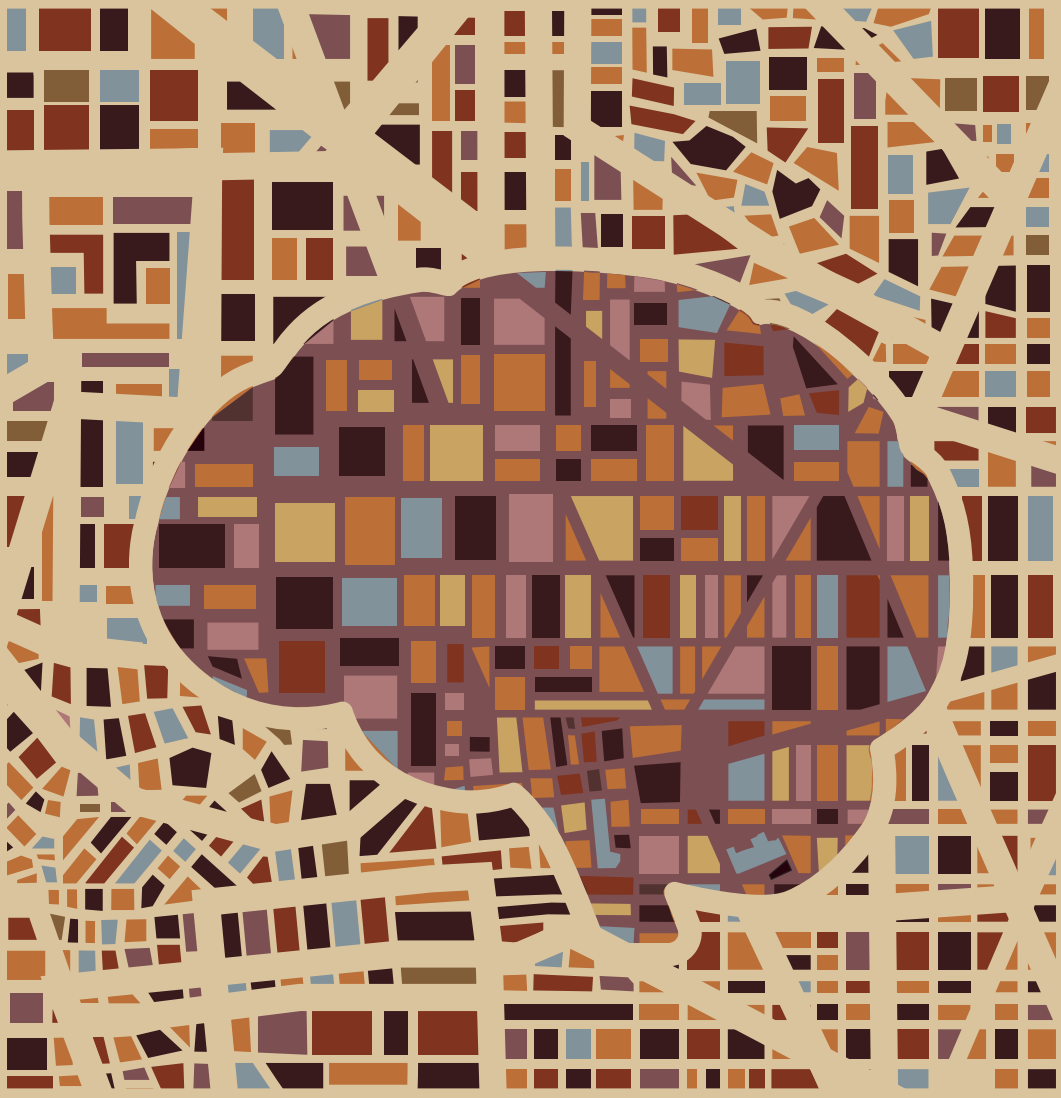
THE BRAIN UNDER PRESSURE

Studying cerebral small vessel disease in adults with hypertension



Esther Janssen

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Contents

Part I – Introduction

Chapter 1: General introduction

Cerebral Small Vessel Disease

Cerebral small vessel disease (SVD) is a term describing pathological processes affecting the smallest blood vessels (arterioles, capillaries and venules) in the brain[1]. There are different subtypes of SVD, including cerebral amyloid angiopathy (CAA) where amyloid-β deposits damages the vessels and genetically caused SVD such as cerebral autosomal dominant arteriopathy with subcortical ischemic strokes and leukoencephalopathy (CADASIL)[2]. This thesis will focus on the most common form of SVD, commonly called sporadic SVD, where the underlying pathology is arteriolosclerosis caused by aging, hypertension and other cardiovascular risk factors[1]. SVD is estimated to contribute to up to 25% of all ischemic strokes and 80% of intracerebral haemorrhages[1]. Moreover, SVD is the leading cause of vascular cognitive impairment and dementia[1, 3]. Other complaints attributable to SVD include gait problems and mood disorders such as apathy and depressive symptoms[4]. Because SVD causes widespread damage across the brain, often affecting multiple neuronal networks, the presenting symptoms and clinical course of SVD are highly heterogeneous[5]. SVD can present acutely as stroke or haemorrhage, but can also follow a more insidious clinical course characterized by progressive cognitive decline, motor and gait problems and development of mood disorders[2, 3].

Since SVD occurs in the smallest blood vessels, it is very challenging to visualize the pathological processes that occur and eventually lead to damage in the brain. In clinical practice, SVD is therefore diagnosed based on pathological lesions in the brain presumed to be caused by SVD that can be detected with magnetic resonance imaging (MRI). These MRI markers of SVD include white matter hyperintensities (WMH), lacunes, microbleeds, enlarged perivascular spaces and recent small subcortical infarcts[6]. These markers are very common with aging and present in almost every older individual over 60 years old[7]. However, MRI markers of SVD are considered to reflect late-stage SVD and do not capture the full spectrum of damage in the brain[5]. MRI markers can be clinically "silent" or "covert", since their appearance do not always lead to acute symptoms and there is large heterogeneity in clinical symptoms among patients with a similar radiological degree of