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# Magnetic resonance imaging markers of cerebral small vessel disease in an elderly population – association with cardiovascular disease and cognitive function

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**Abstract**

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Cerebral small vessel disease (SVD) is identifiable by clinical, neuroimaging, neuropathological and cognitive findings.

The aim of this thesis was to assess SVD and cerebral perfusion on magnetic resonance imaging (MRI) in a 75-year-old population and compare the findings with scars of myocardial infarctions, cardiovascular risk markers and cognitive function. In addition, the evolution of SVD over 5 years was studied.

The study population included subjects from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. The subjects had been chosen in a randomized manner from the register of the municipality. MRI of the brain and the heart, cognitive tests and blood tests for cardiovascular risk factors were performed in 406 subjects at age 75 years and 250 of them were re-examined 5 years later at the age of 80.

Paper 1 showed that unrecognized myocardial infarctions (UMIs) were found in 120 subjects (30%) and recognized myocardial infarctions (RMIs) in 21 (5%). Men with RMIs displayed an increased prevalence of cortical and lacunar cerebral infarctions, whereas women with UMIs more frequently had cortical cerebral infarctions.

Paper 2 showed that one or more brain infarcts were seen in 23% of the subjects (20% had only lacunar infarcts, 1% had only cortical infarcts and 2% had both). Hypertension and obesity were significantly associated with an increased risk of infarction. The newer risk markers investigated were not significantly associated with brain infarcts.

Paper 3 showed that MRI manifestations of SVD progressed over 5 years. Relative cerebral blood flow (rCBF) was not associated with WMH volume or progression of WMH volume.

Paper 4 showed that moderate to severe WMHs and incident lacunar infarcts on brain MRI were associated with a mild impairment of executive function.

In conclusion, this longitudinal population based study compares MRI manifestations of SVD with clinical data, providing knowledge that may be used in further investigations of preventive interventions and for identification of disease in early stages.

*Keywords:* Magnetic resonance imaging, cerebral perfusion, small vessel disease, cognitive function

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*To my Family, with all my love.*

# List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Ebeling Barbier C, **Nylander R**, Themudo R, Ahlström H, Lind L, Larsson EM, Bjerner T, Johansson L.: Prevalence of unrecognized myocardial infarction detected with magnetic resonance imaging and its relationship to cerebral ischemic lesions in both sexes. J Am Coll Cardiol. 2011 Sep 20;58(13):1372-7.

II. **Nylander R**, Lind L, Wikström J, Lindahl B, Venge P, Larsson A, Ärnlöv J, Berglund L, Ahlström H, Johansson L, Larsson E-M.: Relation between cardiovascular disease risk markers and brain infarcts detected by magnetic resonance imaging in an elderly population. J Stroke Cerebrovasc Dis. 2015 Feb; 24(2):312-8.

III. **Nylander R**, Fahlström M, Rostrup E, Kullberg J, Damangir S, Ahlström H, Lars Lind L, Larsson EM.: Quantitative and qualitative MRI evaluation of cerebral small vessel disease in an elderly population – a longitudinal study.

IV. **Nylander R**, Kilander L, Ahlström H, Lind L, Larsson EM.: Small vessel disease on neuroimaging in elderly individuals: association with cognitive and executive tests.

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# Contents

# Abbreviations

| | |
|---|---|
| CBF | Cerebral blood flow |
| CI | Cortical infarct |
| CT | Computed tomography |
| BMI | Body mass index |
| DSC | Dynamic susceptibility contrast |
| FLAIR | Fluid attenuated inversion recovery |
| Gd-DTPA | Gadolinium diethylenetriaminepentaacetic acid |
| GM | Gray matter |
| LI | Lacunar infarct |
| MCI | Mild cognitive impairment |
| MB | Microbleed |
| MRI | Magnetic resonance imaging |
| MMSE | Mini-mental state examination |
| MI | Myocardial infarction |
| NT-pro BNP | N-terminal pro-brain natriuretic peptide |
| RMI | Recognized myocardial infarction |
| ROI | Region of interest |
| OR | Odds ratios |
| PD | Proton density |
| PIVUS | Prospective investigation of vasculature in Uppsala seniors |
| SVD | Small vessel disease |
| SD | Standard deviation |
| SWI | Susceptibility weighted imaging |
| T | Tesla |
| TE | Echo time |
| TR | Repetition time |
| T1W | T1-weighted |
| T2W | T2-weighted |
| TMT | Trail Making Test |
| UMRI | Unrecognized myocardial infarction |
| WHO | World Health organization |
| WMH | White matter hyperintensity |
| WM | White matter |

# Introduction

## Cerebrovascular disease (CVD)

Cerebrovascular disease (CVD) refers to a group of conditions that affects blood circulation in the brain. It affects large vessels, causing ischemic and hemorrhagic infarcts, and small vessels, causing subcortical small vessel disease (SVD). CVD continues to be a significant public health burden in the elderly population and may be present in demented and non-demented individuals. CVD accounts for 6.5 million deaths worldwide (ischemic stroke 3.3 million, hemorrhagic stroke 3.2 million), which is equivalent to 11.6% of all deaths (1).

The etiology of CVD involves several risk factors, such as hypertension, diabetes, smoking, obesity and atrial fibrillation (2). The risk factors affect vessels (causing stenosis, clot formation, embolism, damaging vessel wall, rupture hemorrhages) with disruption of the blood supply to the brain tissue, causing ischemia.

## Small vessel disease (SVD)

SVD is a part of a cerebrovascular disease process and is an important cause of cognitive decline and functional loss in the elderly (3, 4). It contributes to up 45% of dementias and is responsible for approximately a fifth of all strokes worldwide. SVD may cause small deep infarctions, white matter changes, cerebral microhemorrhages and prominent perivascular spaces associated with vascular ectasia and brain atrophy (3).

Included in SVD are all small vascular structures, small arteries, arterioles, capillaries, venules and small veins, located in the parenchyma or in the subarachnoid space (5) Figure 1. By definition, SVD involves cerebral vessels less than 500 µm in diameter and located deeper than in the cortex (6). Usually, the disease involves the arterial tree, small arteries or arterioles, which differ from the capillaries due to the presence of a lamina elastica. There are two arterial origins: superficial, which are terminal branches of medium sized arteries (originating from large arteries) in the subarachnoidal space and deeper, at the base of the brain as a perforating arteries (originating from the large vessels).Those two arterial systems tend to merge in the deepest areas of subcortical white matter, the so-called watershed zone (5).
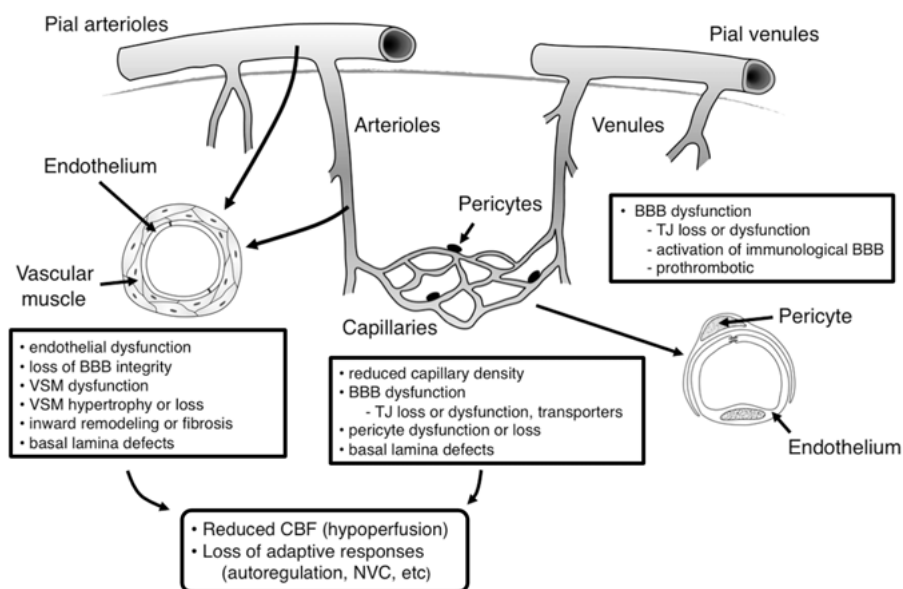
Figure 1. Schematic diagram of key elements of the cerebral microcirculation of SVD. BBB = blood-brain barrier; CBF = cerebral blood flow; NVC = neurovascular coupling; TJ = tight junction; and VSM = vascular smooth muscle From Joutel et al., Stroke. 2014; 45:1215–1221. Reprinted with permission from the journal.

In current standard neuroimaging terminology , the term SVD refers to pathological changes in the brain parenchyma rather than an underlying disease of the vessels (6). The following is an etiological classification of cerebral small vessel disease, modified from Pantoni (5):

Type 1.   Arteriolosclerosis (age, vascular risk factors-related SVD).
Type 2.   Cerebral amyloid angiopathy (sporadic and hereditary).
Type 3.   Inherited or genetic SVD (CADASIL, CARASIL, Fabry's disease, SVD due to COL4A1 mutations, etc.)
Type 4.   Inflammatory and immune-mediated SVD (systemic and cerebral vasculitis, central nervous system vasculitis secondary to infection).
Type 5.   Venous collagenosis.
Type 6.   Other SVD: post-radiation angiopathy, non-amyloid microvessel degeneration in Alzheimer's disease.

