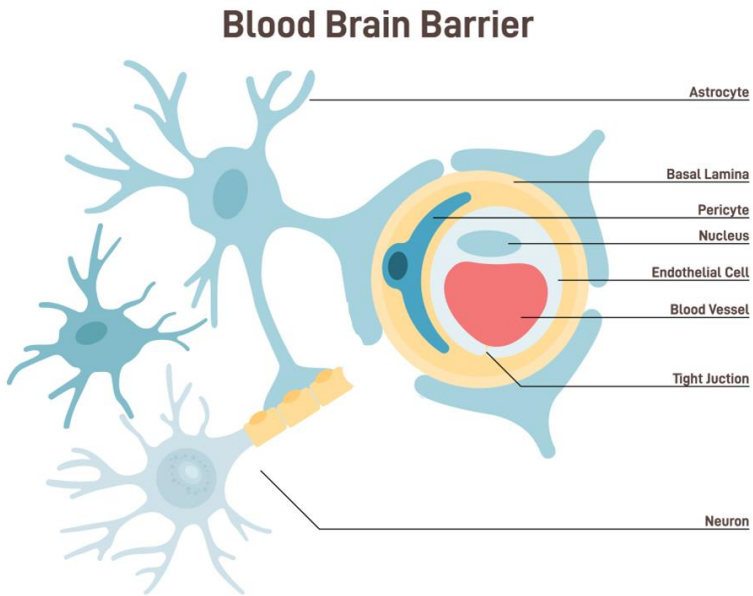


Figure 4. Schematic of the *blood brain barrier* as well as the constituents of the *neurovascular unit*. A single layer of endothelial cells surround the blood vessel lumen, connected by tight junctions. A relatively thick basal lamina encircles the vessel. Mural cells are smooth muscle cells or pericytes depending on location. Feet processes of astrocytes support and anchor blood vessels and neurons, aiding in controlling the microenvironment and communication between cells.

The *endothelium*, the one-cell-thick lining of the blood vessels in the body, plays a critical role in maintaining health and function of the vasculature and the tissue. Functions include control of vascular tone, hemostasis, angiogenesis, control of transport of molecules across the vessel wall, as well as regulation of leukocyte adhesion, thrombosis and antioxidation. The surface of the cerebrovascular endothelium is approximately as large as a tennis court (17). In the brain, the endothelium has an additional role as a vital part of the blood-brain-barrier (BBB) and a leading role in crosstalk with the cells involved in neurovascular coupling. Endothelial cells also play a role in stimulation of neuroglia, such as involvement in oligodendrocyte maturation (24).

The *blood-brain barrier* is a highly selective barrier between the blood and the brain, keeping the milieu of the neurons as constant as possible The BBB consists of four layers: 1) the single cell layer of endothelial cells of the vessels, tightly sealed by *tight junctions*. 2) a relatively thick basal lamina 3) pericytes (the mural cells of the capillaries) or smooth muscle cells (in the artery or venous level) 4) feet processes of the astrocytes, that support the structures and stimulates and regulates the other cell types of the CNS, including the endothelial cells (24, 33).

The *neuroglia* are the supportive cells of the nervous system. They are smaller in size than a neuron, but outnumber neurons by about 10 to 1, and make up about half the mass of the brain. Different glial cells have different functions; there are four in the CNS and two in the PNS. *Oligodendrocytes* form protective myelin sheaths around nerve fibers with their processes to protect them and to increase the speed of transmission. *Astrocytes*, are star-shaped cells with numerous of radiating processes that support neurons, synapses and capillaries, anchoring them in their place. They are a part of the BBB, and also control the microenvironment around neurons, aid and participate in communication between neurons, guide migration of newly forming neurons, and regulate capillary permeability. The *microglia* are the macrophages of the CNS. Microglia can migrate between cells of the CNS, supporting the environment, and can phagocytize microorganisms or cell debris. The *ependymal cells* line the central cavities of the brain and spinal cord. Cilia help circulate cerebrospinal fluid (33).

The *glymphatic system* regulates brain fluid movements and play a critical role in clearing wastes from the brain parenchyma (24). Recently, the important role of p*erivascular spaces* in the transportation of fluid, waste and probably also electrolytes and glucose, has been recognized, but is still debated (24, 66). Perivascular spaces surround arteries and arterioles, but disappear on the capillary level, to reappear on the venous side (66). The system is modulated by the sleep-wake cycle and mainly operates during sleep (66). The accumulation of amyloid-beta in perivascular spaces might impair this function, and is proposed as a mechanism involved in the pathogenesis of AD.

Theories on CSVD pathophysiology

The most consistent risk factors for non-amyloid CSVD appears to be age and hypertension, which have been associated with all manifestations of CSVD. Other risk factors include diabetes, hyperlipidaemia, obesity, inflammation, smoking, and low education level, but results are inconsistent between studies and between CSVD manifestations.

The visible vascular changes described in previous sections (thickening and reorganisation of vascular walls, SMC loss, hyaline deposits, parenchymal oedema, whitematter rarefaction) are signs of severe vessel dysfunction, but what initiates the changes, and how this leads to tissue damage is not fully understood. Mechanistically, endothelial dysfunction leading to impaired BBB integrity and impaired vasodilation seems to be cornerstones in pathophysiology, alongside impaired autoregulation on the arteriolar level, and impaired function of neuroglia and perivascular fluid drainage on the parenchymal level. Recently the contribution of chronic, low-grade inflammation as a contributing factor in pathogenesis has attracted attention (67). Described mechanisms are visualized in Figure 5.

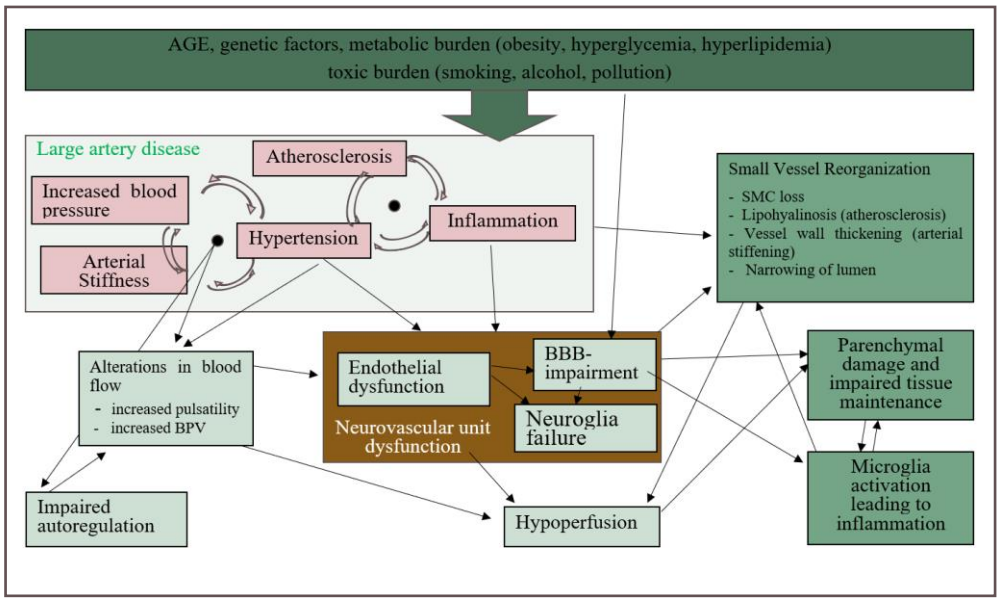


Figure 5: Visualization of described vascular mechanisms involved in development of CSVD.

Endothelial dysfunction results in a number of adverse effects in the cerebral tissue. An important effect is dysregulation of cerebral blood flow. Impaired neurovascular coupling leads to reduced vascular reactivity and failure to dilate vessels in response to tissue demands (24). Compromised cerebral blood flow is a hallmark in CSVD as well as in dementia. Both hypoperfusion per se and BBB impairment have been suggested as causes of CSVD. Recently, a study showed that normal appearing white matter with reduced CBF and increased mean diffusivity became abnormal at follow up. Both measures were independently associated with WMH growth, but mean diffusivity was the more sensitive predictor (68). However, causal pathways are not elucidated, and reduced CBF has been seen both preceding and following cerebral lesions (2), and impaired BBB can not always be shown in conjunction with WMH (69).

The BBB becomes more permeable by advancing age (17), but this process can be accelerated in a number of conditions, including several inflammatory states and brain injury. It is also seen in the concept of CSVD and cognitive decline (69). The exact mechanism leading to BBB-impairment is not known, and several different mechanisms have been found. Microscopically, the endothelial cells seem to be unaffected in endothelial dysfunction, and cellular loss is not seen even in severe cases of CSVD (69). However, the tight junctions between the endothelial cells are believed to be affected in, leading to increased extravasation and an uncontrolled passage of molecules between the endothelial cells. Furthermore, recent animal

studies have shown increased transcytosis by effects of dysregulated proteins, and pericyte loss has been shown following chronic hypoperfusion (69).

Impaired function of the BBB leads to leakage of electrolytes, fluids and proteins into the perivascular tissues. This might by the underlying mechanism involved in the increased interstitial fluid seen in normal appearing white matter in individuals with CSVD (69). Leakage of blood constituents into the interstitium is believed to have a toxic effect on both neurons and neuroglia, as well as inhibit maintenance and repair (69). Furthermore, the foreign substances can activate microglia, which in turn can recruit peripheral macrophages and promote inflammation (2).

Hypertension is associated with increased levels of circulating inflammatory markers such as CRP and IL-6, as well as markers for specific vascular inflammation such as von Willebrand factor and homocysteine (18, 69). This is believed to contribute to vascular remodeling and might negatively affect endothelial function and BBB integrity. Increased levels of these proteins have also been found related to CSVD pathologies (18). The relative contribution of central versus peripheral inflammation on CSVD pathology is still under investigation, but evidence exists for the contribution of both (18). Key inflammatory proteins released by activated microglia are interleukin-1beta (IL-1beta), TNF-alpha and IL-6. These, in turn, activate other microglia, astrocytes and endothelial cells (18). Activated endothelial cells upregulates the expression of adhesion molecules, such as intracellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1), recruiting peripheral immune cells (18).

Dilated perivascular spaces are visible on neuroimaging as well as in histological samples and are included as a manifestation of CSVD. They are proposed to be connected to impaired clearance of fluid and waste products from around the vessels and adjacent interstitium (17).

Alternative causes of CSVD

Sporadic CSVD is mainly associated with vascular risk factors, and the vascular/hypertensive contribution to CSVD is the main focus of our work. However, alternative explanations of CSVD exists, although beyond the scope of this thesis. Briefly, two commonly explored implications to CSVD include:

Cerebral amyloid angiopathy

CAA is the buildup of protein aggregates consisting of amyloid-beta (Aβ) in cerebral vessels, primarily in cortical and leptomeningeal locations. With the exception of some forms of CAA that are hereditary, CAA seems to be sporadic and associated with age (70). Other risk factors include male sex, uncontrolled hypertension, diabetes and brain injury (71). Prevalence is estimated to 10-40% in

older adults, 48-80% in individuals with AD, and 57% in those with lobar intra cerebral haemorrhage (70, 72). CAA is associated with cognitive decline, especially in individuals with AD (71). It is also associated with increased risk of haemorrhagic stroke and cerebral microbleeds (primarily lobar (52)). Definite diagnosis is postmortem, but the Boston-criteria (73, 74) aids in neuroimaging diagnosis.

Hereditary forms of CSVD

Hereditary small vessel diseases represent a small but research-wise significant subset of CSVD. The most well-characterised of these is cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). CADASIL is caused by mutations in the *NOTCH3* gene, leading to the loss of smooth muscle cells and the buildup of NOTCH3 protein in blood vessels (75). Monogenic causes of CSVD are estimated to account for about 2% of cases, and CADASIL stands for about 80% of these. 15 monogenic causes of CSVD have been identified, including CARASIL, a recessive condition caused by mutations in *HTRA1* (76).

Other causes

Other causes of CSVD include infections and autoimmune disorders, radiation damage, environmental or toxic damage such as air pollution, smoking or excessive alcohol consumption and more.

Arterial stiffness and vessel dynamics

- A man is as old as his arteries

Thomas Sydenham (1624-1689)

The concept of vascular aging affecting tissues and organs is not new; the blood vessels have never been viewed as passive tubes through which the blood simply passes. The important influence of the blood flow for health has been recognized already by the ancient Chinese 2000 years BC (77). In the 17th century the British physician Thomas Sydenham stated the famous quote expressed above (78). Brachial-femoral pulse-wave velocity was described in a scientific paper already 1922. With the invention of the cuff sphygmomanometer in the early 20th century, the value of brachial BP was soon recognized, and the measurement was included in routine medical examination (79).

Determinants of blood flow include fluid physics, vessel dynamics, autonomic regulation from the nervous system, hormonal regulation from the kidneys and the adrenal glands, heartbeat properties, tissue demands and more. The large arteries are more elastic than the more peripheral muscular arteries, a change that occurs gradually. The central arteries, primarily the aorta, have an important cushioning effect on the pulsatile waves of blood from the beating heart, delivering a nearly steady flow in the microvasculature of the tissue. This is called the *Windkessel effect*, and in a young individual the aorta dilates around 10% with each heartbeat. As we age, the dynamic properties of the larger vessels decrease, resulting in a stiffer vessel with less cushioning of the pulsations. As the vessel stiffens and less of the motion energy is cushioned by the artery, the pulse wave travels faster through the artery, and at higher pressure. The pulse wave is reflected backwards as it encounters vessels with a lesser diameter or of lesser elasticity, a bifurcation or other disruptive elements. This is part of the natural vessel dynamics; it protects the more distal arteries and microvasculature from the strain of high pressure and pulsatile motion, while the returning wave reflection helps in maintaining an adequate blood pressure also in diastole, which is important for cardiac perfusion (33, 80, 81).

However, with stiffer vessels and a faster travelling pulse wave, the reflection is smaller and returns earlier; instead augmenting the wave while still in systole (81). This results in a greater workload (afterload) for the heart, and in the absence of the augmented pulse wave as well as the lesser blood volume from the ”recoil” of the greater vessel, a decrease in diastolic blood pressure. Large scale longitudinal studies such as the Framingham Heart Study (82) have shown that systolic BP increases until the eighth or ninth decade, but diastolic BP peaks at age 50-60 and

then, attributed to arterial stiffness, begins to decrease. This is the reason for the isolated systolic hypertension often seen in older individuals.