SVD on MRI[5]. Moreover, histopathology studies show that markers with a similar appearance on MRI can have different aetiologies and severities[5]. SVD-related brain damage is not limited to these macrostructural lesions observed on MRI. Diffusion Tensor Imaging (DTI) is an MRI technique that is used to measure the diffusion of water molecules in white matter tracts and quantify the integrity of these tracts[8]. This technique can be used to study early-stage pathological alterations in the white matter that are not (yet) visible on conventional MRI. Using DTI, it has been demonstrated that WMH

are surrounded by regions with altered diffusion properties that are not visible on conventional MRI[9].

Despite the major impact SVD has on human health, there are no specific treatments, despite treating risk factors such as hypertension. This reflects our limited understanding of pathophysiological mechanisms underlying SVD. By the time these markers become visible on MRI, decades of pathological processes in the small vessels have likely already taken place. It is likely that pathological mechanisms are heterogeneous and depend on the risk factor profile, subtype of SVD and anatomical location[10]. Understanding the processes that precede these markers is essential to develop treatment strategies aimed at preventing or slowing down disease progression.

Blood-brain barrier integrity

One of the early pathological changes in SVD is suggested to be dysfunction of endothelial cells. These endothelial cells constitute the blood-brain barrier (BBB), together with astrocytes and microglia[11]. The BBB is a selective barrier that allows homeostasis of essential molecules into the brain, while blocking the passage of potentially harmful substances from the blood[11]. Dynamic contrast-enhanced (DCE) MRI can be used to measure BBB integrity in vivo. Using this technique, it is possible to measure extravasation of gadolinium-based contrast agent into the brain parenchyma, providing a quantitative measure of subtle BBB leakage[12]. In the past years, several studies have demonstrated disruption of the BBB in patients with SVD and dementia compared to age-matched controls[13-15]. When the BBB becomes leakier, harmful plasma proteins and inflammatory molecules may infiltrate the brain and accumulate, leading to demyelination and axonal injury[16]. Over time, this damage may manifest as structural brain damage detectable on MRI, such as WMH. This is supported by studies that have shown that BBB disruption was associated with more microstructural damage, cognitive decline and even poor functional outcome later[17-20].