The history of SVD includes more than 100 years of research, starting with the work of Alois Alzheimer and Otto Binswanger, who described subcortical vascular pathology of demented subjects and called it "atherosclerotic dementia" and "atherosclerosis senilis" (7). Later, detailed information on the etiology and pathology of the disease came from vascular post-mortem examinations by C. Miller Fisher in 1955–1973. His pathological studies were focused

on the lacune (a fluid-filled cavity occurring long after the patient's original stroke), which he considered to represent a LI (3).

The pathological process of SVD is complicated and still not completely understood. The pathophysiological process of vessel wall endothelium damage involves a complex of mechanisms leading to increased permeability with leakage of material into the vessel wall and perivascular tissue. Also, in combination with inflammation, demyelination, glial scarring, thickening and stiffening of the vessel wall, SVD will lead to a late stage of focal brain parenchymal ischemia and infarction (3). It has been hypothesized that parenchymal damage to the brain is caused primarily by chronic ischemia and impaired autoregulation related to the structural changes in the walls of small vessels due to endothelial dysfunction (5).

According to STRIVE (standards for reporting vascular changes on neuroimaging), there are six subtypes of SVD, which include (4): 1) recent small subcortical infarcts, 2) lacunes of presumed vascular origin, 3) white matter hyperintensity of presumed vascular origin, 4) perivascular spaces, 5) cerebral microbleeds (MBs), 6) brain atrophy.

SVD is related to aging and several cardiovascular risk markers, hypertension, diabetes, smoking, obesity, all thought to be causal factors related to SVD as well as to CVD (5). Among all risk factors, hypertension is most consistently associated with SVD (3, 8, 9).

## Neuroimaging of small vessel disease

Neuroimaging techniques play an important role for the evaluation of SVD. Based on imaging of brain parenchymal abnormalities and vasculature impairment or cerebrovascular supply, it is possible to diagnose the severity of the disease and monitor it.

### Morphological imaging

Computed tomography (CT) and magnetic resonance imaging (MRI) are techniques for the evaluation of parenchymal markers of SVD (2).

CT is commonly used for the evaluation of changes in the white matter and gray matter. It is a valuable routine neuroimaging technique for the assessment of chronic infarctions (cortical and lacunar) and WM lesions, mostly confluent. SVD-related white matter changes are seen as less pronounced hypodensities but never more pronounced than cerebrospinal fluid due to a decrease in X-ray attenuation. White matter changes tend to be symmetrical throughout the brain in the periventricular and deep white matter (6). The hypodensity is due to myelin and axonal loss (10).

LIs appear as low attenuation small lesions (3–15 mm). Only about 50% of recent lacunar infarcts can be detected on CT (3). Accidentally detected lacunar infarcts without neurological symptoms are called silent lacunar infarcts.

Smaller and more discrete parenchymal lesions are difficult to assess by CT due to insufficient soft tissue contrast.

MRI plays a leading role in brain imaging (4, 11). The heterogeneous parenchymal changes seen in SVD are better seen on MRI than on CT due to the higher soft-tissue contrast, allowing improved discrimination of different tissue types. It is useful for determining evidence of stroke and for assessing age (acute or chronic) and severity of white matter changes, which on MRI are called WMHs. Assessment of MBs on MRI, with hemosiderin-sensitive sequences (SWI or T2*), has great diagnostic importance. MRI is also a valuable tool to assess local or general cerebral atrophy (2, 4, 5).

WMHs of presumed vascular origin tend to be symmetrically distributed in the periventricular (adjacent to the ventricles) or in the "deep" white matter (located away from ventricles up to 1 cm in the subcortical white matter). The periventricular white matter (WM) is in a terminal zone of arterial supply and is vulnerable to decreased blood flow (12). The pathogenesis of WML is often ischemic due to small vessel arteriosclerosis. However, it may be a complicated cascade of mechanisms which affect ependymal lining, with increased CSF transependymal passage and accumulation of interstitial fluid and subependymal gliosis, which generally occurs before deep WMHs as an aging phenomenon. (12). Deep WMHs appear due to ischemic damage in small vessels, causing hypoperfusion and incomplete infarction (6, 13). As WMHs tend to progress they become confluent. WMHs appear bright on T2-weighted, fluid-attenuated inversion recovery (FLAIR) and proton density (PD) MRI sequences. The size of WMHs is variable, and in the deep WM ranges from punctuate over patchy to confluent changes with mostly indistinct and irregular borders (6). On T1-weighted images, SVD-related WMHs are not visible. This is different from multiple sclerosis, where patchy WML may turn into " black holes" on T1-weigthed images (6).

Hyperintense changes in the subcortical gray matter or in the brain stem are not included in the term WMHs of presumed vascular origin (4), and the collective term recommended is subcortical hyperintensities (4).


## Qualitative evaluation of SVD

Visual assessment on conventional MRI is a rapid and relatively simple assessment technique. To evaluate the severity of WM changes, there are several morphological rating scales, such as the Fazekas scale and modified LADIS scale.

The Fazekas rating scale is divided into periventricular hyperintensities (PVH) and deep white matter hyperintensities (DWMH). PVH: 0 = absence, 1 = caps or pencil-thin lining, 2 = smooth "halo" or 3 = irregular PV extending

deep into the WM. DWMH: 0 = absence, 1= punctate foci, 2= beginning of confluence foci, 3 = large confluent areas. A simplified version of the Fazekas scale is often used (12): score 1: punctate lesions, score 2: early confluence of lesions, score 3: confluent lesions.

The LADIS rating scale is based on the visual Fazekas scale. Grade 1: (mild changes), single lesions <10 mm and /or areas of grouped lesions <20 mm in any diameter. Grade 2: (moderate changes), single hyperintense lesions 10–20 mm and hyperintense areas linked by no more than "connecting bridges" >20 mm. Grade 3: (severe changes), both single and confluent hyperintense areas ≥20 mm in any diameter (14) (Figure 2 LADIS grade 1-3).

Lacunar infarctions of presumed vascular origin (LIs) are focal areas of rounded, ovoid or tubular shaped cavities, located in the deep gray matter or WM. LIs have a diameter of at least 3 mm but less than 15 mm. LIs can be embolic, atheromatous or thrombotic.

On magnetic resonance imaging (MRI), LIs appear as lesions with reduced signal intensity on T1-weighted images, increased signal on T2- weighted images and fluid attenuated inversion recovery imaging (FLAIR) and can have a central CSF like hypointensity surrounded by a rim of hyperintensity (4, 5), which would be specific for a lesion of ischemic origin (15, 16) (Figure 3 lacunar infarct T1, T2).

If LIs are acute, they appear as increased signal on diffusion-weighted imaging (DWI) and reduced signal on apparent diffusion coefficient (ADC) map because they have decreased diffusion and are detected at least 70% of the time as acute LIs (3). Many LIs are silent, i.e., without neurological symptoms (3).

Cerebral MBs are seen on MRI as small punctuate lesions up to 10 mm diameter of low signal on gradient echo sequences (T2* or susceptibility weighted images = SWI sequences) and are often not seen on conventional morphological scans (Figure 4 microbleeds). The signal drop is usually over-estimating the size of the lesion due to the susceptibility effect. These lesions correspond to small perivascular collections of hemosiderin-containing macrophages (3).They are commonly located in the cortico-subcortical junction and deep gray matter or WM in cerebral hemispheres, brain stem or cerebellum (4). Lobar MBs are more common in cerebral amyloid angiopathy, Alzheimer's disease and vascular dementia, while a central location is more characteristic of systemic vascular disease (12).