Measuring arterial stiffness

Pulse wave velocity (PWV), has been recognized as the gold standard of large artery arterial stiffness (83). For practical purposes, the PWV is most often measured as carotid-femoral (cf-PWV). The clinical value of cf-PWV has been recognized by the European Society of Hypertension and the European Society of Cardiology, which recommend its use in guidelines (84, 85). The difference between systolic BP and diastolic BP, known as pulse pressure (PP), is a noninvasive proxy of arterial stiffening. However, this measure is more reliable in the older population, and not a recommended method in younger patients (83). In younger individuals, the blood pressure in the elastic aorta, central BP, is lower than that of the muscular, more peripheral arteries, such as the brachial BP. As the central arteries stiffen, this difference is reduced. Central BP is also a proxy for arterial stiffness.

Mechanisms and implications of arterial stiffness

The elastic properties of the aorta and the greater vessels are due to the particular organization of the extra cellular matrix of the *tunica media*, the middle layer of the three layers that constitutes the vessel wall. The main constituents of the tunica media are smooth muscle cells (SMC), load-bearing collagen fibers, and elastic fibers, composed of elastin. The increased stiffness of the arteries has been attributed to in part a mere mechanical effect of an increased mean arterial pressure (MAP), stretching the arterial wall and redistributing the load from the elastic elastin fibers to the stiffer collagen (86-88). This explains most (but not all) of the reduction in PWV seen in antihypertensive treatment. However, reorganization of the vessel wall is also a key factor in arterial stiffening, with thickening of the tunica media, widening of the vessel lumen, the loss of elastin, and the increase of collagen and SMCs (89).

The two strongest determinants of the progression of arterial stiffening are BP and age (90, 91). Additionally, dysglycemia, insulin resistance, male sex, waist circumference in women and genetic predisposition have been identified as risk factors. Environmental aspects also seem to have a great effect on arterial stiffening on a group level, as well as markers of inflammation (79, 89). However classical risk factors other than age and hypertension have shown variable associations (91).

PWV is strongly associated with cardiovascular (86) and cerebrovascular events (89). PWV also appears to be a powerful prognostic tool, as it improves the risk prediction of cardiovascular events beyond that achieved by conventional risk scores such as the Framingham Risk Score or SCORE system. This seems to be especially true in younger individuals (92), or in individuals of lower risk according to SCORE (93).

Blood pressure

Blood pressure (BP) reflects the pressure exerted on the vessel wall by the blood inside it. It is typically measured in millimeters of mercury (mmHg) by a cuff sphygmometer on the brachial artery, but can be assessed at other sites, however the values are not interchangeable. In young healthy individuals, the pressure is lower centrally and increases further down the arterial tree because of wave amplification and less elastic vessels. Regulation of blood pressure is a complicated interplay involving several key mechanisms, with the main purpose of maintaining perfusion to meet tissue demands. Determinant factors include cardiac output, systemic vascular resistance, blood volume and arterial compliance. The autonomic nervous system, the renin angiotensin aldosterone system (RAAS), and local factors such as nitric oxide modulate these parameters to maintain homeostasis (33).

Endothelial dysfunction has been established another key player in vascular aging, strongly linked to hypertension as well as arterial stiffness (94), although as with other vascular manifestations, temporal associations are not fully known. Endothelial cells have numerous important functions, including regulation of thrombosis, inflammation and vascular tone and remodelling. Endothelial dysfunction leads to increased peripheral resistance, which in turn contributes to increased blood pressure, while increased blood pressure is regarded a driver of endothelial dysfunction. In the same manner, endothelial dysfunction is associated to vessel remodelling and arterial stiffening (94).

Hypertension

Hypertension is defined as a pathologically increased resting blood pressure and is referred to by the WHO as “the silent killer”, affecting over one billion people worldwide (95). Hypertension is an important risk factor for most of the non-communicable diseases, including cardiovascular disease, heart failure, cerebrovascular disease, kidney disease and premature death. More importantly, the reduction of BP has a positive effect on these outcomes (96). However, data is inconclusive regarding the effectiveness of a strong BP lowering regime in the very old, or in patients with CKD or dementia (96, 97).

Risk factors for primary/essential HT include age, genetics, obesity, physical inactivity, salt and alcohol intake, diabetes and kidney disease. Mechanisms behind HT are believed to be multifactorial, including increased peripheral resistance because of reduced vascular reactivity, vasoconstriction and vessel remodeling, RAAS- and Na/volume dysregulation by the kidneys, altered sympathetic and parasympathetic tone, and large artery stiffness (98). Men have higher blood pressure than women in younger years, while women have higher blood pressure and a higher prevalence of hypertension in older years (96). Systolic blood pressure increases throughout life, while diastolic blood pressure can decrease during aging

(82). In adults 35 years or older, SBP is a more important risk factor for CVD than DBP (96).

Carotid Ultrasonography

Carotid Ultrasound (US) is a widely used, non-invasive imaging technique that provides information on vessel morphology and blood flow dynamics. In brief, acoustic energy transmits through tissues in various degree. The reflection of the soundwave can be detected by the probe and the interference pattern displayed as an image. By Doppler technology, the moving blood causes a change in "pitch" in the sound wave (the doppler effect), and this "echo" of the moving blood cells can be transformed into flow velocities (99, 100).

Anatomically, the carotid artery arises from the aorta on the left side, and the right brachiocephalic artery (that arises from the aorta) on the right side. The CCA bifurcates into the external carotid artery (ECA) and the internal carotid artery (ICA). The ICA enters the skull through the carotid canal, and branches into the anterior cerebral artery (ACA) and the middle cerebral artery (MCA), as well as several other arteries.

The brain, like the kidneys, is dependent on a high blood flow to ensure tissue perfusion, hence the vascular resistance is low while systolic, and especially diastolic flow velocities are relatively high (99). In spectral analysis, the difference in resistance between ICA and ECA gives rise to different wave form images (more rapid drop in PSV and lower or diminished EDV in the higher resistance artery, ECA (101)), which can be used to differentiate between the two. Approximately 80% of the blood that passes through the CCA proceeds to the ICA, therefore the waveform of the CCA resembles that of a low resistance artery.

Determinants of flow velocities

Apart from the downstream vascular resistance and the upstream incoming pulsatile force, a few other variables should be considered when assessing carotid flow parameters. Blood pressure influences carotid flow, but compensatory mechanisms and vessel dynamics alter the influence. In physiological terms, blood flow (Q) through a vessel is determined by the pressure gradient (ΔP) divided by vascular resistance (R):

$$Q = \frac{\Delta \mathrm{P}}{R}$$

However, Doppler ultrasound measures flow velocity (V), rather than total volumetric flow. Velocity is determined by the volumetric flow (Q) divided by the area of the lumen (A):

$$V = \frac{Q}{A}$$

This implies that, assuming constant resistance and vessel diameter, an increase in blood pressure would elevate flow velocity. However, in the vascular system at large and particularly in the cerebral circulation, autoregulatory mechanisms compensate for fluctuations in blood pressure in order to maintain a stable cerebral perfusion across a range of systemic blood pressures (typically between 60 and 120 mmHg in mean arterial pressure). In young individuals, an increase in blood pressure would not necessarily affect flow velocity. However, in the aging individual the autoregulatory capacity may be diminished, and blood pressure might have a greater effect on flow velocity than anticipated. Moreover, in stenotic lesions, flow velocity is strongly influenced by the degree of luminal narrowing (again, fluid mechanics: to maintain flow when the lumen narrows, velocity is increased.) A peak systolic flow velocity of 120 cm/s indicates a moderate stenosis of about 50% (102).

Measures of vascular resistance

Resistivity index can be calculated from the Pourcelot formula as follows:

$$RI = \frac{PSV - EDV}{PVS}$$

Resistivity is a measure of peripheral vascular resistance. In a low resistance system such as the brain, the EDV decreases less in diastole, and therefore the relative reduction in flow velocity to peak flow is small, resulting in a low RI.

Goslin´s index calculates the pulsatility index (PI) as follows:

$$PI = \frac{PSV - EDV}{V(mean)}$$

PI reflects both resistance and pulsatility, accounting for more of the wave form by incorporating mean velocity instead of peak flow, making it more sensitive to overall compliance of the peripheral vessel and upstream pulsatile force, as well as downstream resistance.

Increased PI is seen in age and hypertension, probably reflecting both upstream and downstream vascular changes (103). CSVD-related changes in the microvasculature, such as lipohyalinosis or micro atherosclerosis, narrows the lumen, which increases the resistance. Conditions like impaired autoregulation and impaired endothelial function controlling microvascular dilation would also increase the resistance. Increased PI in MCA has been associated with MRI markers of CSVD (103) and cognitive impairment (104).

The kidneys – the gatekeepers of hemodynamics

The kidneys are paired organs with many important functions, one of which is filtering the blood through a multitude of small glomeruli, excreting water-soluble waste as urine. The kidneys are also involved in blood volume- and blood pressure regulation, electrolyte- and water homeostasis, activation of vitamin D, stimulation of red blood cell production via the hormone erythropoietin and regulation of acid-base homeostasis (33). In adults, they receive about 20-25% of the cardiac output, equivalent to 1000-12000 ml of blood per minute, and approximately one fifth of this is filtered through the glomeruli (105).

Kidney function is measured by the glomerular filtration rate (GFR), which is an assessment of the volume of blood filtered by the kidneys per minute. This can be measured in an absolute manner by assessing the clearance of an exogenous substance from the blood, such as iohexol or inulin. But for the most part, an estimation of GFR is sufficient, and this is ascertained by measuring the concentration of endogenous substances such as creatinine (crea) or cystatin C (cysC). Chronic kidney disease (CKD) is defined as eGFR<60 ml/min per 1.73 m^2 or albuminuria (urine albumin-to-creatinine ratio >30 mg/g) persisting for 3 months or more (KDIGO12).

Kidney function declines naturally with age due to loss of nephrons, the functional unit of the kidney containing the glomeruli (106). CKD is estimated to affect more than 10% of the population worldwide (107), being more prevalent with advancing age. In the western world, the most common causes of CKD are hypertension and diabetes (108). However, a multitude of non-CVD-related causes can also lead to CKD; infections, autoimmune disorders, nephrotoxic substances such as certain pharmaceuticals, severe urine-retention and more. CKD is initially an asymptomatic disease, often diagnosed en passant by laboratory testing or urine analysis. As CKD progresses, symptoms can be fatigue, itching, hypertension, anemia or edema (KDIGO12). Decline in kidney function leads to accumulation of toxins and metabolites, as well as a dysregulation of blood pressure and overall homeostasis of the body.

CKD is an independent risk factor for cardiovascular disease and cognitive dysfunction (109). Like the brain, the kidneys are high blood-flow, low vascular resistance organs, susceptible to hemodynamic changes associated with arterial stiffness. The association between hypertension, arterial stiffness and CKD has been shown repeatedly and bi-directionally (110).

Proteins as risk factors & risk markers

The study of CSVD began macroscopically long ago with the observation of clinical symptoms in patients, later followed by microscopic examination of the tissue by histologic samples and culminating in imagery as the cornerstone of CSVD definition and research. However, proteomic research allows organ- and tissue-level understanding of CSVD pathophysiology to be complemented by insights into cellular-level mechanisms.

The proteome is the total protein content of an organism. In proteomics a large portion of these proteins are assessed simultaneously. The study of proteins and their functions is highly complex. Proteins exist in many modified forms, and can be activated, inactivated, cleaved or bound, act locally or systemically. Despite the greater complexity of the proteome in comparison to the genome, like the Human Genome Project that was completed in 2004 (111, 112), there are attempts to identify and classify all human proteins. Examples of this is The Human Proteome Project (HPP) that was initiated in 2009 by the Human Proteome Organization (HUPO), aimed at integrating results from many research groups with the daunting task of identifying the entire human protein set (113, 114). Another example is the Swedish initiative The Human Protein atlas initiated in 2003 (115), that, among other techniques, uses antibody-based profiling of tissue microarrays by immunohistochemistry to categorize proteins based on tissue representation.

It is estimated that our ~20,000 protein coding genes, can give rise to an estimated one million different protein isoforms after alternative slicing of primary transcripts, DNA-recombination, and numerous post-translational modifications (113, 114). By example, multiple splice variants with different protein sequences are encoded by about 72% of the genes (115). As of 2024, HPP estimates that proteins corresponding to 93% of the ~20,000 protein coding genes have been detected by mass spectrometry (MS), antibody-based methods or other proteomic techniques (114).

Despite the diversity of the approximately ~230 distinct cell types that make up the human body (113), research reveals a surprising commonality beneath the surface: nearly half of our genes are expressed across all analyzed tissues (115). And on that note, around 30% of the approximately 600 proteins targeted by clinically approved drugs are also expressed in all analyzed tissue (115). Many proteins have a vast array of functions throughout the body; fine-tuned for each specialized purpose by post-translational modifications, receptor availability, downstream interactions or other modifiers. This prompts for a humble approach in our attempts to draw conclusions about biological effects of proteomic or drug target research.

The challenge is to create real biological understanding of the vast amount of available data, where protein-protein interactions is becoming a new research interest. Bioinformatics and the processing of large amounts of data have been

provided by a number of different databases. By example, the STRING (116) database, among others, is commonly used for protein interactions, and links to various other databases for literature mining.

Although mass spectrometry is still a leading method for large scale protein *discovery*, a number of alternative proteomic approaches has emerged during the 21st century that are designed for clinical validation of the discovered proteins. Proximity Extension Immuno-Assay (PEA) is a dual-recognition antibody based method, that has proven high sensitivity, specificity and reproducibility (117). Compared to other immuno-assays, problems with antibody-cross reactivity, which exponentially increases with higher degree of multiplexing, are minimized due to the dual-recognition system and subsequent proximity induced extension of base-pairs (118). PEA is visualized in Figure 6.

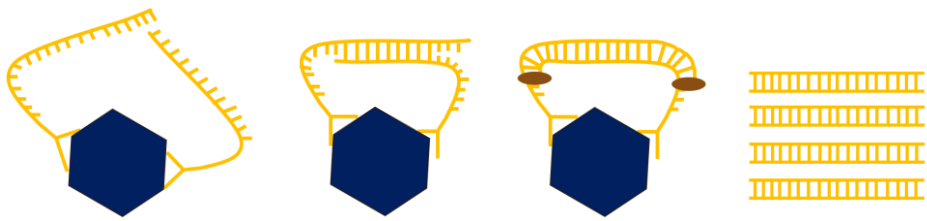


Figure 6. In Proximity Extension Immuno-Assay two different antibodies recognize each protein. As their unique tales of DNA-oligonucleotides comes into proximity of each other, they can hybridise and are then extended by DNA-polymerase, creating a unique DNA-barcode that can be amplified by polymerase chain reaction (PCR) and subsequently quantified.

By coarse classification, proteins can be divided into intra-cellular (or soluble), membrane-bound, or secreted. Though many genes have splice variants that allow alternative locations (115). Examples of membrane bound proteins include ion channels, molecular transporters, enzymes, receptors, anchors for other proteins or proteins associated with the Golgi apparatus or mitochondrial membranes. Examples of important secreted proteins "the secretome" include cytokines like interleukins, interferons or chemokines, coagulation factors, growth factors, hormones, and vesicular proteins like proteases.