The studies described above were cross-sectional and mostly conducted in patients with end-stage SVD or dementia, which limits our ability to draw conclusions about causality and the earliest pathological changes that occur in the brain. Shifting the focus to younger populations at risk of SVD may offer a unique opportunity to investigate the sequence of events from BBB leakage to visible SVD. If BBB dysfunction indeed precedes evident SVD, the BBB may represent a promising target for early intervention in SVD.

Studying SVD in young patients at risk

Studying young and middle-aged adults, especially those at risk of developing SVD is therefore crucial to advance our understanding of the disease's initiation and progression. Hypertension is one of the most prevalent cardiovascular risk factors worldwide, affecting an estimated 1.3 billion adults in 2019[21]. In a recent report, the World Health Organization (WHO) describes hypertension as a "silent killer"; high blood pressure is considered to be the single most important risk factor for early death worldwide, causing approximately 10.8 million avoidable deaths every year[21]. This number is evidently higher than deaths caused by other major risk factors such as smoking. Since hypertension is the strongest modifiable risk factor for SVD, young individuals with hypertension are a particularly valuable population to investigate early, potentially reversible brain changes associated with SVD.

Blood pressure variability and SVD

Having high blood pressure is one of the major risk factors to develop SVD. Blood pressure is however not a stable physiological parameter, but fluctuates continuously in response to internal and external factors[22]. This is referred to as blood pressure variability (BPV). BPV can be measured over short-term periods (within 24 hour) or longer periods such as days, weeks or even years[23]. Increased BPV, thus larger fluctuations in blood pressure within a given time period, is a strong risk factor for cardiovascular diseases and mortality independent of mean blood pressure levels[24, 25]. The brain is also vulnerable to fluctuations in blood pressure. In cross-sectional studies in SVD patients, it has been demonstrated that increased BPV is associated with more WMH and lower microstructural integrity of the white matter[22, 26]. It remains unknown whether increased BPV is a causal factor for SVD or merely a consequence of blood vessel wall alterations that occur in SVD. Investigating the impact of BPV on disease progression, rather than relying on cross-sectional data, helps clarify its role in both the onset and progression of SVD. If BPV is found to accelerate SVD progression, it would represent a modifiable target for intervention.

Aims of this thesis

This thesis has three major aims.

- First, we aimed to examine structural, microstructural and functional brain changes in young patients with hypertension at risk for SVD compared to normotensive controls using MRI.
- Second, we examined changes in BBB integrity following blood pressure increase and decrease after antihypertensive medication withdrawal.
- Finally, we studied study the effects of BPV on progression of SVD.

Outline of this thesis

After this introduction, we describe the protocol of the Hyperintense study in **chapter 2**. In part II of this thesis, we investigated several imaging features of SVD in young patients with hypertension. In **chapter 3**, we examine the prevalence of common SVD MRI markers, such as WMH, in young patients with hypertension and normotensive controls. In **chapter 4** we investigated BBB permeability in both groups and in **chapter 5** we studied changes in BBB integrity following blood pressure increase and subsequent decrease. **Chapter 6** describes whether this BBB permeability is associated with microstructural integrity of the white matter. In part III, we examined the association between visit-to-visit BPV with progression of SVD over 14 years in **chapter 7**.

Finally, we provide a summary and general discussion of the findings in part IV.