Brain atrophy (BA). Many studies report an association between SVD and BA, general or focal, affecting only particular lobes or regions (4). In the context of neurovascular diseases, cortical atrophy could be due to neuronal loss, cortical thinning, subcortical vascular changes, atherosclerosis or venous collagenosis and/or WM rarefaction (4, 17).
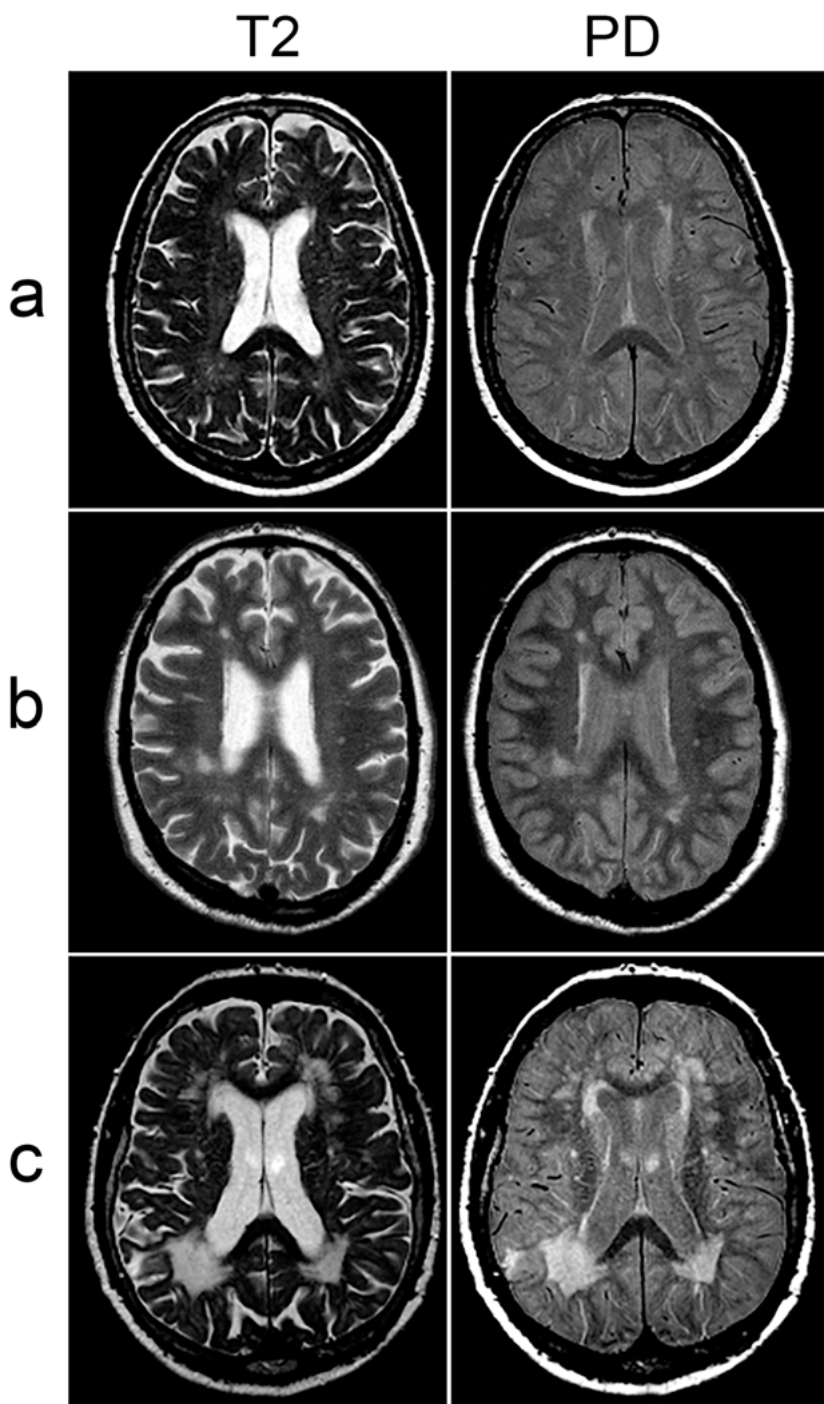
Figure 2. WMHs on MRI of the brain: T2-weighted and PD weighted images show-ing three grades of severity according to the LADIS visual scoring scale (modified Fazekas scale): a, mild; b, moderate; c, severe.
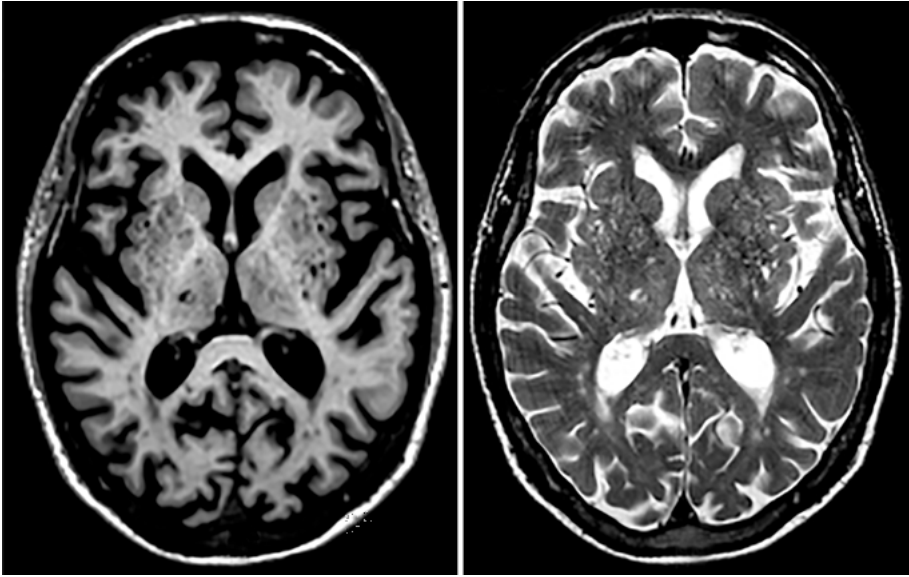
Figure 3. Lacunar infarcts and enlarged perivascular spaces on T1-weighted and T2-weighted MR images.
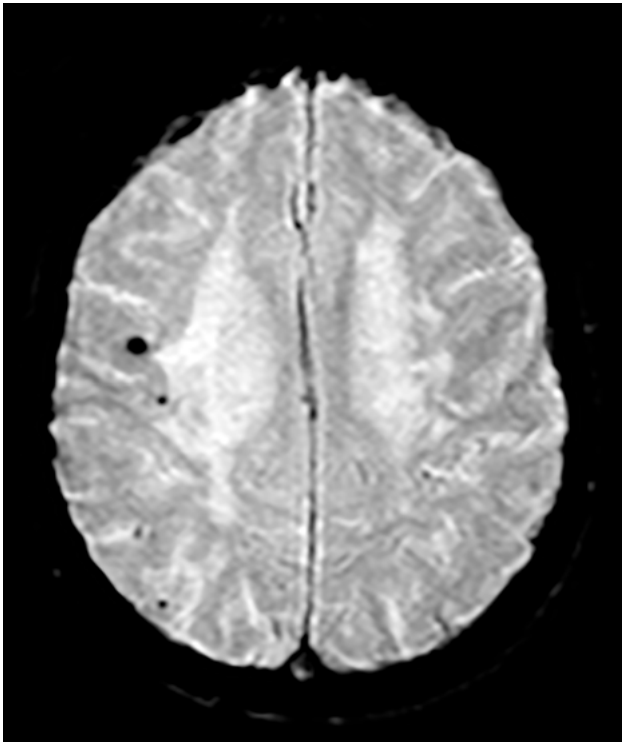


Figure 4. Microbleeds on T2*-weighted MR image.

## Quantitative evaluation of SVD

WM, gray matter and CSF segmentation could be performed using different automated segmentation methods, e.g., Freesurfer (V5.3. http://surfer.nmr.mgh.harvard.edu/). Intracranial volume, WM and gray matter (GM) volumes are extracted from the segmentation results. An increasing interest over past the decade has been to find accurate segmentation methods for WMHs (18). In the current study, we used the Cascade method for WMH volume. Quantitative susceptibility mapping (QSM) is used for the assessment of MBs using gradient-echo SWI or T2* imaging.

## Imaging methods for perfusion evaluation

Cerebral perfusion imaging compromises methods to assess the cerebral blood flow (CBF) through the capillary bed. Different perfusion techniques include single photon emission computed tomography (SPECT), positron emission tomography (PET), xenon-based computed tomography (Xenon CT), CT perfusion and MRI perfusion (6).

SPECT uses lower energy photon emitting radionuclides. Radio-isotopes used in this technique emit gamma rays. The source of radiation is detected by photomultiplier crystals after filtering through collimators to locate the origin of the radiation in three dimensions. The ligands are highly lipophilic. They are taken up and bound in the brain and by metabolism into hydrophilic compounds that give a snapshot of CBF within a minute after injection (6). The most common clinical use is in the differential diagnosis of Alzheimer's disease, but also to investigate perfusion differences in SVD and rare types of dementia.

PET uses coincidence radiation employing positron-emitting radionuclides. The most widely used compound for CBF imaging is water, using positron-emitting oxygen-15 ($^{15}$O) to produce $H_2$$^{15}$O. This allows accurate measurement of oxygen utilization and the oxygen extraction fraction (OEF) for directly repeated measurements of arterial and venous levels of $^{15}$O. Oxygen-15 needs to be produced in a cyclotron and has a short half-life (2 min) why this method can only be used in centers with a cyclotron close to the PET-scanner room (6). In PET perfusion studies, fluorine-18-labelled deoxyglucose ($^{18}$F-FDG) is often used to measure regional glucose uptake, which represents aerobic and anaerobic glucose metabolism, thereby giving an estimate of relative cerebral blood flow (rCBF). The half-life of $^{18}$F is 110 minutes allowing also fast transportation to sites without a cyclotron (6).

Xenon gas-based CT perfusion is used to measure CBF using a tracer, xenon gas, which can be inhaled. It dissolves and diffuses rapidly through the blood-brain barrier A washout mechanism is used, and initial equilibrium of the gas is reached in the tissue, after which the supply is discontinued and the concentration decreases, which corresponds to cerebral perfusion (6).