By proteomic studies, we can try to understand more about the cellular interactions leading up to the visible changes we observe with neuroimaging. With further understanding, we can search for biomarkers or targetable proteins that can help us predict, or prevent, the risk of CSVD in presymptomatic individuals.

Aging, arteries, and atrophy

This thesis explores the possible connection between a multitude of systemic manifestations and CSVD. Four separate lines of investigation were chosen – hypertension, carotid artery hemodynamics, kidney function, and circulating proteomic markers – which all represent interrelated yet distinct aspects of vascular aging. They are all, just like CSVD, associated with extremely severe outcomes, but in the process of aging the difference between health and pathology is not always clear.

Using data from the large, population-based study Good Aging in Skåne with detailed clinical, vascular and neuroimaging data, the overarching aim of this thesis is to provide a more integrated understanding of the factors contributing to CSVD. This thesis hopes to contribute to the understanding of how systemic processes converge on the cerebral microvasculature to drive the structural brain changes that underlie cognitive and functional decline. By expanding our knowledge of the mechanisms underlying CSVD we might find better ways to predict and prevent cerebral aging.

We wonder: is small vessel disease of the brain connected to large artery aging of the body?

Aims

The overall aim of this thesis is to gain a better understanding regarding cerebral aging in the older general population by investigating the prevalence of MRI markers of CSVD and atrophy. Additionally, the aim is to explore the association between MRI-markers of cerebral aging with signs of hemodynamic changes and hypertensive-related organ dysfunction, as well as circulating biomarkers of cardiovascular disease.

Paper I

The aim of this paper was to investigate the prevalence of CMB in the general population, and the association between hypertension and CMB, with a particular interest in the spatial distribution of CMB.

Paper II

The aim of this paper was to investigate the prevalence and inter-relations between MRI markers of CSVD and brain atrophy. Additionally, the aim was to investigate the association between carotid artery flow parameters and the MRI lesions.

Paper III

The aim of this paper was to investigate the association between chronic kidney disease and MRI markers of CSVD, with special reference to hypertension status.

Paper IV

The aim of this paper was to explore circulating biomarkers previously associated with cardiovascular disease or inflammation, and their association with MRI markers of CSVD and brain atrophy.

Methods

GÅS – Good Aging in Skåne Study

The Good Aging in Skåne (GÅS) study is one of four participating sites in the longitudinal multi-purpose population study Swedish National Study on Aging and Care (SNAC) (4, 119). The other included sites are SNAC-Blekinge, SNAC-Nordanstig in Gävleborg, and SNAC-Kungsholmen in Stockholm. The SNAC study was initiated by the Swedish government with the aim of collecting data from a large, representative panel of older adults during a long time (30 years or more). The purpose was to enhance the knowledge of medical and psychosocial aspects of aging, study risk factors, prevalence and progression of chronic diseases in the older population, collect data on all health-care utilization in the included areas, and attempt to predict future health and healthcare needs of the older population.

The GÅS study was initiated in 2001 and is still ongoing. Participants were randomized from the National Municipality Register in five municipalities in Skåne County, located in the southern part of Sweden. Included municipalities were Malmö, Eslöv, Ystad, Hässleholm and Osby; representing both rural and urban areas. Men and women from nine age cohorts (60, 66, 72, 78, 81, 84, 87, 90, and 93 years) were invited for participation in Baseline examination during 2001-2004. Participants were re-invited for follow-up visits every 6th year if they were younger than 81 years, and every 3rd year if they were 81 years or older. Additional cohorts of 61-year-olds and 81-year-olds have been recruited every 6th year to keep the number of participants at a high level. Recruitment for additional waves occurred during 2006-2012 (Wave 2), 2012-2016 (Wave 3) and 2017-2022 (Wave 4). Wave 5 was initiated in 2024 and is still ongoing (Figure 7).

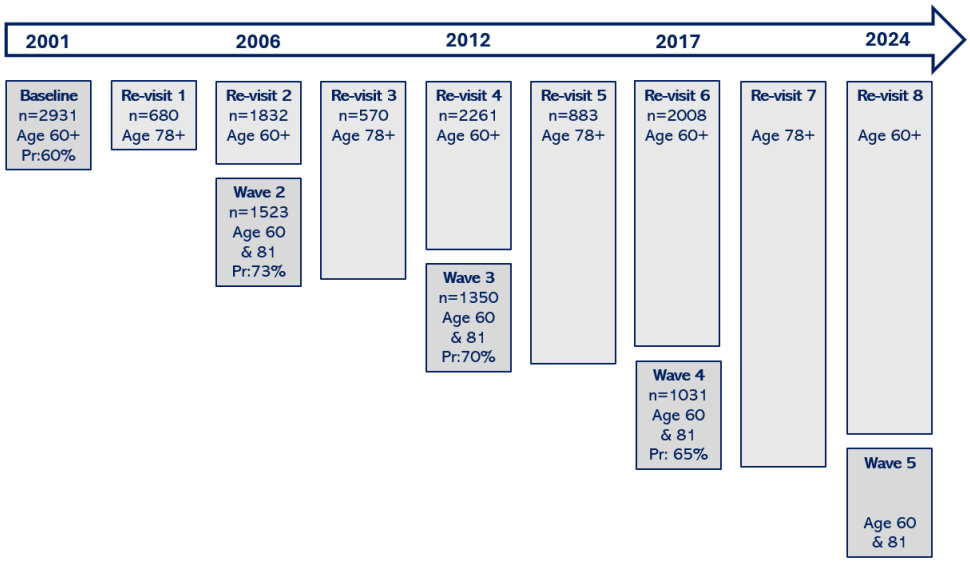


Figure 7. GÅS study design, displaying number of participants and age cohorts. Participation rate (Pr) defined as n (participants)/n(eligible) is displayed for each of the waves.

Participants were invited by letter, and non-responders were contacted again by letter and/or telephone. The only exclusion criteria was inability to speak and understand the Swedish language in absence of a friend or relative that could interpret. Having dementia or living in a nursing home was not an exclusion-criteria. Home-visits were offered to those unable to attend the research centre in attempts to increase participation-rate of older or more fragile subjects.

Data was collected by research staff including a registered nurse, physician, test leader, and medical secretary. Examinations normally took place during a full day at one of the study centres but could be split into two occasions. Participants were examined with a comprehensive clinical examination (including electrocardiogram, cardiopulmonary examination, blood pressure measurements, and neurological examination), assessment of medical records, assessment of current and former medication, anthropometric measurements, blood laboratory sampling for direct analyzation and to be saved in biobank, spirometry, functional tests, a battery of cognitive tests, and self-reporting questionnaires.

A sub-study was deployed starting 2013, where all GÅS-participants aged 70 - 87 from the Malmö cohort were invited for a comprehensive examination of flow dynamics in the greater vessels. 647 subjects were examined in the “vascular physiology” sub-study. All examinations were performed by the same biomedical analyst and included central and brachial blood pressure; Doppler ultrasonography of aortic artery, carotid artery, and middle cerebral artery; heartbeat variability by

finapress Finometer; 24-hour blood-pressure measurements and more. Plasma from the biobank was harvested and examined by multiplex immuno-assay proteomic analysis by Olink Proteomics, Uppsala. Analysis described in further detail below.

Participants that underwent the "vascular physiology" examination were invited for 3T MRI of the brain between the years 2016 and 2018. In all, 408 subjects accepted the invitation. Two MRI-examinations were non-completed and have missing data. Inclusion criteria in both sub-studies were being able to independently come to the study centre or MRI examination and endure the examination, excluding individuals with pronounced physical illnesses, movement difficulties or dementia (Figure 8).

Subjects that had undergone the MRI-brain investigation were subsequently invited for a follow-up scan during 2022-2023, of which 241 individuals attended.

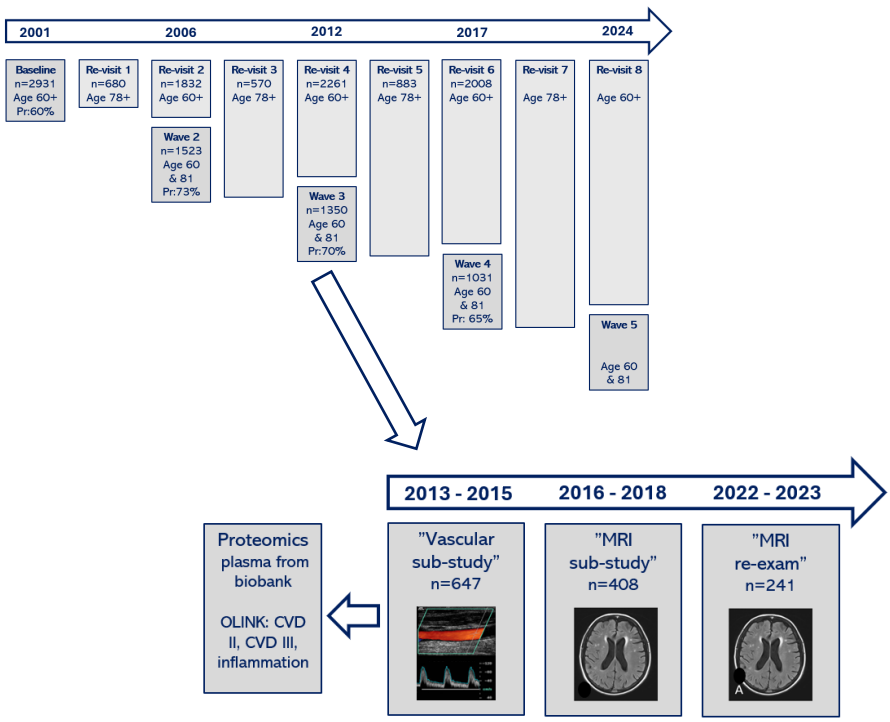


Figure 8. GÅS sub-studies initated in 2013.

Study samples

Paper I

Although all MR-images were completed by March 2018, the image processing, a time-consuming manual process performed systematically by a neuroradiologist, were not completed for all individuals for the first paper. Subjects were included in the first paper without a predetermined selection criteria or processing order, however comparison of demographic data and CSVD prevalence reveals no major difference between the cohorts. Consequently, for *Paper I*, 344 GÅS participants from the Malmö cohort, who by March 2018 had completed the "vascular physiology" sub study as well as MRI examination and MR-image processing were included in the study.

Paper II

A total of 406 subjects with carotid Doppler ultrasound data from the "vascular physiology" sub-study completed the MRI examination. Subjects with peak systolic velocity (PSV) ≥ 120 cm /s in any location (indicating stenosis >50%) were excluded from analysis, in all 15 subjects. Consequently, for *Paper II*, 391 GÅS participants from the Malmö cohort - with data from "vascular physiology"- and MRI sub studies, and free of stenosis in the carotid arteries - were included in the study.

Paper III

406 subjects had eligible data from the GÅS MRI sub-study. 17 subjects had missing data regarding kidney function from the main study follow up and were excluded from analysis. Consequently, for *Paper III*, 390 GÅS participants from the Malmö cohort - with available MRI data and blood samples regarding kidney function - were included in the study.

Paper IV

406 subjects had eligible data from the GÅS MRI sub-study. Multiplex proteomic analysis of plasma from the biobank had been performed on all individuals in the "vascular physiology" sub study with samples available in the biobank. In the MRI sub-study 5 individuals had missing data and were excluded from analysis. Consequently, for *Paper IV*, 401 GÅS participants from the Malmö cohort - with available MRI- and proteomic data - were included in the study.

Variables and data collection

Magnetic resonance image acquisition and processing

MRI examination

MRI examination of the brain was performed 2016-2018 in the Radiology Department at Skåne University Hospital in Malmö, using a 3 Tesla MRI (General Electric discovery MR 750w).

Examination included:

- Sagittal T1-weighted 0.9-mm isotropic 3D fast spoiled gradient echo (3D-FSPGR) images were performed and reconstructed in the axial and coronal planes
- Axial T2-weighted fluid-attenuated inversion recovery (T2-FLAIR)
- Axial diffusion weighted images (DWI)
- Axial susceptibility-weighted angiography (SWAN), acquired at two settings: 3-mm-thick images and 5-mm-thick phase images in order to differentiate blood from calcifications.

MR image assessment

MR-images were systematically assessed by the same experienced neuroradiologist regarding:

- **White matter hyperintensities (WMH/WMC)** as assessed on T2-FLAIR sequence, using the Fazekas scale (29) ranging as follows: absent (0); mild/punctate (1); beginning confluence (2); severe/confluent (3).
- **Pontine white matter hyperintensities (WMP)** as assessed on T2-FLAIR sequence, by visual examination by the neuroradiologist.
- **Lacunar infarcts (LAC)** as assessed on T2-FLAIR sequence, defined as one or more small infarction (<1.0cm) in the deep white matter, the basal ganglia or pons (31).
- **Cerebral microbleeds (CMB/MB)** as assessed with thin slices on the SWAN sequence defined as hypointense lesions (0.2-0.5 cm).
- **Global cortical atrophy (GCA)** as assessed with the FSPGR-sequence evaluating sulcal and ventricular dilation in 13 brain regions using the Pasquier scale (120), ranging as follows: absent (0); mild (1); moderate (2); severe (3).

- **Medial temporal lobe atrophy (MTA)** as assessed on the coronal T1 weighted sequence, evaluating a) width of the choroid fissure, b) width of the temporal horn of the lateral ventricle, c) hippocampal height. and scored according to the Scheltens' scale (121) ranging as follows: absent (0); mild (1); moderate (2); severe (3); pronounced (4).
- **Parietal lobe atrophy (PAR/KPA)** as assessed on FSPGR-sequence on sagittal, coronal and axial planes using the Koedam score /posterior atrophy score (122) ranging as follows: absent (0); mild (1); moderate (2); severe (3).
- **Precuneus atrophy (PREC/PA)** as assessed with the FSPGR-sequence.
- **Central atrophy (CA)** as assessed with the FSPGR-sequence.
- **Specific atrophies,** including frontal cortical, temporal cortical, frontotemporal, cerebellar, and midbrain atrophy.

Nomenclature

While stringency in nomenclature is ideal, it is not always feasible. Expressions, abbreviations and compound variables used to describe the MRI variables have varied slightly between the papers, as they were selected based on the specific research questions of each study, rather than with the overall aim of the dissertation in mind.