CT perfusion involves repeated sequential acquisition during intravenous iodinated contrast agent injection. Analysis of contrast enhancement curves, according to the central volume principle, allows calculation of CBF and mean transit time (MTT), and the method can potentially be used for absolute CBF value calculation if information regarding arterial input and venous outflow curves can be calculated (6).

Perfusion MRI techniques are based on the use of an exogenous or an endogenous tracer. The exogenous tracer method is most widely used and relies on the intravenous injection of a paramagnetic gadolinium-based contrast agent.

The most commonly used MR perfusion technique is dynamic susceptibility contrast (DSC) imaging. The DSC method was first described in 1988 as a method which uses dynamic tracking of an intravenously injected paramagnetic contrast agent bolus with echo planar imaging (EPI) to capture the first pass of the injected contrast agent (19, 20).

This technique includes both a short examination time and a high signal-to-noise ratio. The contrast agent (a gadolinium chelate) is injected intravenously, causing changes in the MR signal as the agent passes through the vasculature. Deconvolution of the measured signal-time curves is performed using singular value decomposition with arterial input function (AIF) using the method described by Tikhonov (21).

The vessel and tissue concentration of contrast agent during one single transit of contrast agent through the tissue is calculated. The signal intensity is then measured and perfusion maps are calculated, most commonly of rCBF, relative cerebral blood volume (rCBV), mean transit times (MTT) and time-to-peak (TTP).

CBV refers to the volume of blood in a given region of brain tissue, measured in mL per 100 g of brain tissue. Deconvolution using the AIF is used for calculation of CBF and MTT. CBF refers to the volume of blood per unit of time passing through a given region of brain tissue (measured in mL per minute per 100 g of brain tissue). MTT refers to the average time it takes for blood to pass through a given region of brain tissue, measured in seconds. TTP refers to the time it takes for an intravenously injected bolus of contrast material to arrive at a given region of the brain, measured in seconds. Temporal resolution is usually set to 1.5 seconds or faster. It is difficult to obtain absolute CBF values using this DSC perfusion method. Hence, rCBF values are determined by measuring signal intensity on the calculated CBF map in a region of interest (ROI), for example in an ischemic region, and dividing it by the rCBF in normal appearing tissue, e.g. contralateral white matter or the cerebellum.

The dynamic contrast-enhanced (DCE) perfusion MRI technique uses calculation of perfusion parameters by repeated T1-weighted scans with signal changes induced by paramagnetic contrast bolus passage in tissues over time. The most commonly calculated parameter is k-trans (transfer constant). An

intravenous contrast bolus is injected, and rapid repeated T1-weighted imaging is performed. Regional signal increase on T1-weighted images (T1 shortening) is due to the gadolinium concentration, which can be a result of intravascular gadolinium (i.e. true perfusion) and accumulation of gadolinium in the extravascular space (i.e. permeability). With pharmacokinetic modelling, a number of regional values can be derived: k-trans, rate constant, fractional volume of extravascular-extracellular space, fractional volume of the plasma space (22).

Arterial spin labeling (ASL) perfusion MRI uses an endogenous tracer. ASL works by applying a magnetic tag to the blood flowing toward the skull. By measuring signal changes between tagged images and baseline untagged images, qualitative and quantitative CBF images can be obtained (6). This technique is entirely non-invasive and can be repeated often; however, multiple acquisitions may be required as the signal-to-noise ratio is low. The technique is less well developed for routine clinical use; however, it is mostly used for research. ASL needs further development with regard to CBV, flow quantification measurements and reproducibility of absolute measures of brain perfusion (23).

# Cardiac MRI

Myocardial infarction (MI) is defined as death of myocytes due to ischemia. MI can occur with typical, atypical or without symptoms and could be detected by ECG, biochemical markers or cardiac imaging (24).

Late enhancement (LE) (delayed enhancement) MRI is used to image myocardial scars. Late enhancement MRI has been specifically developed to image myocardial viability. Delayed enhancement cardiac MRI was first described more than 20 years ago. Currently, delayed myocardial enhancement MRI is used as a standard for the evaluation of scar formation after MI (25). After administration of gadolinium-based contrast media, the technique uses an inversion recovery (IR) sequence pulse to null normal myocardium, followed by segmented k-space gradient-echo acquisition. Retention of contrast material increases the signal intensity on T1W imaging relative to normal myocardium. This technique is capable of detecting myocardial infarctions and could be used to identify a variety of other disorders, inflammatory/infections, cardiomyopathy, cardiac neoplasms. However, this technique was developed primarily for characterization of scarring after myocardial infarction. (25).

Normally, the technique requires gadolinium chelate (Gd) contrast material, 0.1–0.2 mmol/kg followed 10–30 min later by a cardiac-gated T1-weighted pulse sequence. Myocardial enhancement is present when intravascular Gd concentration declines, which refers to late (or delayed) enhancement. This is due to increasing Gd concentration in the interstitial space between collagen fibers in scar tissue (compared to the densely packed myocytes

in normal tissue) or due to change of integrity of myocyte cell membranes in acute MIs (26). Irrespective of when the imaging is performed within this time-frame, the size of the enhanced region does not change provided that the inversion time is adjusted appropriately in order to 'null' normal myocardium (26). However, optimal demarcation of a chronic MI is seen at two time points: 6 to 9 minutes after contrast agent injection (bright blood and dark normal myocardium), and 25 to 30 minutes after contrast agent injection (bright scar and dark normal myocardium) (27). Late enhancement that involved the subendocardial layer was considered to represent an MI scar and should be visible in short and long axis images (28, 29). The MI scar or fibrosis refers to an area of high signal intensity and is subendocardia or transmural in a coronary artery distribution (25).

# Cardiovascular risk factors/markers

Established cardiovascular risk markers are, for example, hypertension, diabetes, obesity and smoking. Potential new markers are for example N-terminal pro-brain natriuretic peptide, troponin C-reactive protein, cystatin C and I.

## Established cardiovascular risk markers

Hypertension is a well-established risk factor and is strongly associated with all types of brain infarct (30-32), cognitive decline and dementia (33). Thus, hypertension is an important factor for the pathogenesis for LIs (30) in small vessel disease.

Diabetes is associated with an increased risk for stroke and is associated with silent brain infarctions (34). Diabetes is related to cognitive impairment, Alzheimer Disease and all dementias, including vascular dementia (35).

Smoking was one of the risk factors for recognized LIs (31), but there is no strong association with silent brain infarcts (32).

HDL cholesterol has shown an inverse relation to stroke in most epidemiological studies (36). A previous study showed that serum cholesterol (total cholesterol, LDL cholesterol and non-HDL cholesterol) showed a significant association with silent brain infarcts (37).

## New cardiovascular risk markers

NT-proBNP is a marker released in response to myocardial stretch and is valuable for the diagnosis of heart failure and as a marker of increased mortality and morbidity in cardiovascular events (38). It has also been shown to be strongly associated with cardioembolic stroke (39). Cardiac troponins, intracellular proteins involved in heart muscle contraction, are biochemical markers of myocardial damage (39). Troponins are used as biomarkers to detect

acute coronary syndrome and may also be elevated in other diseases such as renal failure, atrial fibrillation and acute stroke (3).

CRP is a marker of inflammation, involved in the endothelial inflammatory response, that predicts stroke and cardiovascular events (40, 41).

Cystatin C is mainly used as a marker of renal function and has been reported as a strong predictor of cardiovascular death in elderly people (42).

# Cognitive function

As people age, they change both biologically and psychologically. Aging causes neuroanatomical and neurophysiological changes in the brain, in the vasculature and in cognition. The brain changes may affect molecular aging, intracellular aging, tissue aging and organ aging. Cognitive impairment, depression, walking difficulties and urinary incontinence are the main contribution to loss of functional independence in aging (14). The complexity of both the neural and cognitive functions, however, makes exact mapping between brain and behavior extraordinarily difficult (43).

The most widely cognitive changes in aging are associated with memory decline. Memory is divided into four subgroups: episodic, semantic, procedural and working memory. Episodic memory, where information stored "with mental tags", and semantic memory "memory of meanings" are most important in aging (43).

Mild cognitive impairment (MCI) is a stage between the expected cognitive decline of normal aging and the more serious decline of dementia. It is usually but not necessarily progressive. Most commonly it is associated with memory, language, thinking and judgment that are greater than normal age-related changes. Many of the symptoms that cause dementia have a progressive insidious course (5–10 years) and a prolonged end-stage period. Age-related incidence are the most common cause of dementia (12). SVD, particularly WMHs and LI, is associated with cognitive function. WMHs show a relationship with cognitive decline, motor performances, depressive symptoms, urinary disturbances and various neurological abnormalities in aging in the LADIS study (44).

## Cognitive tests

Several tests are used to evaluate cognitive function, e.g. mini-mental state examination (MMSE), trail making test (TMT), seven minute screen test (7MS). The MMSE test is best known and most widely used (45). Performance on these tests decreases with age and is poorer in individuals with a low level of education (46).

MMSE includes 11 questions, requires only 5–10 min to perform, and is a composite test mainly of memory and verbal functions (25). The test is designed to differentiate organic from functional psychiatric syndromes, identifying patients with mild cognitive impairment and Alzheimer´s disease (45).

Trail Making Test A and B (TMT-A and TMT-B) give information on visual search, scanning , speed processing, mental flexibility and executive functions (46).

TMT has two parts: TMT-A requires subjects to draw the lines connecting 25 encircled numbers, distributed on a sheet of paper, as fast as possible. The TMT-B is more demanding since the subject is asked to draw lines in the right order as fast as possible between targets that alternate between figures (1–13) and letters (A–L). The score on each part represents the time required to complete the task: normal test performing time should be less than 50 sec. If the test time reaches 180 sec, it suggests the presence of pathologic changes. The TMT provides information on visual search, speed of processing, mental flexibility and executive functions (46).

7MS is a compilation of the temporal orientation test involving cued recall, clock drawing and verbal fluency. It has been used for patients with memory complains and is highly sensitive to AD (45).

# Aims of the Thesis

## General aim

The overall aim of this thesis was to assess the prevalence of SVD and cerebral perfusion in a 75-year-old population and correlate the findings with cardiovascular risk factors, biochemical markers of arteriosclerosis, silent myocardial infarctions and cognitive function and to study the evolution of SVD over the course of 5 years.

## Specific aims

**Paper I**: To investigate the prevalence of MRI-detected unrecognized myocardial infarction (UMI) and whether it is related to cerebral ischemic lesions on MRI in an elderly population-based cohort.

**Paper II**: To investigate the associations between established and new cardiovascular risk markers and brain infarcts detected on MRI in a 75-year-old population.

**Paper III**: To assess the imaging manifestations of cerebral SVD and their evolution over the course of 5 years in an elderly population by qualitative as well as quantitative MRI and to investigate whether cerebral blood flow (rCBF) at baseline is related to the progression of WM lesions.

**Paper IV**: To evaluate imaging manifestations of SVD and cerebral perfusion by magnetic resonance imaging (MRI) and their relationship to cognitive function in a 75-year-old population. In addition, to study the relationship between progression of SVD and decline of cognitive function during the following 5 years.

# Materials and Methods

## Study population

The study population included subjects from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study (47), Figure 5. The subjects had been chosen in a randomized manner from the register of the municipality. At the age of 70 years, 2025 subject were invited and 1016 of them agreed and gave written informed consent to participate in the study. Subjects with a history of disease or with current disease were not excluded. Five years later, 52 subjects had died, and the remaining 964 were invited again. From this cohort, 827 agreed to participate. MRI of the brain was performed in 406 randomly selected subjects (210 men) at the age of 75 years. The major measurements performed at the age 70 were repeated at age 75. In addition, there was a focus of interest on infarcts in the brain and heart, so both of these items were investigated by brain and myocardial MRI. Also, cognitive tests were included in the reinvestigation. The first MRI brain cohort was scanned at the age of 75 from 2006 to 2009. Five years later, 250 of these subjects were re-examined with a shorter MRI protocol during the years 2011 to 2014.
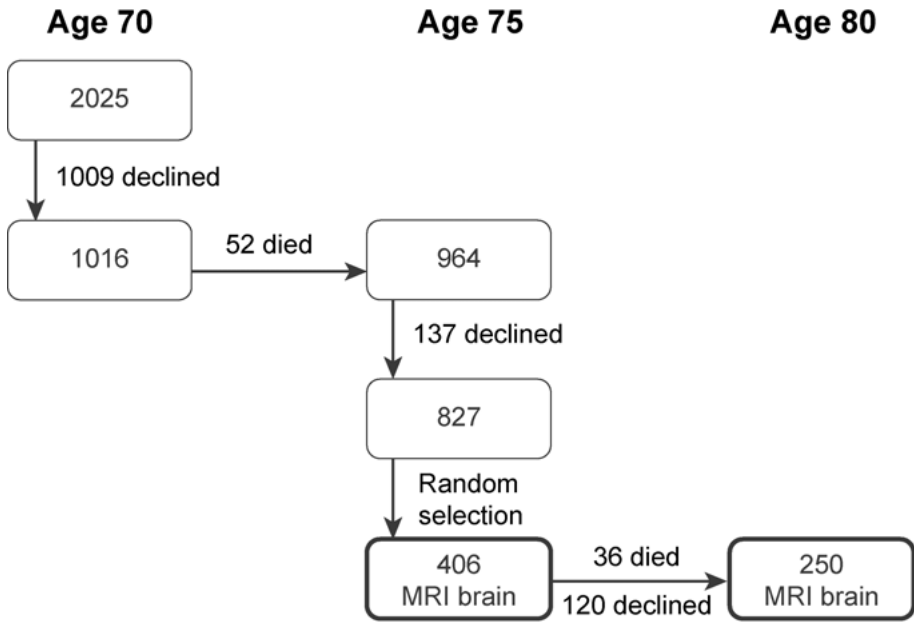
# PIVUS - MRI brain flowchart



Figure 5. Schematic overview of the study design showing the number of subjects examined by MRI of the brain at age 75 and 80 years

## MRI

MRI was performed in the 75-year-old subjects on a 1.5 Tesla MRI system (Gyroscan Intera, Philips Medical Systems, Best, the Netherlands) with a 25 mT/m gradient system.

MRI of the heart (paper I only) was performed using the standard SENSE cardiac coil in the supine position with retrospectively gated vector ECG for cardiac triggering.

Approximately 20 to 40 min after contrast injection, late-enhancement images were acquired using a 3-dimensional inversion recovery gradient echo sequence covering the entire heart in short and long axis views. The inversion time was individually adjusted to null viable myocardium for every subject.

MRI of the brain (papers I, II, III, IV) was performed using a quadrature head coil (receive only). The protocol included sagittal 3-dimensional T1-weighted (T1-w) gradient echo images, transverse proton density weighted (PD) and transverse T2-weighted (T2-w) turbo spin echo (TSE).

Cerebral perfusion MRI (papers III, IV) was performed using DSC MRI with contrast agent injection in the 75-year-old subjects. Perfusion MRI was not repeated in the 80-year-old subjects due to patient age and potential risk of contrast-induced nephropathy.

## Image analysis

For assessment of the images, a PACS workstation (Carestream PACS, Carestream Health Inc., Rochester, NY, USA) was used.

Cardiac LE-MRI was assessed by a radiologist who was blinded to information on any previous disease and to the cerebral MRI findings. Left ventricular myocardium nulled by the inversion pulse was visually assessed as viable, whereas left ventricular myocardium showing late enhancement (i.e., hyperintense myocardium) was classified as nonviable. To classify myocardium as nonviable, late enhancement had to be visible in short and long axis images. Late enhancement that involved the subendocardial layer was considered to represent an MI scar (14,15) and is hereafter referred to by that term.

The cerebral MR images were assessed by a neuroradiologist who was blinded to information of any previous disease. LIs were defined as hypointense foci (3–15 mm size), on 3D T1-w images. They were further defined to be isointense on PD-weighted images and hyperintense on T2-weighted images, surrounded by WM or subcortical gray matter, and not located in areas with a high prevalence of widened perivascular spaces. Recognized brain infarcts were infarcts seen on MRI and associated with clinical symptoms reported by the patients in the questionnaire and thereafter verified by data in the clinical patient record by an experienced clinician. Silent brain infarcts were infarcts seen on MRI without reported clinical symptoms.

Calculation of perfusion data and the corresponding maps of relative cerebral blood flow (rCBF) were done using the software NordicICE (NordicNeuroLab AS, Bergen, Norway).

## Cognitive tests

Cognitive function was assessed using the Mini-Mental State Examination (MMSE), the Trail Making Test A (TMT-A) and the Trail Making Test B (TMT-B) in the 75- and 80-year-old subjects.

## Cardiovascular risk factors/markers

Markers of cardiovascular risk factors were available at both ages (48). Systolic and diastolic blood pressure, hypertension prevalence, smoking, diabetes mellitus, serum total cholesterol and body mass index were used to adjust the statistical analyses.

## Data analysis and visual image evaluation

WMHs were assessed qualitatively (visual scoring) and quantitatively (semi-automatic volume calculation). LIs and MBs were assessed visually, and the perfusion maps were evaluated with regard to regional relative CBF and supratenotrial relative CBF, Freesurfer (V5.3. http://surfer.nmr.mgh.harvard.edu/) was used for the classification of tissue types and segmentation of different regions in the brain. Three tissue classes were defined: WM, gray matter and CSF. Segmentation of gray and WM and different regions and calculations of total WM and GM volumes were made.

# Ethical considerations

The Regional Ethical Review Board in Uppsala Sweden approved the study and all subjects provided written informed consent.

# Statistical analysis

In **paper I**: StatView version 5.0.1 (SAS Institute, Cary, NC, USA) was used for statistical analyses. The Fisher exact test was used to estimate differences between groups. Logistic regression (LR) was performed to test interactions between gender and the 2 MI types and to test interactions between CRF and the 2 MI types. The significance level was set at 0.05 in all analyses.