- White matter hyperintensities, WMH, are called white matter changes, WMC, in *Paper II*. The two names are used interchangeably in the literature (although WMC might be viewed as a somewhat broader term encompassing all changes, not only hyperintensities). However, WMH is the term used in the STRIVE-criteria, and the term that has been used in the *papers I, III* and *IV*, and in this thesis.
- Cerebral microbleeds, CMB, are called microbleeds, MB, in *Paper II*. However, CMB is the term defined in the STRIVE-criteria, and the term that has been used in the *papers I, III* and *IV*, as well as in this thesis.
- In *Paper II,* parietal atrophy was abbreviated KPA and precuneal atrophy was abbreviated PA. However, in the literature, the more commonly described parietal atrophy is oftentimes abbreviated PA, which might open for confusion. In *Paper IV* and in this thesis, parietal atrophy is abbreviated PAR and precuneal atrophy is abbreviated PREC to avoid misunderstanding.
- In *Paper III* we explored two different definitions of CSVD; the SVD score presented by Staals et al in 2014 (123) and a variable based on the definitions of CSVD that were encompassed by the STRIVE-criteria presented by Wardlaw et al in 2013 (19). We used modified versions of the

definitions since we lack assessment of "perivascular spaces" in our material. However, in *Paper IV*, it was not of interest to assess CSVD widely but rather to keep the number of variables to a minimum. The variable "modified STRIVE" from *Paper III* was chosen to represent a composite CSVD-variable. Since it was not entirely according to the STRIVE-criteria, it was renamed to "CSVD-score". Consequently, the variable "modified STRIVE" in *Paper III* and "CSVD score" in *Paper IV* are in fact the same variable.

Variable definitions

- **WMH** (WMC in *Paper II*): mild white matter hyperintensities was considered a normal finding in this elderly cohort aged 70+. Therefore, upon dichotomization, Fazekas grade 0-1 was defined as normal/un-pathological, while Fazekas grade 2-3 was considered pathological in all four papers.
- **MTA** mild media temporal lobe atrophy was considered a normal finding in this elderly cohort aged 70+. Therefore, upon dichotomization, Scheltens' scale 0-1 was defined as normal/un-pathological, while Scheltens' scale 2-3 was considered pathological. This variable was used in *papers I, II* and *IV*.
- **CMB** (MB in *Paper II*): When dichotomized, one or more CMB was recorded as "yes". CMBs were further divided into "strictly lobar" and "deep/mixed" depending on location in *Paper I*.
- **Number of MRI pathologies** was calculated as the sum of how many different types of MRI-pathologies (WMH, CMB, LAC, WMP, KPA/PAR, PA/PREC, MTA, CA or GCA) were found. (Mild WMH categorized as Fazekas grade 1, and mild MTA categorized as Scheltens´ scale 1 were considered normal/un-pathological and coded as "no pathology"). This variable was used in *Paper II*.
- **MRI burden score** was calculated as the sum of the severity of each MRI-pathology found, as following: Scale variables (WMH, MTA, KPA/PAR, GCA) were coded according to the visual rating scales: no (0); mild (1); moderate (2); severe (3). MB/CMB was divided into o severity scale: no CMB/MB (0); 1 CMB/MB (1); 2-9 CMB/MB (2); ≥10 CMB/MB (3). The maximum score for dichotomous variables (LAC, PA, WMP, CA) was one.
- **Cortical Atrophy** was defined as the presence of one or more of the following: frontal atrophy, temporal atrophy, frontotemporal atrophy (all three as assessed visually by a neuroradiologist), parietal atrophy (by the Koedam scale) or one or more points on the Pasquier scale for Global Cortical Atrophy (GCA). This variable was used in *papers III* and *IV*.

- **Composite CSVD** was defined as the presence of one or more of the following: CMB, LAC or Fazekas grade ≥ 2. This is a modified version of the cerebral small vessel disease (SVD) score presented by Staals et al in 2014, since we lack assessment of "perivascular spaces" in our material. *Composite CSVD* was used in *Paper III*.
- **Modified STRIVE/CSVD score** was defined as the presence of one or more of the following: 1 CMB, LAC, Fazekas grade ≥ 2, Cortical Atrophy or Central Atrophy. This is a modified version of Standards for reporting vascular changes on neuroimaging (STRIVE) presented by Wardlaw et al 2013. *Modified STRIVE* variable was used in *papers III* and *IV*. In *Paper IV*, for reasons explained above, the term was *CSVD score*.
- **Primary Atrophy** was defined as the presence of white- or grey matter atrophy (Cortical atrophy and/or Central Atrophy) without signs of vascular burden (individuals with Fazekas grade ≥ 2 were excluded from analysis). *Primary Atrophy* was used in *Paper IV*.
- **AD-related Atrophy** was defined as the presence of MTA, PAR and/or PREC. These atrophy patterns have previously been associated with Alzheimer´s Disease (AD). *AD-related Atrophy* was used in *Paper IV*.
- **Healthy Brain** was defined as absence of all the defined MRI-manifestations, except for WMH Fazekas grade 1, and MTA Scheltens´ scale 1, which were both considered normal/non-pathological. *Healthy Brain* was used in *Paper IV*.

Blood pressure and hypertension variables

In conjunction with the original study protocol, **blood pressure (BP)** measurements were taken by the study physician using a manual sphygmomanometer of appropriate size (a smaller 9 cm, standard 12 cm and a wider 15 cm). Measurements included **systolic blood pressure (SBP)** and **diastolic blood pressure (DBP).** Subjects were in a supine position and in a restive state, with the arm supported to be levelled with the heart. Measures were taken bilaterally, and the highest value was used.

Orthostatic hypotension (OH) was tested immediately after standing from a restive supine position, and repeated after 1, 3, 5 and 10 minutes (124). Orthostatic hypotension (OH) was defined as a fall in systolic blood pressure (SBP) greater than 20 mm Hg and/or diastolic blood pressure (DBP) greater than 10 mm Hg after 1 to 10 minutes stand and greater than 40- and 20-mm Hg SBP/DBP immediately upon standing. Subjects were asked if they experienced symptoms (such as dizziness or impaired vision) in conjunction with the test, or during the previous year. Affirmative answer was defined as **orthostatic intolerance (OI)**.

Systolic ankle pressure was determined bilaterally by applying a doppler probe on the posterior tibial artery and a manual BP-cuff on the lower calf. **Ankle-brachial index (ABI)** was defined as increased if the quota between brachial and ankle- BP was higher than 1.3 and decreased if it was below 0.9 (125).

Pulse pressure (PP) was defined as the difference between systolic and diastolic brachial BP.

Hypertensive BP was defined as values SBP ≥ 140 and/or DBP ≥ 90, according to WHO criteria.

Hypertension (HT) was defined as current or previous diagnosis of hypertension according to ICD-10 as assessed by a physician from medical records and interview with the subject. Medical records were reviewed in retrospect by the authors for ICD-10 diagnosis of primary or secondary hypertension by date of MRI examination. In *Paper III*, current use of antihypertensive treatment (ATC codes 02 – antihypertensive agents, 03 - diuretics, 07 - beta-blockers, 08 - calcium antagonists, 09 – renin-angiotensin-system inhibitors) was classified as hypertension.

In *Paper I*, subjects were divided into groups of **BP phenotypes** regarding status of HT diagnosis (as reported by the subject, reviewed by the physician in medical charts, and retrospective review by the authors of national registers of ICD-10 diagnosis), medications (ACT codes 02,03,07,08,09 as reported by the subject, reviewed by study physician in medical review, and compliance affirmed by the subject) and resting blood pressure (taken by the physician at the study centre), as follows in Table 1:

Table 1. Chart of BP phenotypes.

BP-phenotypes	Normal BP	Hypertensive BP
No HT diagnosis, no BP lowering drugs	**Healthy Controls**	**Untreated HT**
HT diagnosis and antihypertensive drugs	**Controlled HT**	**Uncontrolled HT**
No HT diagnosis, takes BP lower drugs with other indication than HT	Excluded from analysis	
Reports previous but not current HT diagnosis and treatment		**Previous HT**

BP: blood pressure; **HT**: hypertension

Carotid duplex ultrasonography

Participants in the "vascular physiology" sub-study were examined by grey-scale, color Doppler. The Doppler waveform was obtained with an angle of insonation less than or equal to 60 degrees. The sample volume was positioned within the area of greatest stenosis and sampled through the region of stenosis completely until the distal end of the plaque. Examinations took place 2013-2015, all within a year prior to MRI examination, and were conducted systematically by one and the same laboratory technician in a controlled environment. Examination included vessel diameter, peak **systolic velocity (PSV)** and **end diastolic velocity (EDV)** at the following sites: left and right common carotid artery (CCA) and left and right internal carotid artery at proximal (ICAp) and distal (ICAd) sites.

For each of the locations, mean velocity (126), **resistivity index (RI)** and **pulsatility index (PI)** (127) was calculated manually with the Pourcelot formula and Goslins' index, respectively.

Pourcelot formula: $RI = \frac{PSV-EDV}{PSV}$

Goslins' index: $PI = \frac{PSV-EDV}{V(mean)}$ $V(mean) = \frac{PSV-EDV}{3} + EDV$

Kidney function

Blood samples were collected non-fasted by a nurse in conjunction with original study visits. Samples were cryopreserved and later analyzed for creatinine (crea) and cystatin-c (CysC) in the laboratory of Skåne University Hospital, Malmö, Sweden. Analyses were performed using a modified Jaffe method with a Beckman Coulter LX 20 traceable to isotope-dilution mass spectrometry for crea, and Gentians reagent with a Beckman Coulter LX 20 for CysC (128). Blood samples closest in time to the MRI-examinations were chosen (mean 738 (SD 366) days). Estimated glomerular filtration rate (eGFR) can be calculated from crea, cysC or both, using the well-established and reliable chronic kidney disease epidemiology collaboration (CKD-EPI) formula (129), in which consideration for age and sex is taken. CKD-EPI crea/CysC was used for non-underweight subjects, while CKD-EPI CysC was used on underweight subject (defined as BMI <23 (130)) to minimize risk of overestimation of GFR due to low muscle mass (131).

CKD, chronic kidney disease, was defined as eGFR ≤ 60 ml/min/1.73m^2, based on CKD-EPI crea/CysC or CKD-EPI CysC depending on BMI (see above).

Proteomics

All participants that attended the extended "vascular physiology" sub-study were examined by proteomic analysis of plasma from the biobank. After sampling of blood, the vials have been centrifuged immediately for 10 minutes (2000 G), and the plasma have been stored in 2 ml cryo vials in -80 ° C freezer. Samples have not been thawed prior to retrieval for proteomic analysis. The biochemical analysis was conducted at the Clinical Biomarkers Facility at the Science for Life 2 Laboratory at Uppsala University, Uppsala, Sweden.

Cardiovascular panels CVD II and CVD III and inflammation, each containing 92 proteins, were chosen. The biomarkers included in the panels were pre-selected by Olink Proteomics based on prior research, published literature, and clinical relevance.

For each protein of interest there are two antibodies, each linked to oligonucleotide-tails with a slight affinity to one another. As the dual antibodies attach to the same protein their oligonucleotide tails come in proximity to one another. Upon this, they are extended by a DNA-polymerase, forming a unique DNA sequence representing the specific protein. This sequence is amplified and later quantified by quantitative real-time PCR (qPCR) (118). Quality control was performed by Olink Proteomics and included internal and external controls in each run, normalization of data and assessment of deviation from median value of controls. Data was presented as normalized protein expression (NPX), which is an arbitrary unit on log2 scale.

Other variables from original study protocol

Original study protocol includes questions on smoking status, education level, height, weight and other demographic variables.

Age and age cohorts

Age refers to age at date for MRI-examination. Age cohorts were determined based on sample sizes and encompassed three groups: 70-74. 75-80 and 80+. The older group is the smallest in size, including approximately ¼ of subjects, depending on study.

Cognitive status

General cognitive status was examined by the mini-mental-state-examination (MMSE), with a range from 0-30 (132). Scoring 28-30 was considered normal; 25-27 was considered intermediate; and ≤ 24 was considered impaired.

Data on medications and medical diagnosis

Data on medication and medical diagnosis was retrieved from the County Patient Medical Records Registry containing outpatient and inpatient records, National Inpatient and Outpatient Registry, and Swedish National Board of Health and Welfare covering all medical inpatient and outpatient visits coded with diagnosis according to the International Classification of Diseases (ICD) 9 and 10 (133). Drugs were coded according to the Anatomic Therapeutic Chemical (ATC) classification system (134) and assessed by the physician in medical records and together with the participant. Indication for drug and treatment duration as well as compliance was documented.

Records were assessed by the authors and current or previous diagnosis or medication on date of MRI was coded as "yes".

Statistical methods

Paper I

The study had a cross-sectional design. Independent variables were measures of BP, PP, ABI, OH, BP phenotypes and duration of antihypertension treatment. Multivariable logistic regression analyses were performed with CMB as the dependent variable, with age and sex as covariates. Analyses were repeated in sub-cohorts stratified according to CMB location; strictly lobar or non-lobar/mixed. For descriptive purposes, the outcome variable was also investigated with multivariable regression analysis against background characteristics and other MRI-findings.

Paper II

The study had a cross-sectional design. Co-occurrence between MRI markers were investigated by crosstabulation using Pearsons´chi^{2,}, and by assessing combinations of pathologies. Pairwise correlation was established by Yule´s Q formula for colligation, and covariance was investigated by hierarchical cluster analysis based on Yule´s Q. Association between carotid flow parameters and MRI-markers were investigated by logistic or linear regression analysis. Independent variables were carotid flow parameters (PSV, EDV, PI and RI), and dependent variables were MRI markers (WMH, WMP, CMB/MB, LAC, KPA/PAR, PA/PREC, MTA, CA, GCA) and the composite variables "Number of MRI pathologies" and "MRI burden score". Primary, unadjusted calculations were performed on carotid flow parameters of CCA, ICAp, and ICAd on both left and right sides. To test the robustness of the findings, analyses were repeated in sub-cohorts stratified according to age and sex.

For brevity, subsequent calculations were performed using the mean of the left and right CCA only, adjusted for age and sex.

Paper III

The study had a cross-sectional design. Independent variable was CKD defined as eGFR $\leq$ 60 ml/min/1.73m^2. Dependent variables were WMH, LAC, CMB, Cortical Atrophy, Composite CSVD and Modified STRIVE. Univariable and multivariable logistic regression models were performed. In the multivariable model, age and sex were included as covariables. HT is intimately associated with CSVD as well as CKD, and in the case of CKD causal pathways are complicated and might be bidirectional. For this reason, we chose not to include HT as a confounder because of risk of overfitting. To adjust for HT, analyses were repeated in sub-cohorts stratified according to HT status.

Paper IV

The study had a cross-sectional design. Independent variables were the normalized NPX values of 257 proteins. Dependent variables were WMH, CMB, LAC, Cortical Atrophy, CSVD score, AD-related atrophy, MTA, KPA, PA and Primary Atrophy. Primary univariable logistic or linear regression analyses were performed for each of the 257 proteins and each MRI-variable. The Benjamini-Yekutieli (135) method was used to keep false discover rate (FDR) at 5%. Benjamini-Yekutieli method was chosen over the Benjamini-Hochberg (136, 137) method since it is expected to control the FDR regardless of the correlation among the p values. Proteins that passed FDR correction were analyzed by multivariable regression analyses, with age and sex as covariables. Analyses were repeated against a smaller cohort consisting of only the "Healthy Brain" variable.