In **paper II**: Data were presented as means, standard deviations (SD) and min and max values for continuous variables and as numbers and percentages for dichotomous variables. Univariate associations and associations adjusted for gender, between outcomes and risk markers were assessed with LR models. Further, in multiple LR models we sought to find independent risk markers among statistically significant risk markers in the univariate analyses. Results from the LR models were presented in forest plots as estimated odds ratios with 95% confidence intervals (CI) of a one SD increase for continuous risk markers and of a one unit increase for dichotomous variables.

All statistical tests and confidence intervals were two-sided, and a statistically significant result was declared if the CI did not include unity. No adjustments for multiple tests were made in this exploratory study. All analyses were performed with the statistical program package, SAS 9.3 (SAS Institute, Inc., Cary, NC, USA).

In **paper III**: STATA14 (Stata Inc, College Station, TX, USA) was used for calculations. $P<0.05$ was regarded as significant. Relationships between two categorical variables were assessed by chi-square tests. Relationships between categorical variables and continuous variables were assessed by one-way ANOVA. These analyses were adjusted for gender. Relationships between changes between age 75 and age 80 in one variable vs. changes in another variable (e.g., change in lacunar infarction status vs. change in WM lesion volume) were assessed by random-effects mixed models with random intercept adjusting for the baseline value of the exposure and gender.

In **paper IV**: the relationships between the cognitive function tests and markers of cerebral small vessel disease at age 75 (cross-sectional) were evaluated with Tobit regression (since all three tests were censored at high levels). These models were adjusted for sex, education level, systolic blood pressure, HDL and LDL cholesterol, BMI, smoking and diabetes.

The relationships between changes in the cognitive function tests and changes in markers of cerebral small vessel disease over 5 years (longitudinal) were evaluated with mixed models with a random intercept. Also, these models were adjusted for sex, education level, systolic blood pressure, HDL and LDL cholesterol, BMI, smoking and diabetes.

# Results in summary

**Paper I**: Unrecognized myocardial infarctions (UMIs) were found in 120 subjects (30%) and recognized myocardial infarctions (RMIs) in 21 (5%). The prevalence of UMIs (*P*=0.004) and RMIs (*P*=0.02) was greater in men than in women. Men with RMI displayed an increased prevalence of cortical and lacunar cerebral infarctions, whereas women with UMI more frequently had cortical cerebral infarctions (*P*=0.003).

**Paper II**: One or more infarcts were seen in 23% of the subjects (20% had only LIs, 1% had only cortical infarcts and 2% had both). Hypertension (odds ratio [OR] 2.6, 95% confidence interval [CI] 1.4, 4.7) and obesity (OR 1.3, CI 1.0, 1.8) were significantly associated with increased risk of brain infarction. The newer risk markers were not significantly associated with the brain infarcts.

**Paper III**: A significant progression of the WMH score and WMH volume occurred over the 5 years (*P*<0.0001). New LIs were seen in 10%. Subjects with new LIs at age 80 showed a more pronounced increase in WMHs (*P*<0.0001).

MBs were present in 14% at age 75. The visual WMH score was significantly associated with the presence of MBs (*P*<0.0001). There was no relationship between total WM rCBF and WMH volume at age 75, and no significant associations between regional or total rCBF at age 75 and changes in WMH volume over 5 years. The total WM and GM volume decreased significantly between ages 75 and 80 (*P*<0.0001).

**Paper IV**: TMT-B at age 75 was significantly related to WMH visual scoring comparing moderate and severe with no WMH following adjustment for sex, education level and baseline levels of systolic blood pressure, HDL and LDL cholesterol, BMI, smoking and diabetes (*P*<0.05).

MMSE and TMT-A were not significantly related to WMH visual scoring, WMH volume, WM volume or the number of MBs nor to brain perfusion at the age 75.

After 5 years, there was progression of WMHs and LIs. Those subjects, who had had at least one LI 5 years later showed a greater increase in TMTA

(= decreased cognition) than those who did not have any LI (*P*=0.003) follow-ing adjustment for risk factors as above. The same trend was seen for TMTB (but not MMSE) (*P*=0.24).

The change in WMH volume, WMH visual scoring, or cerebral perfusion at baseline were not significantly related to the cognitive function tests.

# Discussion

## General discussion

SVD has multiple etiologies and manifests in a variety of disorders, typically as a WM lesions, LIs, MBs, vascular cognitive impairment and other vascular condition, affecting small vessels (5).

Histopathologic studies described non-ischemic and ischemic WMHs theories. According to the distribution of WMHs, the origin could be divided into vascular or nonvascular. It is believed that periventricular smooth WMHs are due to a disruption of ependymal lining, and early confluent and confluent WMHs are due to incomplete ischemia, demyelination and axonal loss (49). Atherosclerosis (thickened vessels walls of small penetrating arteries) was seen in all brains in which WMHs were present. The primary hypothesis regarding WMH etiology is that these lesions are due to chronic partial ischemia secondary to diffuse arteriosclerosis, which is probably due to hypertension. Apart from this "ischemic" hypothesis, other mechanisms are possible: blood-brain barrier breakdown and leakage of toxin fluid to the brain parenchyma (it was proposed due to raised proteins content in the CSF) (6). One study explained the association between WMHs and venous collagenases as possibly being due to small venous ischemia and edema (50). Another hypothesis regarding the etiology of periventricular WMHs suggests that they are caused by transependymal leakage and gliosis and that the deep WMHs are caused by reduced CBF with hypoxia (50). Another study showed that reduced CBF as a result of hypoperfusion affected the development of periventricular WMHs (51).

Confluent WMHs progress over time and could progress rapidly, especially in hypertensive subjects. (52, 53). WMHs induce cognitive, motor, mood and micturation disorders. Moreover, subjects with WMHs showed increased risk of cardiovascular events and death (54).

Lacunar stroke is a widely accepted consequence of SVD. LIs are mainly located in the thalamus, basal ganglia, internal capsule, corona radiate brain stem and cerebellum. LIs may be symptomatic, causing neurological symptoms or asymptomatic, referred to as "silent" LIs. Both types of infarcts share the same risk factor profile and impact (55). Lacunar strokes account for about ¼ of the total number of ischemic strokes. The incidence reported in previous studies reached up to 59/100 000 per year and increased, especially in young patients, less than 65 years of age (56, 57). Occasionally single LIs in strategic

locations, particularly the thalamus, can result in cognitive impairment, but more often multiple LIs in the basal ganglia will be the cause of cognitive impairment (58). The Prevention of Small Subcortical Strokes (SPS3) study showed that nearly half of the patients with LIs had mild cognitive impairment (59).

MBs represent the hemorrhagic MRI expression of SVD (6). Cortical-sub-cortical, lobar MBs are more closely related to amyloid angiopathy, also to Alzheimer's disease pathology, whereas deep and infratentorial MBs are the result of hypertension (60). Preliminary data suggest that MBs could be considered as an independent marker of the risk of deterioration in global functioning (6).

Vascular cognitive impairment (VCI) includes all forms of cognitive loss related to vascular disease (58). SVD has an important role in VCI and partly overlaps with other conditions (Figure 6).

When patients have Alzheimer's disease in conjunction with with SVD, the condition of mixed dementia is present. Inherited forms of SVD (ISVD) are a separate category of SVD. One of them is CADASIL: cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy.
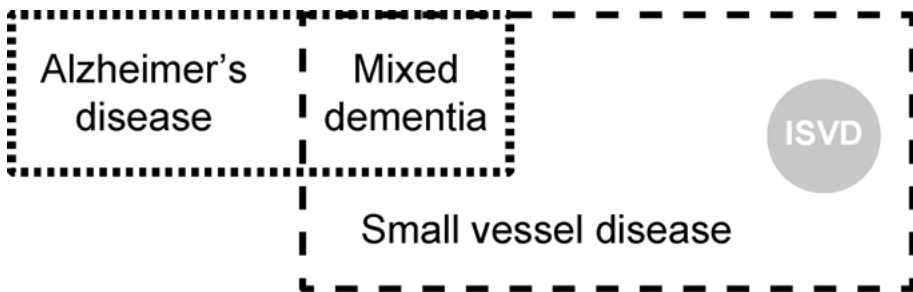


Figure 6. Modified from G.A. Rosenberg, et al., Journal of Cerebral Blood Flow and Metabolism 2015. Consensus statement regarding the diagnosis of subcortical small vessel disease (SVD). Diagram of the various categories of small vessel vascular cognitive impairment (VCI). ISVD=inherited forms of SVD.

Cerebral hypoperfusion could alternatively be seen as a result of circulatory failure and cardiac arrest. Severe hypoperfusion may cause border-zone infarcts. Cardiac arrest may also result in selective neuronal death in specific regions of the brain; particularly vulnerable are the hippocampi, thalamic nuclei, cortex (61). Hypoperfusive-hypoxic manifestations may contribute to the damage in patients with MI. Also, global ischemia resulting in cognitive impairment is noted in some situations following circulatory impairment such as MI with profound hypoperfusion (6).

# Paper I

It is well-known that men have about twice the total incidence of RMIs compared with women (62), which is confirmed by the results of the present study. Moreover, it was observed that UMIs were more frequent in men than in women. This is somewhat in contrast with the observation in our subjects at 70 years of age, where women constituted 45% of the UMI group and 18% of the RMI group (63). At 75 years of age, they constituted 37% of the UMI group and 24% of the RMI group, suggesting that MIs in women are recognized to a larger extent at age 75 years than they were 5 years earlier. This corresponds to the Framingham study, in which it was found that MIs in women are more likely to be unrecognized than MIs in men, but that this difference tends to diminish after the menopause and to be eliminated around the seventh decade of life (62).

The association between UMIs and cortical infarcts in women and that between RMIs and cortical infarcts in men remained despite sex differences in blood pressure, heart rate and serum lipid levels, whereas the association between RMIs and LIs in men was slightly impaired.

In women, cortical cerebral infarctions were more frequent in subjects with UMI than in those without MI scars. Even though there were few subjects in these groups (5 of 44 in the UMI group and 2 of 139 in the no MI group), the difference is statistically significant, and this observation might indicate an increased risk of stroke for women with MRI-detected UMIs.

However, cortical cerebral infarcts are considered to be a manifestation of atherosclerosis (64), but the pathogenesis of LIs has been suggested to differ from the atherothrombotic process (65). Thus, a hypothetical association between UMIs and LIs would further have supported the interpretation that UMIs are not caused by atherosclerosis, whereas an association between UMIs and cortical infarcts would have suggested that UMIs are caused by atherosclerosis.

Because an association between UMIs and cortical infarcts was observed in women, their UMIs might be caused by atherosclerosis. This could imply that MRI-detected UMIs have a different pathogenesis in women than they have in men. However, it may be more likely that the vascular vulnerability differs between women and men, so that atherosclerosis would manifest itself earlier in cerebral vessels in women and earlier in cardiac vessels in men.

# Paper II

In our study population, one or more brain infarcts were seen in 23% of the subjects. Out of all subjects, 4% had recognized and 19% had silent brain infarcts on MRI. This is in agreement with previous studies showing a prevalence of silent brain infarcts from 8% to 28% within the age range 34–97 years (30). Both silent and recognized LIs were in our subjects predominantly located in the basal ganglia, thalamus, and the corona radiata. It has been shown that the main location of acute symptomatic (recognized) LIs is the primary motor and sensory pathways, and LIs in other locations are usually silent (31).

In our population, 9 subjects (2%) had a verified history of stroke without any infarct seen on MRI, not even at a second evaluation when the clinical findings were revealed to the neuroradiologist who had performed the image assessment. This is expected because it has been shown that up to 30% of patients with lacunar stroke syndromes do not have a visible small infarct on imaging. Small subcortical infarcts may evolve into a lacunar cavity or a hyperintense lesion without apparent cavitation on T2-w images or can even regress totally without any visually detectable lesion on MRI (66).

It is also more likely that cortical and LIs have different risk factor profiles because of the presumed different etiologies of large-vessel and small-vessel disease (perforating arteriolar abnormality) (67). Many embolic infarcts have a cardiac origin, and these infarcts are often large and multiple and predominate in the middle cerebral artery territory, but cardiac embolism may sometimes result in lacunar infarction (in up to 5% of cases) (68).The patients with small-vessel disease have a higher risk, especially of recurrent nonfatal stroke.

## Established Cardiovascular Risk Markers

In our study, *Hypertension* was related to brain infarcts. This is in agreement with previous studies, where hypertension is a well-established risk factor, which is strongly associated with all types of brain infarcts (30-32). Other studies have shown that hypertension is strongly associated with silent brain infarcts, which are most often lacunar (30, 32, 37, 69, 70) and also with recognized LIs (31).Thus, hypertension and small-vessel disease are important factors for the pathogenesis of LIs (30).

Subjects with *Diabetes* showed an increased OR for risk of brain infarcts in our study, but this was not statistically significant. In a previous study, hypertension, diabetes and smoking were associated with recognized LIs (31). Diabetes was a significant risk factor for all subtypes of ischemic brain infarcts on MRI in another study (71).The population-based Rotterdam Scan study showed a significant association between diabetes and silent brain infarctions (34).

*Smoking* was not significantly associated with any type of brain infarct in our study. However, smoking was one of the risk factors for recognized LIs

in a previous study, (31) but there was no strong association with silent brain infarcts. Another study showed that smoking alone was not a significant risk factor for silent brain infarcts (32). The silent brain infarcts were predominant in our study, so our results are in agreement with these studies.

*HDL cholesterol* has shown an inverse relation to stroke in most epidemiologic studies (36), and in agreement with this, we found that low HDL cholesterol was associated with increased for brain infarcts, but it was not statistically significant.

The *LDL cholesterol* was not significantly associated with brain infarcts in our study. A previous study showed that serum cholesterol (total cholesterol, LDL cholesterol, and non-HDL cholesterol) showed a significant association with silent brain infarcts (37).

## New Cardiovascular Risk Markers

*NT-proBNP* is a marker released in response to myocardial stretch and is valuable for diagnosis of heart failure and a marker of increased mortality and morbidity in cardiovascular events (38, 41). It has also been shown to be strongly associated with cardioembolic stroke (39).

*Cardiac troponins*, intracellular proteins involved in heart muscle contraction, are biochemical markers of myocardial damage (39).

*CRP* is a marker of inflammation, involved in endothelial inflammatory response, and predicts stroke and cardiovascular events (40).

*Cystatin C* is mainly used as a marker of renal function and has been reported as a strong predictor of cardiovascular death in elderly people (42). All the 4 markers mentioned earlier significantly predicted death from cardiovascular causes in a longitudinal population-based study of a large male cohort (42).

None of these new risk markers were significantly related to brain infarcts in our study. However, in a recent article, troponin I and NT-proBNP were found to be independently related to increased risk of stroke and mortality in a population with atrial fibrillation, and a review and meta-analysis showed that NT-proBNP has a strong association with cardioembolic stroke (38, 39). Because most of the infarcts found in our study were asymptomatic and lacunar, they were most likely not of cardioembolic origin.

# Paper III

SVD in the elderly population tends to progress over time. We found a progression of WMH score in 5.6% of the individuals examined at both 75 and 80 years ($P<0.001$), but only a small volumetric change over these 5 years (increase 1.8 ml). It is known that the progression of SVD on MRI is represented by a progression of WMHs (72-74). Previous studies have shown that mainly early confluent (moderate) and confluent (severe) WMHs progress over time (74-77). Previous studies have shown that rapid progression of WMHs caused by early confluent to confluent lesions correlates with symptomatic cerebrovascular disease and poor cognitive functioning, i.e., a risk of dementia and certain psychiatric disorders (14, 72, 78, 79).

LIs were associated ($P<0.05$) with moderate and severe WMHs in the current study. Subjects with LIs had a larger WMH volume than subjects without. There was a progression of LIs in 26 (10.4%) subjects over 5 years. This is consistent with previous reports on the progression of LIs over a 3-year period, which was 19% in the LADIS study (75) and 12% in the Rotterdam Scan Study (74).

MBs are part of SVD and were associated with the WMH severity score and WMH volume load in our study. Similarly, a previous study demonstrated that cerebral MBs correlated with total Fazekas scale score and their number and severity correlated with the severity of WMHs (80).

## CBF

In this study, we did not find any relationship between total WM rCBF and WMH volume at age 75, and no significant associations between regional or total rCBF at age 75 and changes in WMH over 5 years. Several studies measuring CBF in patients with SVD have shown conflicting results (3). A previous study showed that cerebral perfusion and cerebral vascular reactivity are reduced within areas of increased WMHs (81). Other studies also show that cerebral perfusion is reduced in patients within WMHs (51, 82) and is correlated with WMH volume (83).The mechanism of cerebral perfusion reduction in association with progression of WMH is not fully understood. It is still an open discussion as to which appears first: WMHs and secondary to them the alteration of the underlying perfusion, or that a reduction in perfusion occurs first and secondary to it, WMHs, or that the two phenomena appear simultaneously without a causal link (10). It should also be noted that the progression of WMHs in the elderly could be related to regional hypoperfusion rather than to a reduction in global perfusion (51, 84, 85).

# Paper IV

## WMHs and cognition

In this study, we found that cognition tested by TMT-B at age 75 was significantly related to WMH scoring and WMH volume at age 75 when individuals with moderate/severe WMHs were compared with those without WMHs. This is in agreement with several previous studies that have shown a relationship between WMHs and cognitive function (86-91). In the LADIS study, the presence of severe age-related WM changes predicted a more rapid decline in global functioning (14). In another study, the subclinical phase of cognitive decline, indicated by the TMT-B test, was associated with increased risk of stroke (92).

WMHs played a role in the development of cognitive impairment as show in the Rotterdam study, where a healthy elderly population with severe WMH had decreased scores on cognitive tests over a 5-year follow-up (89). Two longitudinal epidemiological studies of aging showed that in old age WMHs are associated with the development of MCI and contribute to a progressive decline in multiple cognitive systems (93, 94).

## LI and cognition

LI as a part of SVD has previously been reported to be associated with a higher risk of dementia, mainly in individuals with WMHs, cortical atrophy and recurrent strokes (95). Progression of LIs showed an association with cognitive impairment, mostly with regard to processing speed and executive functions (96). This is in agreement with our study, showing that the appearance of new LIs was associated with an increased decline in cognitive function as assessed by TMT-A over the course of 5 years.