Statistical analyses were performed using the softwares IBM SPSS v24-27, Stata SE 17 and Python.

The authors´ contribution to the papers

The study PI (SE), and main supervisor of the author (KE), designed the study and supervised data collection, which was performed by GÅS staff. Co-supervisor (ASL) participated in design and supervision of sub-studies. KAK performed and and interpreted neuroimaging.

KE was study physician during a year prior to being a PhD-student. During this year she performed medical examinations, performed structured interviews of the subject's medical history and current symptoms, and performed searches in medical records. KE has not participated in data collection for participants in the MRI sub-study.

Paper I

KE and supervisors cooperated in conceptualization of the study. KE conducted the data curation and formal analysis. KE wrote the original draft. All authors reviewed, revised and approved the final version of the manuscript.

Paper II

KE and supervisors cooperated in conceptualization of the study. KE conducted the data curation and formal analysis. KE wrote the original draft. All authors reviewed, revised and approved the final version of the manuscript. KE was corresponding author in the publication process.

Paper III

KE actively participated in conceptualization of the study. Lead author (TM) conducted the data curation and formal analysis and wrote the original draft. All authors reviewed, revised and approved the final version of the manuscript.

Paper IV

Paper not yet published. KE and supervisors cooperated in conceptualization of the study. Statistical methods were planned together with study statistician. KE conducted the data curation and formal analysis. KE wrote the original draft. All authors reviewed, revised and approved the final version of the manuscript. KE will be corresponding author in the publication process.

Ethical considerations

The GÅS study is conducted in accordance with the Declaration of the Code of Ethics of the World Medical Association (Declaration of Helsinki) (138). All papers are part of the GÅS study and was approved by the Ethics Committee of Lund University, Sweden (reference number: LU 744-20 and LU 744-00) and by the Regional Ethics Review Board, Lund University (2015/859). All participants have given written informed consent to participation, biobank participation and retrieval of medical records. All participants have been informed that they can withdraw from the study at any moment. Participants were pseudo anonymized, and personally identifiable information was unavailable for researchers and replaced by a study ID. Research data is stored, analyzed and managed via the internal Lund University data environment tool – LUSEC (139), which is a local data storage platform with high level of encryption and a two-factor identification requirement for access. All access to data is approved by the PI upon retrieval, and researchers only have access to the necessary data approved for their specific project.

Examinations included in the survey are time consuming but generally not uncomfortable. With the exception of the blood draw, participants did not experience other painful procedures. During the examination, if changes in health were discovered, subjects were informed about it and, after consent, referred to a general or specialized health care unit if deemed necessary. MRI examination may be perceived as uncomfortable for some participants, but it was conducted as a standard clinical routine examination. The staff was prepared to help or discontinue the examination if necessary. Every subject received a letter with information about the findings and was offered an opportunity to discuss them. Some of the participants were referred to the Neurology Clinic for further examination and/or treatment considerations.

Results

Paper I

344 subjects were investigated. CMB was present in 26,5% of the participants. A majority of subjects presented with a single CMB, while 8% had more than 10 CMBs. Four subjects had an uncountable number, estimated to >100 CMBs. 59 % of CMBs were lobar, while 37% were non-lobar. Information about location is missing for the four subjects with an uncountable number of CMBs (Figure 9).

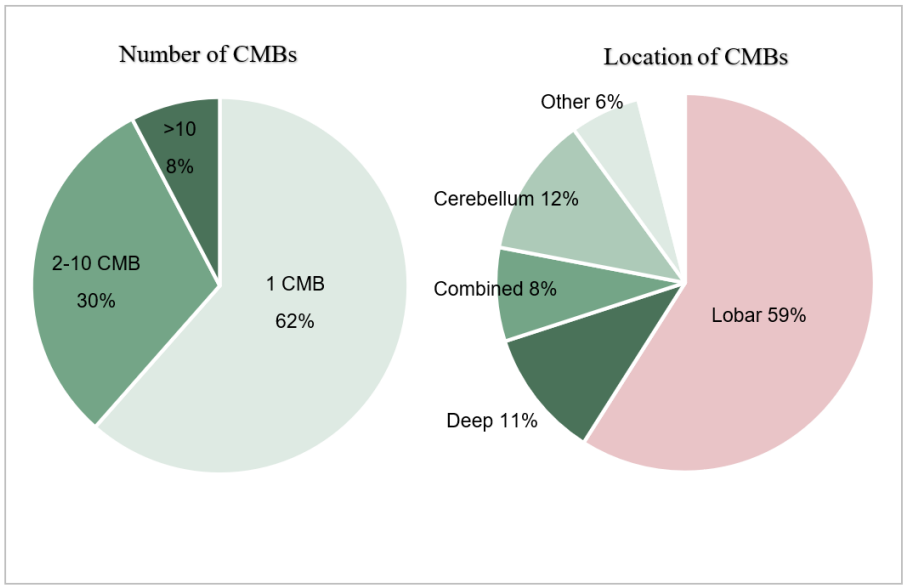


Figure 9. Total number of CMBs presented in CMB positive subjects, and location of CMBs. Location information is missing for 4% of subjects, who all had multiple numbers of CMB.

Odds ratio (OR) for presenting with CMB was 2.27 (1.37 -3.77) times higher in men (prevalence was 34% in males vs 21% in females). Prevalence of CMBs were higher in the older age cohorts (80-87 years: 30.3%; 75-79 years: 31.5%) compared to the

younger cohort (70-74 years 18.8%). This difference was significant when examined with Pearsons´ chi^2, but when entered into a logistic regression model with age and HT as covariates, the association was no longer significant. 23 subjects (7%) scored below 24 on MMSE, reflecting impaired cognition, and 88 subjects (26%) scored intermediate between 25-27. In the impaired group, OR for presenting with CMB was 4.00 (1.66-9.63) compared to normal results (28-30). In the intermediate group, this number was 1.9 (1.10-3.27) (Figure 10).

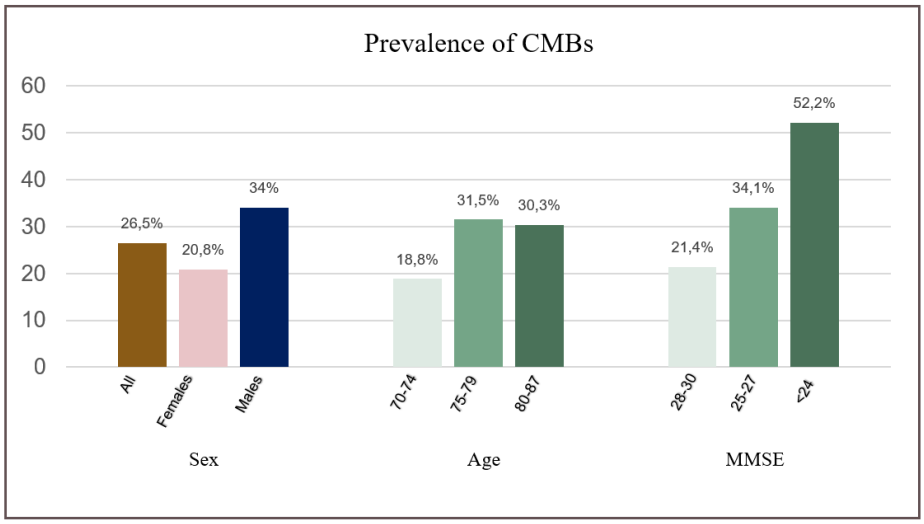


Figure 10. Prevalence (%) of CMB by sex, age and MMSE results.

Associations between CMBs and blood pressure variables were investigated in the whole cohort and repeated in two sub-cohorts stratified according to CMB location. The non-lobar cohort consisted of subjects with mixed or deep CMBs, or no CMBs. The lobar cohort consisted of subjects with strictly lobar CMBs, or no CMBs. Hypertensive SBP or DBP was associated with the presence of one or more CMB, and this association was stronger for most variables in the non-lobar cohort. In the lobar cohort, only diastolic hypertension was associated with CMBs (Table 2).

Table 2 Multivariable logistic regression analyses with blood pressure variables as independent variables and CMB as dependant variable, adjusted for age and sex. Analyses were repeated in cohorts stratified according to CMB location.

	Whole cohort n=344			Non-lobar n=290			Lobar n=311		
	OR	95%CI	p	OR	95%CI	p	OR	95%CI	p
SBP	1.01	1.00-1.03	0.062	**1.03**	**1.01-1.05**	**0.007**	1.01	0.99-1.03	0.258
H SBP	**1.69**	**1.01-2.83**	**0.048**	**2.17**	**1.00-4.69**	**0.049**	1.65	0.89-3.04	0.110
DBP	1.01	0.98-1.04	0.492	1.02	0.98-1.06	0.392	1.01	0.98-1.05	0.452
H DBP	**2.26**	**1.11-4.61**	**0.025**	**2.69**	**1.04-6.96**	**0.042**	**2.51**	**1.11-5.63**	**0.026**
H BP	**1.93**	**1.13-3.28**	**0.016**	**3.11**	**1.33-7.28**	**0.009**	1.67	0.90-3.09	0.105

SBP: systolic blood pressure; **H SBP:** hypertensive systolic blood pressure; **DBP**: diastolic blood pressure; **H DBP**: hypertensive diastolic blood pressure; **HBP**: hypertensive blood pressure

Moderately increased pulse pressure (PP) was associated with CMBs across the three different cohorts. In the non-lobar cohort, but not the in two other cohorts, presenting with severely increased PP had an even stronger association with CMBs. ABI was not associated with CMB in any of the cohorts (Table 3).

Table 3 Multivariable logistic regression analyses with pulse pressure (PP) and ankle-brachial index (ABI) as independent variables and CMB as dependant variable, adjusted for age and sex. Analyses were repeated in cohorts stratified according to CMB location.

	Whole cohort n=344			Non-lobar n=290			Lobar n=311		
	OR	95%CI	p	OR	95%CI	p	OR	95%CI	p
PP	1.01	1.00-1.03	0.083	**1.03**	**1.01.1.05**	**0.014**	1.01	0.99-1.03	0.338
PP ≤ 60	1	-	-	1	-	-	1	-	-
PP 61-80	**2.12**	**1.19-3.78**	**0.011**	**2.72**	**1.12-6.61**	**0.027**	**2.13**	**1.09-4.14**	**0.027**
PP ≥ 81	1.84	0.95-3.56	0.072	**3.17**	**1.25-8.02**	**0.015**	1.43	0.63-3.27	0.392
ABI	0.62	0.12-3.30	0.573	0.13	0.01-1.45	0.098			
ABI < 0.9	1.28	0.49-3.34	0.614	1.83	0.56-6.01	0.322	0.84	0.23-3.09	0.792
ABI 0.9 -1.29	1	-	-	1	-	-	1	-	-
ABI ≥ 1.3	0.57	0.24-1.37	0.209	0.36	0.08-1.67	0.191	0.65	0.24-1.71	0.379

PP: pulse pressure; **ABI:** ankle-brachial index

In the cohort as a whole and compared to subjects without HT diagnosis or elevated resting BP, having a diagnosis of HT was not associated with higher OR for CMB as long as BP was normotensive. Subjects with HT diagnosis presenting with elevated BP however, had an increased risk of CMB. In the non-lobar sub-cohort, having a diagnosis of HT and/or an elevated resting BP was highly associated with increased risk of CMB. Subjects with HT diagnosis and treatment, but who still presented with elevated resting BP had a 14-fold (95%CI 1.82-114.10) increased risk of CMBs compared to healthy, normotensive individuals. In the lobar sub-group, there was no effect of BP-phenotypes on the occurrence of CMB (Table 4).

Table 4 Multivariable logistic regression analyses with hypertension status as independent variables and CMB as dependant variable, adjusted for age and sex. Analyses were repeated in cohorts stratified according to CMB location.

	Whole cohort n=344			Non-lobar n=290			Lobar n=311		
	OR	95%CI	p	OR	95%CI	p	OR	95%CI	p
No HT	1	-	-	1	-	-	1	-	-
Controlled HT	1.49	0.63-3.54	0.368	**9.18**	**1.09-77.14**	**0.041**	0.84	0.31-2.32	0.738
Uncontr HT	**2.30**	**1.05-5.05**	**0.037**	**14.40**	**1.82-114.10**	**0.012**	1.54	0.65-3.64	0.326
Untreated HT	2.02	0.91-4.51	0.085	**9.08**	**1.09-75.52**	**0.041**	1.53	0.64-3.66	0.336
Previous HT	2.23	0.48-10.36	0.307	10.63	0.58-195.82	0.112	1.65	0.29-9.26	0.576

HT: hypertension

Duration of antihypertensive treatment was not associated with CMB in any of the cohorts.

Paper II

391 subjects were included in analysis. The most common manifestations were WMC/WMH (Fazekas ≥2), PAR/KPA and PREC/PA, which were present in roughly 30% of subjects, respectively. CMB/MB was present in 27% of subjects, whereas LAC was present in 8% (Figure 11).

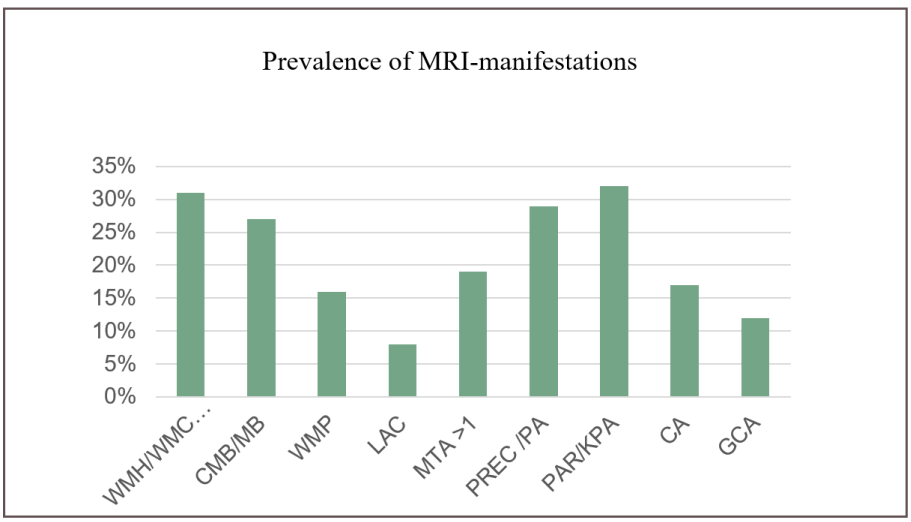
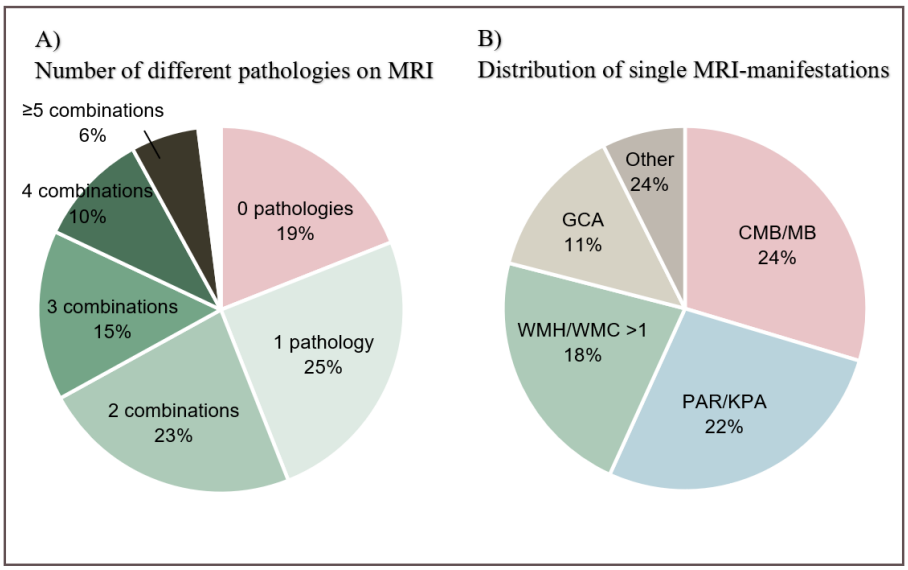


Figure 11. Prevalence of MRI-manifestations
WMH/WMC: white matter hyperintensities; **CMB/MB:** cerebral microbleeds; **WMP:** pontine white matter changes; **LAC:** lacunar infarctions; **MTA:** medial temporal lobe atrophy; **PREC/PA:** precuneal atrophy; **PAR/KPA**: parietal atrophy; **CA:** central atrophy; **GCA:** global cortical atrophy

19% of subjects showed no pathologies on MRI (Fazekas grade 1 and Scheltens´ grade 1 were accepted), while 16% of subjects presented with a combination of 4 or more concurrent findings. Among subjects with only a single manifestation, CMB/MB was the most commonly observed. (Figure 12).



Figue 12. **A)** Number of different pathologies on brain MRI **B)** Distribution of single manifestations of MRI-pathologies.
CMB/MB: cerebral microbleeds; **PAR/KPA**: parietal atrophy; **WMH/WMC:** white matter hyperintensities; **GCA:** global cortical atrophy

In crosstabulation, presence of WMH/WMC >1 was significantly associated with the occurrence of MB, LAC, MTA, WMP and CA. Likewise, presence of LAC was associated with GCA.

Individuals with PSV ≥ 120 indicating stenosis >50% were excluded from analysis. PSV decreased significantly with age, and increased significantly distally from CCA, ICAp to ICAd. There was no significant difference in PSV between the sexes, and PSV was not associated with MRI-manifestations. However, PSV was borderline significantly (p 0.05) associated with LAC. Like PSV, EDV decreased significantly with age, and increased distally from CCA to ICAp to ICAd. EDV was significantly lower, and PI and RI significantly higher in males.

EDV was negatively associated with WMH/WMC, manifestations of atrophy (PAR/KPA, PREC/PA, GCA), and the composite MRI-variables. PI and RI were

associated with PAR/KPA and the composite variables (Table 5). CMB/MB, WMP, LAC, MTA and CA were not associated with carotid flow parameters in this study.

Table 5. Multivariable logistic or linear regression analyses with carotid artery flow parameters as independent variables and MRI-markers as dependent variables, adjusted for age and sex.

	EDV			PI			RI		
	OR	CI	p	OR	CI	p	OR	CI	p
WMH/WMC	**0.92**	**0.87-0.97**	**0.004**	1.09	0.98-1.22	0.132	1.40	0.91-2.18	0.129
PAR/KPA	**0.94**	**0.89-0.99**	**0.039**	1.11	1.0-1.24	0.062	1.47	0.95-2.26	0.081
PREC/PA	**0.94**	**0.88-0.98**	**0.022**	**1.13**	**1.01-1.27**	**0.029**	**1.67**	**1.06-2.61**	**0.027**
GCA	**0.90**	**0.83-0.98**	**0.013**	1.10	0.95-1.28	0.210	1.44	0.78-2.65	0.244
	β	CI	p	β	CI	p	β	CI	p
"number of"	**-0.07**	**-0.11- -0.03**	**<0.001**	**0.120**	**0.05-0.20**	**0.001**	**0.470**	**0.18-0.76**	**<0.001**
"burden score"	**-0.11**	**-0.17- -0.06**	**<0.001**	**0.22**	**0.11-0.33**	**<0.001**	**0.85**	**0.42-1.29**	**<0.001**

WMH/WMC: white matter hyperintensities; **PAR/KPA**: parietal atrophy; **PREC/PA:** precuneal atrophy; **GCA:** global cortical atrophy

Paper III

390 subjects were included in analysis. 206 subjects (53%) presented with hypertension, and 94 subjects (24%) presented with CKD. Mean eGFR was 50.2 (SD 8.4) in the CKD group and 77.3 (SD 10.8) in the non-CKD group. Among subjects with HT diagnosis, 74 subjects (36%) presented with CKD, while 20 subjects (11%) had CKD in subjects without HT diagnosis (Table 6).

Table 6. Prevalence of HT and CKD**,** and co-occurrence of both.

	All	HT	no HT	CKD	no CKD
HT	206 (53%)			74 (79%)	132 (45%)
no HT	184 (47%)			20 (21%)	164 (55%)
CKD	94 (24%)	74 (36%)	20 (11%)		
no CKD	296 (76%)	132 (64%)	164 (89%)		

HT: hypertension; **CKD:** chronic kidney disease

Cohorts that presented with HT or CKD generally had a higher age and disease burden, and higher prevalence of MRI-manifestations than cohorts without these manifestations (Table 7).

Table 7. Characteristics of participants according to hypertension- and CKD-status.

	All	HT	no HT	CKD	no CKD
n	390	206	184	94	296
Age	75.4 (SD 3.6)	76.1 (SD3.9)	74.5 (SD3.1)	76.6 (SD 3.6)	75.0 (SD 3.5)
Female	214 (55%)	110 (53%)	104 (57%)	57 (61%)	157 (53%)
BMI	26.7 (SD4.0)	27.6 (SD 4.0)	25.8 (SD 3.8)	27.6 (SD 4.2)	26.5 (SD 3.9)
DM	62 (16%)	49 (24%)	13 (7%)	19 (20%)	43 (15%)
WMH	120 (31%)	80 (39%)	40 (22%)	37 (39 %)	83 (28 %)
LAC	32 (8%)	23 (11%)	9 (5%)	10 (11%)	22 (7%)
CMB	107 (27%)	66 (32%)	41 (22%)	34 (36%)	73 (25%)
Cortical Atrophy	197 (51%)	103 (50%)	94 (51%)	56 (60%)	141 (48%)
Composite CSVD	198 (51%)	122 (59%)	76 (41%)	58 (62%)	140 (47%)
Modified Strive	309 (79%)	170 (83%)	139 (76%)	80 (85%)	229 (77%)

BMI: body mass index; **DM:** diabetes mellitus 1 or 2; **WMH:** white matter hyperintensities; **LAC:** lacunar infarctions; **CMB**: cerebral microbleeds; **HT:** hypertension; **CKD:** chronic kidney disease

In the univariable model, CKD was associated with WMH, CMB, Cortical Atrophy and Composite CSVD (not shown), but after adjusting for age and sex, these associations were no longer significant. In the hypertensive group, CKD was associated with CMB and Cortical atrophy, after adjusting for age and sex. In the non-hypertensive cohort, CKD was not associated with MRI-markers of CSVD (Table 8).

Table 8. Multivariable logistic regression analyses with CKD as independent variable and MRI-markers as dependent variables, adjusted for age and sex. Analyses were repeated in sub-cohorts stratified by hypertension status.

	All			HT			No HT		
	OR	CI	P	OR	CI	P	OR	CI	p
WMH	1.44	0.88-2.37	0.152	1.15	0.64-2.08	0.633	1.28	0.45-3.70	0.643
LAC	1.57	0.70-3.53	0.273	1.11	0.44-2.82	0.825	1.93	0.36-10.47	0.447
CMB	1.61	0.96-2.70	0.074	**1.93**	**1.04-3.59**	**0.037**	0.54	0.14-2.03	0.359
CortAtr	1.52	0.93-2.48	0.093	**2.45**	**1.34-4.48**	**0.004**	0.63	0.24-1.69	0.360
CompCSVD	1.55	0.95-2.53	0.079	1.5	0.82-2.73	0.186	0.97	0.36-2.63	0.951
ModSTRIVE	1.38	0.71-2.66	0.344	1.56	0.67-3.60	0.301	1.05	0.33-3.39	0.935

WMH: white matter hyperintensities; **LAC:** lacunar infarctions; **CMB:** cerebral microbleeds; **CortAtr:** Cortical Atrophy; **CompCSVD:** composite CSVD; **ModSTRIVE:** Modified STRIVE; **HT:** hypertension

Paper IV

401 subjects were included in the study. After FDR-correction and adjustment for age and sex, 11 plasma proteins were associated with "CSVD score" and 11 plasma proteins were associated with "Cortical Atrophy". MTA was associated with one protein and "Primary Atrophy" with one protein. For brevity, only significant associations are shown (Table 9).

Table 9. Multivariable logistic and linear regression models, with protein-abundance as independent variable, and MRI-manifestations as dependent variables, adjusted for age and sex. Only proteins that passed FDR are shown.

Dependent Variable CSVD score	Protein		β	CI	p
	CTSL1	Pro-Cathepsin L1	0.37	0.07 – 0.68	0.017
	PGF	Placental Growth Factor	0.41	0.09 – 0.72	0.012
	NTpBNP	NtproBrain Natriuretic Protein	0.13	0.04 – 0.21	0.003
	TNFR2	Tumour Necrosis Factor R2	0.31	0.1 – 0.53	0.005
	GDF15	Growth Differentiation Factor 15	0.19	0.01 – 0.37	0.041
	TNF r1	Tumour Necrosis Factor R1	0.30	0.08 – 0.53	0.008
	IL4RA	Interleukin 4 receptor A	0.41	0.13 – 0.69	0.004
	ADM	Pro-Adrenomedullin	0.33	0.10 – 0.56	0.005
	CXCL9	CXC-chemokine ligand 9	0.15	0.05 – 0.26	0.006
	TFF3	Trefoil Factor 3	0.29	0.11 – 0.48	0.002
	BNP	Brain Natriuretic Peptide	0.09	0.01 – 0.16	0.020
Dependent variable Cortical Atrophy	Protein		OR	CI	p
	CDH5	Cadherin 5	4.25	2.1 – 8.59	<0.001
	IL4RA	Interleukin 4 receptor A	3.53	1.76 – 7.13	<0.001
	TNFR1	Tumour Necrosis Factor R1	2.57	1.46 – 4.52	0.001
	PGF	Placental Growth Factor	3.16	1.44 – 6.94	0.004
	TF	Tissue Factor	3.93	2.10 – 8.59	<0.001
	TNFR2	Tumour Necrosis Factor R2	2.20	1.29 – 3.75	0.004
	CD93	Cluster of Differentiation 93	3.31	1.62 – 6.77	0.001
	CTSL1	Pro-Cathepsin L1	2.93	1.40 – 6.15	0.004
	LTBR	Lymphotoxin beta receptor	2.63	1.36 – 5.10	0.004
	TNFRSF11A	TNF receptor superfamily 11A	2.20	1.32 – 3.65	0.002
	TNFRSF10A	TNF receptor superfamily 10A	3.04	1.6 – 5.79	0.001
MTA					
	GDF15	Growth Differentiation Factor 15	1.65	0.98 – 2.76	0.059
	PI3	Peptidase Inhibitor 3	1.57	1.03 – 2.40	0.035
Primary Atrophies					
	IL4RA	Interleukin 4 receptor A	5.00	2.00–12.51	<0.001

Discussion

Summary of main findings and interpretation of results

We have investigated the prevalence of CSVD and brain atrophy in a general cohort of older adults, and explored cross-sectional associations with hemodynamic changes, kidney dysfunction and circulating biomarkers.

White matter hyperintensities

WMH was the most prevalent lesion, present in mild to severe forms in 84% of subjects in *Paper I*. 11% of participants presented with Fazekas grade 3, representing severe/confluent WMH. Moderate to severe WMH was associated with the presence of CMB, LAC, Central Atrophy, moderate to severe MTA and pontine white-matter changes in *Paper II*.

Reduced EDV was connected to moderate to severe WMH, and although not significant, the trend for PSV was also negative (95%CI 0.97-1.00). This might be interpreted as an overall slightly higher total flow velocity in the small group of individuals presenting with none or mild WMH changes. Although no difference could be noted in PI or RI, the reduced systolic and diastolic flow velocity might reflect the slightly lower resistance in the cerebral blood flow that could be expected in the healthier group.

In *Paper III*, in the univariable model of the whole cohort, CKD was associated with moderate to severe WMH. However, age or sex was a confounder in this situation and in the multivariable model there was no significant association. CKD, just like WMH, is a condition that is strongly associated with age, and both might be described as natural occurrences in the aging individual. Moreover, both CKD and WMH are slightly more prevalent in females (39, 107).

WMH has been described as an overall indicator of brain health, and while the milder forms are very common, pronounced WMH is strongly associated with adverse outcomes, such as cognitive impairment, gait disturbances and risk of progression to dementia (140). WMH might be viewed as the "base-indicator" of CSVD – probably appearing in almost all brains with time. In *Paper II*, the significant connection to a majority of the other lesions in crosstabulation is representative of the heterogenic nature of CSVD, where specific patterns of disease progression among lesions have been difficult to identify.

Cerebral Microbleeds

CMB, a risk marker for hemorrhagic and ischemic stroke, was present in 26% of subjects, and 10% of the cohort had multiple CMBs. In *Paper I*, the presence of CMBs was only associated with confluent WMH, not Fazekas grades 1-2. By assessing all CMBs together, the smaller group with diastolic HT had a stronger association with CMB than systolic HT, and this difference prevailed in the lobar sub-cohort.