Also, the longitudinal evaluation in the LADIS study showed a significantly steeper decline of cognitive function and thus an increased risk of developing dementia and medial temporal atrophy in patients with a combination of severe WMHs and lacunes, independent of age, sex and education (44).

## MBs and cognition

Cerebral MBs are part of SVD and the number of MBs increases with age. Recent longitudinal studies showed that MBs have an impact on cognitive impairment which varies with their location in the brain (97-99). In the Rotterdam study, a higher number of MBs was associated with a lower MMSE score and worse performance on tests of information processing speed and motor speed. The presence of lobar MBs was associated with a worse performance on tests measuring cognitive function (98), even after adjustment for vascular

risk factors and other imaging markers of small vessel disease (97, 98). In our study, the number of MBs was not significantly related to cognitive tests at the age of 75, which is not in agreement with these findings.

The MRI markers of SVD, WMHs, LIs and MBs have in other studies been shown to be independently associated with cognitive decline and dementia (96, 100, 101). MBs were associated with cognitive function independent of WMHs and LI, and they were not clinically silent (99).

## Perfusion and cognition

Our findings did not show a significant relationship between cerebral perfusion and cognitive functions at age 75, either totally or regionally. In contrast, another study showed an association between reduced cerebral blood flow velocity in the middle cerebral artery, more severe WMHs and cognitive impairment (102). In a study of patients with hypertension, higher WM lesion volume was related to a regional decrease in perfusion within areas of WMHs but not to a reduction of general perfusion (85).

# Conclusions

**Paper I**: MI scars were more frequent in men than in women at 75 years of age. The prevalence of RMI and UMI was related to that of cerebral infarcts.

**Paper II**: The established risk markers, hypertension and obesity, were associated with a significantly increased risk of brain infarction, whereas the newer cardiovascular risk markers were not associated with the LIs in our study population. Thus, troponin I and NT-proBNP may be associated mainly with cardioembolic infarcts, as recently shown.

**Paper III**: MRI manifestations of SVD progressed over 5 years in an elderly population (age 75 to age 80). rCBF was not associated with WMH volume or progression of WMH volume.

**Paper IV**: In this cognitively healthy community-based population, moderate to severe WMHs and incident LIs on brain MRI in individuals aged 75–80 years were associated with a mild impairment of executive function.

# Future perspectives

## General aspects

SVD provides challenges not only regarding an understanding of its underlying pathogenesis and pathophysiology, but also with regard to its relation to prevention aspects, since SVD is a leading cause of cognitive impairment and functional loss, and it represents 25% of all strokes in the elderly (5). Furthermore, longitudinal studies are needed to promote a better understanding and prevention of "silent" strokes, WMHs, vascular risk factors and cognitive impairment.

## MRI techniques

The brain consists of a complex network of pathways that regulates the functions of the human body. Pathological disruption even at a microstructural level affects not only structural function but disturbs the connectivity of the surrounding network. Combining structural neuroimaging and physiological MRI techniques will probably increase our knowledge about pathological changes and severity status. Multimodality imaging is increasingly used in clinical neuroscience research.

High resolution conventional MRI techniques should be used as follows: 3D Flair (MPR) for WMHs and 3D T1W(MPR) for GM assessment and cortical thinning. It is known that the aging brain shows volume loss and more significantly loss of gray matter, which is affected due to neuronal loss. This could be determined with 3D T1W images.

Patients with WM disorders may present with motor deficits, attention dysfunction, and other cognitive impairments, reducing the feasibility of task-driven fMRI. Resting state (rs) fMRI will help to assess neuronal activity and functional connectivity networks (rsFC) associated with cognitive impairment and evaluate level of severity. Dynamic functional connectivity will be another trend to study rsFC in clinical trials as cognitive fluctuation and may be a sensitive marker of alterations in early stages of brain diseases. Future research is needed to further characterize the clinical significance of rsFC dynamics in WM disorders (103).

# Specific aspects

## Design and data sampling of longitudinal study

The power of a longitudinal study depends on the design of the study. Standardized scanning/protocols should be used for baseline and follow-up studies. Also, the newest techniques should be used for post processing and quantification of data analysis methods. By assessing volumes of WM damage, we could better understand and evaluate associations between regional atrophy and white-matter tract damage and their clinical impact (104). If detailed mapping of the white-matter architecture is possible from diffusion tensor MRI, then it should be feasible to integrate quantitative measures of tissue damage along tracts of known functional significance to form a more relevant assessment of the burden of brain disease in a clinical prospective.

Ultra-high-field MRI increases the sensitivity, but also presents new technical challenges.

Future studies should also incorporate the full spectrum of SVD and associated brain atrophy when the relationship between fMRI and EEG measurements with cognitive impairment is studied. Investigators should also consider including comparison groups with vascular risk factors but no evidence of cognitive impairment; however, those without vascular risk factors may not be representative of the general population. Such an approach can help researchers pursue questions regarding what differentiates those who develop cognitive impairment from those who do not.

Moving forward, functional neuroimaging of SVD should help to resolve the clinical heterogeneity of the disease and provide better evaluation of the effects of novel cognitive and pharmaceutical interventions (103).

# Sammanfattning på svenska

Småkärlssjukdom i hjärnan, "cerebral small vessel disease" (SVD), drabbar små artärer och vener i vit substans och patogenesen är inte helt klarlagd. Sjukdomen är relaterad till åldrande och vaskulära riskfaktor och är den näst vanligaste orsaken till demens hos äldre individer. Avbildning av hjärnan, framför allt med magnetresonans (MR), är essentiell för att diagnostisera, karakterisera och erhålla mer kunskap om SVD.

MR-markörer för SVD inkluderar vitsubstansförändringar med hög signalintensitet (WMH), lakunära infarkter, mikroblödningar och atrofi av framför allt hjärnans vita substans. Dessa markörer har använts i flera epidemiologiska studier med olika inklusionskriterier, men inte i någon studie med helt homogen ålderssammansättning. Flera studier har analyserat cerebralt blodflöde i förhållande till MR-markörer för SVD och presenterat olika resultat. Man har visat att WMH är associerade med lågt CBF men det är oklart om lågt blodflöde förutsäger progress av WMH.

WMH betraktades initialt som ett bifynd relaterat till normalt åldrande, men nyare studier har visat ett samband med kognitiv nedsättning, speciellt för lesioner som progredierar. Relationen mellan MR-manifestationer av SVD och kognitiva testresultat i en icke-dement homogen äldre population är inte etablerad.

Det finns en korrelation mellan symptomatiska ischemiska lesioner i hjärta och hjärna, men relationen mellan icke-symptomgivande hjärtinfarkter och ischemi och infarkter i hjärnan har varit oklar.

Målet med denna avhandling var att klarlägga betydelsen av MR-markörer för SVD påvisade på MR av hjärnan samt cerebralt blodflöde mätt med MR i en stor åldershomogen äldre population av kvinnor och män. Dessutom analyserades progress vid uppföljning efter 5 år, då deltagande individer var 80 år.

Samtliga arbeten baseras på insamlat material i den prospektiva populationsbaserade studien PIVUS, genomförd vid Uppsala Universitet.

MR av hjärnan hade utförts under åren 2005–2009 på 406 individer vid 75 års ålder och 250 av dem accepterade att återkomma för kontroll-undersökning 5 år senare.

**Artikel 1** jämförde MR förekomsten av symptomatiska och tysta (icke symptomgivande) hjärtinfarkter med WMH och infarkter i hjärnan i en 75-årig population. Ärr efter hjärtinfarkter var vanligare hos män än hos kvinnor. Förekomsten av symptomatiska hjärtinfarkter hos män var relaterad till förekomst av kortikala och lakunära hjärninfarkter, medan kvinnor med tysta hjärtinfarkter oftare hade kortikala hjärninfarkter.

**Artikel 2** jämförde etablerade kardiovaskulära riskfaktorer och 4 nya riskmarkörer med kortikala och lakunära hjärninfarkter i en 75-årig population. De etablerade riskfaktorerna var associerade med ökad risk för hjärninfarkter, medan något samband mellan de nya riskfaktorerna och hjärninfarkter inte påvisades.

**Artikel 3** omfattade såväl visuell gradering som kvantitativ volymsmätning av WMH, visuell analys av lakunära infarkter och mikroblödningar samt kvantitativ analys av perfusion. MR visade progress av SVD fynden under 5 år i en äldre population (mellan 75 och 80 års ålder). Cerebralt blodflöde vid 75 års ålder var inte associerat med volym av WMH eller progress av dessa under 5 år.

**Artikel 4** jämförde SVD-fynden på MR samt MR-perfusion med resultat på kognitiva tester vid de 2 undersöknings-tillfällena med 5 års intervall. Kvalitativa och kvantitativa MR-resultat från arbete 3 användes även i denna studie. Måttliga-uttalade WMH och nya lakunära infarkter på MR av hjärnan hos äldre individer var associerade med lätt nedsättning av exekutiva funktioner.

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