Diastolic hypertension can follow systolic hypertension or be a separate manifestation. Furthermore, arterial stiffness can lead to a lower DBP, as discussed previously, but can also yield falsely high readings due to vessels being harder to compress. Isolated diastolic hypertension has been considered more closely related to end organ failure, but the associations with cardiovascular risks are inconsistent, which suggests that implications for diastolic hypertension are heterogenic (141). High DBP indicates a stronger effect from peripheral resistance or vasoconstriction and can also be seen in excessive alcohol consumption (142). On this note, in a study of patients with CSVD, the presence of CMB was associated with excessive alcohol consumption among other risk factors. CMB is associated with intracerebral hemorrhage, and in a meta-analysis alcohol was found to be a risk factor for intracerebral hemorrhage, together with hypertension, male sex and age (143). In a follow up MRI-examination of the GÅS cohort after 6 years (144) (the same cohort as in this thesis), incidence of CSVD, but not CMB specifically, was associated with alcohol intake.

Non-lobar CMBs were associated with all measurements of hypertension, except the linear variable DBP. As discussed previously, DBP can be either elevated or reduced in the concept of HT, depending on the underlying mechanism. The ABI marker however, which represents atherosclerosis as well as muscular artery stiffness, showed no connection to CMBs.

The connection between hypertension and CMB or other manifestations of cerebral damage is highly investigated, but still not completely understood. Possible explanations include mechanical injuries in the microcirculation caused by excessive pulsatile force due to altered vessel dynamics. Arterial stiffness and hypertension is associated with an increase in blood pressure variability (145-147), which in turn is independently associated with CMB burden and progression (146, 148, 149). BPV is believed to enhance stress on the vascular walls and contribute to the vascular remodeling seen in hypertension (145).

On a mechanistic level, hypertension is associated with impaired autoregulation (150), endothelial dysfunction (151), impaired vascular reactivity, inflammation (152) and loss of BBB integrity (153). These are all important contributors to matrix reorganization and vascular remodeling, ultimately believed to result in the deposit of fibrohyaline material, loss of vascular smooth muscle cells, narrowing of lumen, thickening of the vessel wall, and microdissections (152). As a result, the vessel may

become increasingly brittle. Furthermore, functional dysregulation may lead to chronic hypoperfusion of the parenchyma and supportive tissues (152).

Prevalence of MRI manifestations vary between studies and is affected by cohort age, field strength, MRI-techniques, and MR-image assessment (30, 154). A summary of the agreement in CMB detection using neuroimaging (1,5T-3T) and histopathological examination concluded that false positive CMB findings occurred in 11-24% of cases. CMB-mimics on MRI included microdissections, microaneurysms and calcifications (154).

The prevalence of CMB is considerably higher in our cohort compared to other larger population studies. However, cohort age, field strength and MRI sequence differ between studies, rendering the results hard to compare (155-157). Overall, distribution of lobar CMB compared to non-lobar tends to be between 63%-80% (155-157), which is in line with our results (70%). In the population-based Mayo Clinic Study of 1253 older adults examined by 3T MRI with T2*GRE, comparable to our settings, prevalence of CMB was 26.3%, of whom 77% were lobar. In their study, as in ours, CMB was associated with age, male sex and hypertension (158).

Lacunar infarctions

Lacunar infarctions have been described as "the hallmark of manifest CSVD" and are viewed as a slightly more serious find than other more prevalent CSVD-markers. 8% of participants in *Paper II* presented with LAC. Prevalence of LAC was significantly associated with WMH and global cortical atrophy (GCA) in crosstabulation. Borderline significant, LAC was associated with an increased PSV. Increased PSV might represent narrowed lumen because of atherosclerosis, although by study design, stenosis >50% were excluded from our study. This is in line with the literature, as LAC has a stronger connection to atherosclerosis and cerebral large vessel disease (stroke) than other CSVD manifestations. In a study of patients with TIA, carotid stenosis >50% was associated with the presentation of lacunar infarction (159).

Cortical Atrophies

Global cortical atrophy (GCA), assessed by the Pasquier scale, was present in 14% of participants in *Paper I*, and a majority of these had mild GCA. In crosstabulation in *Paper II*, GCA was significantly associated with LAC but not with WMH. In the same study, GCA was associated with lower levels of EDV, which also was significantly associated with the cortical atrophies PAR/KPA and PREC/PA.

In *papers III* and *IV* the variable "Cortical atrophies" was employed instead of the specific cortical atrophies separately. 50.5% of participants presented with atrophy in one or more of the cortical regions. Among hypertensive subjects, but not in the cohort as a whole, cortical atrophy was associated with CKD.

Hypertension is intimately associated with CKD; being both a risk factor and a consequence of CKD. However, CKD can also result from causes other than hypertension-related changes (110). In the literature, there is a strong association between cerebral atrophy and hypertension, probably mediated by CSVD (57). Although borderline significant association could be identified in the unadjusted model, CKD was not associated with cortical atrophy in the cohort as a whole.

By stratification according to hypertension status, we likely also stratified the material by hypertension-related CKD and other causes of CKD. In the normotensive group, non-hypertension related causes of CKD are likely more common. This type of CKD may have a weaker connection to cerebral pathologies. Given the relatively healthy status of the cohort as a whole, the decline in kidney function probably is not pronounced enough to reveal a possible causal connection between CKD and cerebral pathologies.

In the hypertensive group, CKD was associated with cortical atrophy, but not with composite CSVD. CKD was similarly represented in the group with CSVD and the group with cortical atrophy (47.3% and 47.6%, respectively). However, a larger proportion of individuals with CSVD presented with hypertension (59.2% and 50%, respectively). Although there is a great overlap between the two variables; composite CSVD might be seen as an earlier or milder manifestation than cortical atrophy, whereas a greater proportion of these individuals have not (yet) developed CKD.

The brain and the kidney share many commonalities regarding the vascular bed; both being high flow, low resistance organs. It is probable that at least part of the association between CSVD and CKD are explained by common mechanisms of pathophysiology related to hypertension or diabetes mellitus. While pronounced CKD or albuminuria has had an independent association with CSVD or cognitive measures in previous studies (160, 161), the independent association between early-stage CKD and CSVD remains unclear (162, 163), and temporal associations regarding arterial stiffness, hypertension, CKD and cerebral manifestations have not yet been established (110). It is probable that causal associations are bidirectional, emphasizing that these changes should not be viewed as singular entities but rather systemic changes happening in multiple organs simultaneously.

In the hypertensive population, CKD and cerebral changes like CMB and cortical atrophy were connected. In the clinic, monitoring of kidney-status in hypertensive subjects is already routine. Our results emphasize the importance of this, as decline in kidney function might be a warning sign of concurrent progression of CSVD.

Medial temporal lobe atrophy

MTA is not typically regarded as included in the established CSVD-criteria. However, it might play a mediating role in the connection between CSVD and cognitive impairment (164). MTA is very common in the aging brain, present in

mild to severe form in 77% of participants in *Paper I*. However, pronounced MTA is strongly associated with Alzheimer's dementia (60). In this cohort, 8% of participants presented with MTA scores pathological for their age. The most pronounced form of MTA, Scheltens´scale 4, was not present in this community dwelling cohort. In crosstabulation, MTA was associated with WMH and widening of the ventricles (CA). Notably, CA was present in 17% of the whole cohort, but in 51% of individuals with moderate to severe MTA. MTA showed no association with carotid flow variables, and it was not included in *Paper III*, which had a stricter CSVD-profile than the other articles.

In *Paper IV*, MTA was associated with elevated levels of PI3. In the non-adjusted model but not in the model adjusted for age and sex, it was also associated with GDF15. To our knowledge, PI3 has no known connection to dementia. According to The Human Protein Atlas (115), it is primarily found in skin, lymphoid tissue and GI-tract, i.e. epithelial cells subjected to the outside environment. Hypothetically this may support theories on environmental or microbial explanatory theories on brain pathologies.

However, according to the Protein Atlas, PI3 is also found in the brain, although it is not specifically enhanced in the hippocampal formation. It acts as a protease inhibitor, primarily involved in modulating inflammatory responses and regulating tissue degradation (165). In line with our results, a recent proteomic study has associated IP3 with cognitive decline and hippocampal atrophy (166). As discussed previously, MTA is associated with at least two different proteinopathies; AD and the newly described LATE-proteinopathy (62).

CSVD; a whole brain disease

Manifestations of CSVD are common and heterogenous, similar to their clinical presentations. Furthermore, when employed, composite variables of the total CSVD burden showed stronger associations with the outcome than the individual markers. Research has shown CSVD markers can transform into or develop from other CSVD lesions (167). Common for all lesions, as well as their combinations, associations are stronger the more pronounced the lesions are (17).

The composite CSVD-scores in *Paper II* were strongly associated with lower levels of EDV and higher PI and RI. While PI reflects a combination of upstream and downstream flow qualities, RI does not take into account the relative values of EDV and PSV, only the quota between them. Therefore, to a greater deal reflecting the isolated downstream resistance. This is in line with histological research on CSVD showing changes in small vessel morphology and narrowing of vessel lumen, leading to increased peripheral resistance (6). The negative association of EDV and positive association of PI and RI with CSVD are consistent across studies (168, 169). Furthermore, a recent study associated carotid artery flow parameters with

decreased cerebral vascular reactivity, although morphological measures (like IMT or plaque) had a stronger association than hemodynamic ones (170).

However, decreased EDV might also be a reflection of large vessel arterial stiffness, although this association is insufficiently researched. In a community sample free from dementia, high blood pressure was associated with low carotid EDV and PSV flow (171), and EDV and PSV were positively associated with cognitive function. Furthermore, in a population cohort of individuals aged 30 or older and a mean follow up time of 9.85 years, EDV was a better predictor of future ischemic stroke (172) than other carotid ultrasound variables. In another study low EDV predicted future cardiovascular events, implying a connection to systemic arterial changes (171).

In proteomic analysis, the composite variables CSVD-score and cortical atrophies were each associated with 11 circulating proteins, 5 of whom were associated with both variables. It is important to interpret these results in the light of our study design, which specifically targeted proteins associated with vascular health and inflammation. Other mechanisms than those reflected by the chosen proteins may be equally or more important in disease progression. Furthermore, cerebral origin or causal associations to CSVD can not be explored.

The identified proteins that associated with MRI-manifestations include proteins indicative of vascular or metabolic burden (GDF15, ADM, PGF, BNP, pBNP), blood-brain barrier dysfunction (CDH5, CD93), immune dysregulation (CXCL9, IL4RA), markers of inflammation and apoptosis (TNFR1, TNFR2, TNFRSF11a, TNFRSF10a, LTBR), and proteins mostly associated with anti-inflammatory or beneficial effects on tissue (TFF3, PI3). Although the associations to markers of CSVD is novel for most of the identified proteins, associations to cerebral pathologies such as stroke or neurodegenerative dementias have been shown for a majority of them. GDF15, LTBR and TFF3 have previously been associated with markers of CSVD in epidemiological proteomic studies (173). GDF15 and ADM have been found to be important mediators of cardiovascular health on risk of dementia (174).

Figure 13 displays known and hypothetical associations between the identified proteins based on experimental data, gene location, tissue co-expression and other interactions, curated by the STRING database (175). As can be expected, multiple associations exist between the proteins of the TNF-system and the immunomodulatory proteins CXCL9 and IL4RA.

PGF - believed to regulate vascular permeability and to be a marker of vascular burden (176) - associated by text-mining and co-expression with the BBB-associated protein CDH5, which in turn associated with CD93. The latter are proteins involved in formation and regulation of adherens junctions of endothelial cells.

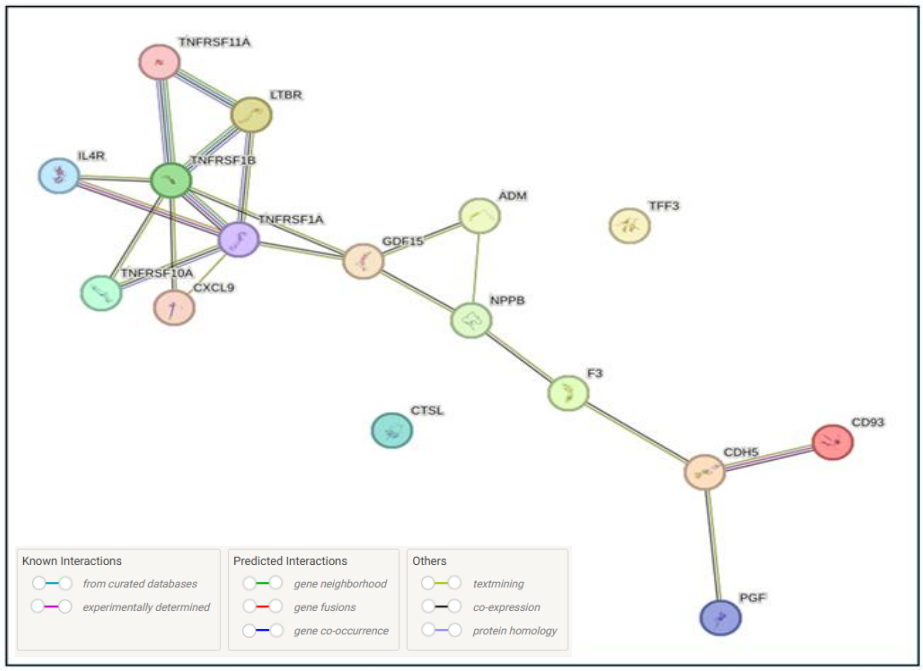


Figure 13. Functional and physical protein associations in homo sapiens, curated by the STRING databese. Line color indicates the type of interaction evidence, evidence confidence requirements set to medium. (https://string-db.org)

GDF15 is a promising emerging biomarker. It has a strong association with age and is a very strong predictor of adverse outcomes and disease progression across the disease spectra (177). It has been associated with dementia, especially vascular dementia in previous studies, meanwhile thought to have mostly beneficial - including neuroprotective - properties (177). It might not be suitable as a specific biomarker for cerebral pathologies given its diversity, but it may prove to be a sensitive and versatile clinical biomarker of metabolic or inflammatory stress, to evaluate cardiovascular risk in patients, or in evaluation of drug benefits.

CSVD score and cortical atrophy both were associated with an increased number of several different proteins belonging to the TNF-superfamily; consisting of both ligands and receptors. TNF-proteins are mainly involved in regulation of the immune-system and regulation of cell apoptosis and proliferation. TNF-receptors are membrane bound, but can be released by different mechanisms such as receptor activation or as decoy receptors in a feedback mechanism (178). The increase in several TNF proteins, in our case with counteracting effects in some cases, might

indicate a dysregulation of inflammation and apoptosis in the setting of CSVD and atrophy.

Methodological considerations

External validity

External validity refers to which extent the results of a study can be applicable or *generalized* to the larger population.

The GÅS study is a randomized population study, stretching over five municipalities in the south of Sweden in the effort to cover both a rural and urban population. As a part of SNAC, the whole study covers southern and northern parts of Sweden; larger cities and smaller communities. This is planned in an effort to adequately reflect the whole Swedish population. Randomization occurs from the Swedish Population Register, which has almost complete coverage over citizens. However, residents who are undocumented, asylum seekers or lack an address are not included. Effort is made so that as many of the invited subjects as possible chose to join the study, e.g. by repeated invitations, offering of home visits and a possibility to split examinations between days, or the option to only participate in selected parts of examination. For very ill or demented residents, relatives are asked to give what information they could.

Despite careful planning and great effort, there is always some degree of *selection bias* in all studies. Participation rate in waves 1-4 was 60%, 73%, 70% and 65%, respectively. This is to be viewed as a high participation rate. However, one should keep in mind upon interpretation of the results that 30-40% of the population lack representation. We do not know anything about the individuals that chose not to participate, but it is probable that physically and mentally ill individuals, individuals with intellectual or physical inabilities, individuals with trouble speaking and reading the Swedish language or otherwise marginalised individuals are underrepresented. It is equally probable that highly productive individuals that lack the time for a full day of examination are underrepresented. Sweden is a country in northern Europe with a predominately Caucasian population. This might affect the applicability of our results to other populations around the world. On the other hand, the relatively small genetical variation that is to be expected in this cohort might result in lifestyle factors having a larger impact on variability.

In the “vascular physiology” and MRI sub-studies there was by design a selection bias favouring healthier individuals that could endure examinations. There is also the possibility that the examination would attract individuals with higher resilience to cerebral pathological changes, underestimating the clinical impact of CSVD-

pathologies (in future studies). The third alternative would be that the examination attracts individuals that have a high degree of engagement in their own health – the reason for this could be a concern about early symptoms of cognitive impairment, or in the other end of the spectra highly health-conscious individuals.

Consequently, the results of our study should be viewed as representative of a somewhat healthier population than the age-equivalent general public.

Internal validity

MR image acquisition

MRI was performed in a clinical setting by experienced personnel and interpreted by the same experienced, and clinically and scientifically active, neuroradiologist. Despite this, there are some methodological considerations to address.

MRI sensitivity to pathological findings differs with field strength, MR-sequence, and slice thickness (53). 3T is a good field strength for an epidemiological study, as most large-scale studies use 1,5T-3T. Higher field strengths, like 7T, are mainly used in more specific research with high demands on resolution, approved for human clinical use in Europe and the USA as late as 2017 (179). However, 7T require longer scan times, and as an effect are more sensitive to movement artefacts (179). Additionally, its findings might less easily be generalizable to clinical settings that rely on more commonly available technologies.

MR image interpretation

All MRI manifestations were assessed by visual rating and no volumetric measurements were performed. We have not had the opportunity to validate our MRI findings by a second rater. When applicable, rating scales were used for defining MRI pathologies. Previous studies have shown good to excellent inter- and intra- rater reliability and good correlation with volumetry regarding Fazekas scale for white matter hyperintensities (180, 181) and Scheltens´scale for medial temporal lobe atrophy (182, 183).

We have identified signs of atrophy in the subjects based on visual features such as widening of sulci or ventricles. However, by lack of longitudinal neuroimaging comparisons, atrophy as the explanation is only assumed, not confirmed. Moreover, visual assessment of CSVD and atrophy will inevitably result in a less sensitive assessment of mild pathological changes, and less pronounced atrophy and WMH changes might be misclassified as absent. However, firstly, visual rating scales are used in clinic and therefore reflect the clinical reality which might make results more generalizable to a clinical setting. Secondly, it can be argued that visually undetectable changes lack clinical significance. For *papers I-III* the sensitivity of visual rating scales are considered sufficient to answer the research question. For

Paper IV, misclassification might have a slightly more significant impact on results. In both cases there is a slight risk of increasing variability and weaken or even miss a true association.

Cut-off values for "pathological" MRI-findings were discussed within the group. By example, MTA 1-2 is considered normal in individuals <75, and 1-3 is considered normal in individuals > 75. These cut-off values are based on the prognostic value of MTA on risk of dementia and therefore found less suitable for our research questions. On the other hand, mild MTA is increasingly common in the aging population and including MTA grade 1 as a pathological finding would not add value in differentiating accelerated cerebral aging from normal. Therefore, MTA 0-1 was considered normal in this high age cohort. While this suits our research questions, it is less conservative than most studies (182, 184).

By the same rationale, cut-off for pathological WMH was set to Fazekas scale ≥2. This was similar to the procedure used in the SVD score (123) by Staals et al. In the CSVD score, Fazekas scale ≥2 regarding deep WMHs and Fazekas scale ≥3 regarding Periventricular WMH was compatible with score for presence of WMHs. In the Rotterdam score for CSVD, assessment of WMH was volumetric, and WMH volume in third or fourth quartiles was compatible with point for score (185).

Misclassification

Chronic kidney disease (CKD) is defined as eGFR<60 ml/min per 1.73 m^2 or albuminuria (urine albumin-to-creatinine ratio >30 mg/g) persisting for 3 months or more (KDIGO12). In the GÅS database we did not have access to urinary analysis of the participants. For this reason, eGFR only is used for CKD classification. Furthermore, the temporal acquirement was not met, since we only assessed one measuring point for eGFR. This might have led to misclassification of subjects with proteinuria but eGFR >60 as healthy, when they in fact should belong to the CKD group. Furthermore, we might have falsely classified some participants as CKD because of temporarily decreased eGFR that would have reverted back to normal within three months, had we measured it. Both of these misclassifications would result in increased variability in the data, resulting in the risk of weakening the associations.

In *Paper III*, sensitivity analyses were performed for all statistical models with eGFR estimated by the CKD-EPI formula based on krea/cysC for all subjects regardless of BMI. Low muscle mass overestimates eGFR (131), and resulted in the decrease of subjects with CKD from 94 to 87 individuals. Most associations remained; however, CKD was no longer significantly associated with CMB in the hypertensive group.

In *Paper II*, for calculation of Goslins´index for pulsatility, mean velocity was not measured by the apparatus, but instead estimated by the formula (126):

$$V(mean) = \frac{PSV-EDV}{3} + EDV$$

This is a well-established calculation of mean velocity; however, it will not provide absolute mean velocity. It is not known to us if there is a systematic error in this method that would unevenly underestimate or overestimate mean velocity based on, by an example, the different appearances of the resistance waves. If this is the case, results of our study could be significantly affected.

Handling of large numbers of data

In the effort to understand the molecular mechanisms of disease, high throughput proteomics can be addressed in a multitude of ways. We chose to search for single proteins associated with the outcome and to perform a literature search to validate the possible clinical implications of each protein. However, it is becoming increasingly common to move beyond the study of individual proteins and instead examine the characteristic signaling networks associated with specific conditions, often with the aid of machine learning. The underlying rationale is that combinations of proteins, rather than isolated biomarkers, are more likely to reflect ongoing mechanisms in the individual. While this may indeed be a better approach for predicting disease outcomes, we chose to assess biomarkers individually to support hypothesis generation and facilitate reflections on pathophysiological mechanisms.

We chose the Benjamini-Yekutieli (BY) method (135) to keep false discovery (FDR) rate at 5%. This method is less conservative than the Bonferroni method, and we considered it better suited than the Benjamini-Hochberg (BH) method. The key difference lies in their assumptions about the dependency between the variables. BH is only valid under independence or positive dependence, while BY is valid under arbitrary dependence.

Handling of missing data

For all papers, we chose to exclude subjects with missing data on a per-variable basis in order to maximize cohort size. The number of missing observations is accounted for in the results when needed. In the proteomic study, a few of the analytes had less than 75% accepted values due to careful quality control, and these variables were excluded altogether. In datasets where groups of proteins are analyzed together it is not uncommon to impute data (186). In our setting however, this would not add any value.

Causality, confounding and mediators

As all four studies are cross sectional, *causal* relationships between independent and dependent variables cannot be explored. A *confounder* is associated with both the

exposure variable and the outcome and might influence the association between these, even if there is no true association. Confounding factors can be managed in the effort to minimise their influence on results, however, even after handling of known confounders there might be residual confounding from unknown or unmeasured confounders. In an observational study, exposure to the explanatory variable is never random; it is the effect of a multitude of known and unknown confounders. Confounders can be handled in different ways; one way is to adjust for them in a multivariable model. This approach was used in all four papers. Another way is to remove the effect of the confounder by stratifying the material, by example performing sensitivity analysis stratified by age, sex, or hypertension status, as was the case in *Paper II*, and to some degree in *Paper III*. A third way to deal with confounding is by restriction. This was performed in *Paper II*: to control for the effect of carotid stenosis on brain pathology and carotid flow velocity, individuals with these conditions were excluded from analysis. A *mediator* is a factor that does not influence the exposure variable but influences the relationship between the exposure variable and the outcome. It is possible that the effect of arterial stiffness on CSVD or CKD might be mediated, at least partly, by hypertension.

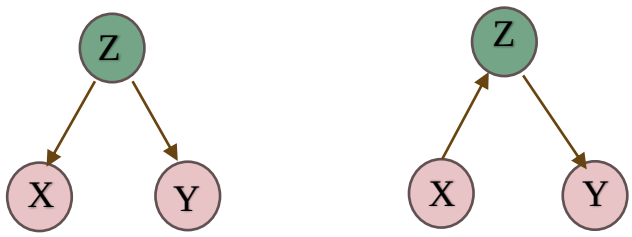


Figure 14 A) A conufounder (Z) effects the exposure (X) and the outcome (Y), mimiking an effect between X and Y. **B)** There is no *direct* effect of X on Y; X effects Z, the mediator, which in turn effects Y.

In the four papers, we have - after careful consideration for each of the papers separately - chosen to limit the number confounders included in the models to minimize the risk of overfitting. While diagrams like the one above are pedagogical, they fail to capture the complexity of biological systems. If hypertension *mediates* the effect of arterial stiffness on CSVD, but simultaneously affects the degree of arterial stiffness, is it a mediator and a confounder? The *independent* association of our exposure variables and the outcome are of limited interest in the context of our specific research questions, where causal associations are difficult to establish and are likely bidirectional.

Clinical implications and future directions

CSVD is the second most common cause of dementia and an important cause of morbidity worldwide. Moreover, beyond the scope of dementia and stroke, CSVD can contribute to depression, apathy, balance issues and incontinence, greatly affecting the everyday life of the aging individual.

Collectively, these studies highlight the multifactorial nature of CSVD and the importance of addressing both traditional risk factors and emerging markers of vascular dysfunction. They emphasize the importance of multifactorial management of hypertension; attention should also be given to arterial stiffness, renal function and systemic inflammation. Ultrasonography, although operator dependent, is relatively inexpensive and, most importantly, non-invasive and free from ionizing radiation. It may serve as a valuable tool for evaluating vascular function beyond the traditional assessment of atherosclerosis common in neurological settings.

Looking ahead, the results presented here have several implications for future research. In the setting of the GÅS study, further exploration of arterial stiffness, hemodynamic properties and connections to specific organ functions are warranted.

Data is scarce on the relationship between arterial stiffness and carotid ultrasound markers, and temporal associations are to our knowledge unexplored. It is of interest to explore the relationship between aortic PWV and flow parameters in the cerebral arteries, such as CCA, ICA and MCA. The role of hypertension in these relationships are also of interest.

In *Paper I*, duration of anti-hypertensive treatment did not significantly alter the risk of CMB. This calls for further exploration of the acute events associated with incident hypertension. A proteomic assessment of incident hypertension might shed light on mechanisms leading to increased risk of CMB, beyond those posed by longstanding hypertensive remodeling of the small vessels.

By the longitudinal design of the GÅS study, it is possible to explore temporal associations between hemodynamic manifestations and decline in organ function. The predispositions to and implications of cerebral aging are far from fully understood, and further research is warranted. The association of MRI markers of pathology and not only cognitive performance, but also movement disorders, incontinence, autonomic dysfunction, apathy and depression – as well as potential mediators of these links – requires further investigation. This field has been researched before, but larger epidemiological studies with brain MRI are scarce, and this is especially true for the older cohorts. Given the heterogeneity of lesions and manifestations, results are inconclusive.

The identified proteins in *Paper IV* have mostly been researched in biomedical settings; proper epidemiological research regarding risk factors and clinical

implications across the clinical spectra is called for. Longitudinal studies for each of the proteins are warranted in order to establish causal associations.

Our specific findings regarding the proteins associated with CSVD, call for further validation in separate cohorts.

It becomes increasingly clear that in evaluation of new drugs and treatment regimens for manifestations such as DM2, CVD, HT or CKD, diseases should not be viewed as separate manifestations but rather a continuum of related conditions. Clinical outcome measures should not focus on progression or regression of just the primary outcome, but rather the combined effect on all these manifestations. CVD is already an important secondary outcome in the evaluation of new drugs. It is time to make CSVD equally important.

Conclusion

The results presented in this thesis conclude that systemic hemodynamic properties are important risk markers for CSVD. Altered flow properties in the carotid arteries can be associated with MRI markers of CSVD, probably reflecting both increased cerebral vascular resistance and large vessel arteriosclerosis. The association of hypertension and blood pressure variables with deep CMB confirm previous studies suggesting that the pathophysiology of CMB differ depending on location. Moreover, it seems that in the hypertensive population, organ dysfunction such as CKD and CSVD might develop in concert. Finally, our findings on the circulating proteome associated with CSVD and atrophy builds upon previous theories of pathophysiological mechanisms. Although cerebral origin of the proteins cannot at all be assumed, they might reflect ongoing systemic or cerebral mechanisms. Increased levels of proteins crucial for BBB integrity, together with proteins previously associated with endothelial dysfunction and markers of vasodilation and angiogenesis might be reflective of the vascular remodeling associated with CSVD. Increased levels of apoptotic signals and immune modulators are in line with theories of CSVD and especially neurodegeneration being influenced by neuroinflammation. Among the identified proteins was also general markers of metabolic and ischemic burden and proteins mostly known to be tissue protective. The results of the proteomic analysis require validation in other cohorts.

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First and foremost, I wish to express my gratitude to my main supervisor Sölve Elmståhl. Your curiosity and never-ending enthusiasm enable the valuable research conducted on floor 13, and I am confident that it will lead to a better aging for many generations to come. I am inspired by your optimism and your scientific reasoning. I will take care not to go on scientific fishing-expeditions, and in life and in future research I will try to remember to (figuratively) kill my darlings.

Thank you, Arkadiusz Siennicki-Lantz, my co-supervisor, for your support and profound knowledge of the field. To every mechanistic reasoning you could always bring more valuable input, and everything I thought I discovered you somehow already knew. I believe that your involvement in the GÅS-project has been a key for building the rich foundation of data that can contribute to our understanding of cerebral aging for years to come.

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About the author

KATARINA ELLSTRÖM is a medical doctor currently undertaking her specialty training in general practice. This thesis explores cerebral aging in the general population, with a special interest in vascular contributions. All data comes from the ongoing population study Good Aging in Skåne (Gott Åldrande i Skåne, GÅS).

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