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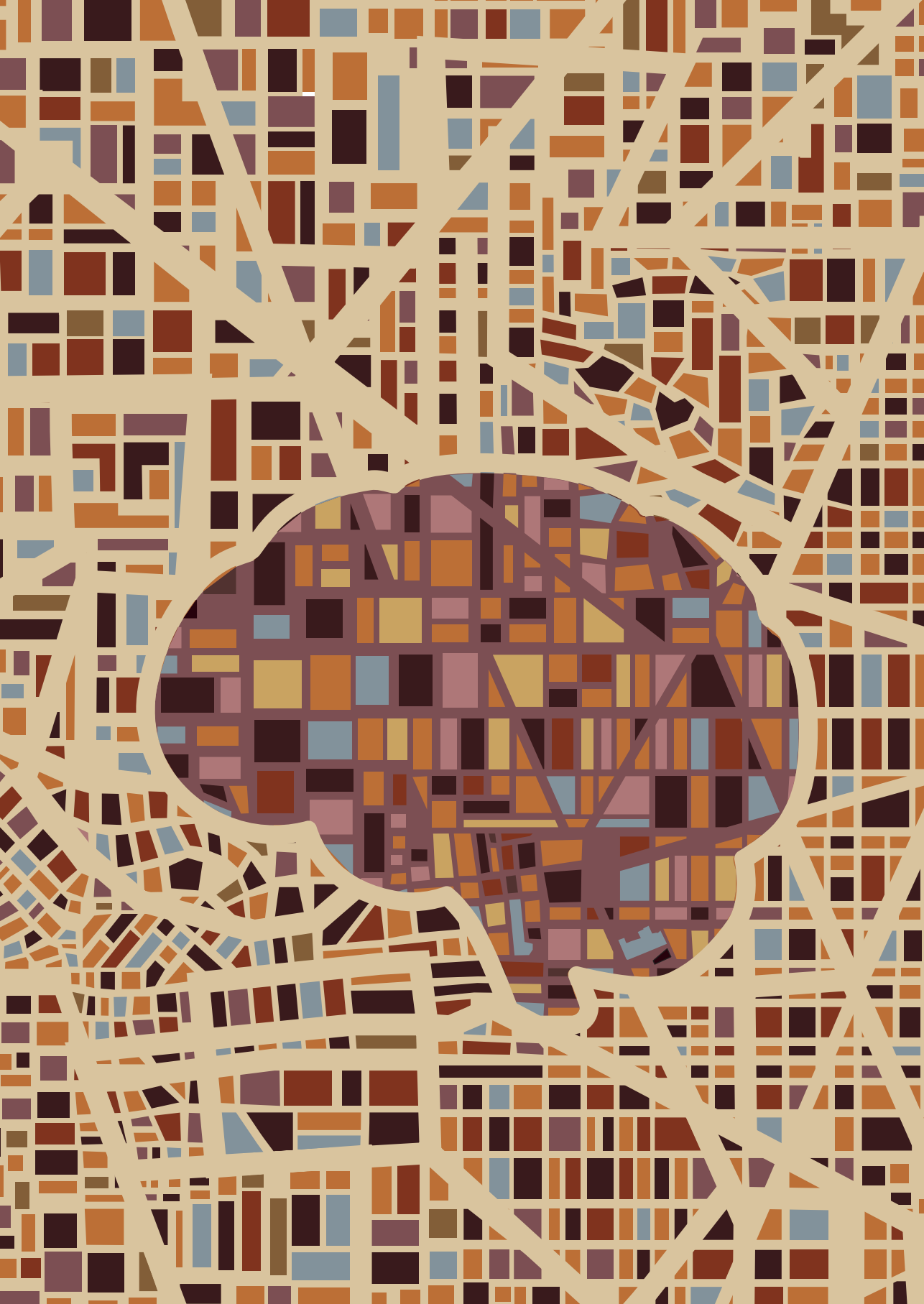

Chapter 2: The Hyperintense study: Assessing the effects of induced blood pressure increase and decrease on MRI markers of cerebral small vessel disease: study rationale and protocol

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Abstract

Background

Neuroimaging markers of cerebral small vessel disease (SVD) are common in older individuals, but the pathophysiological mechanisms causing these lesions remain poorly understood. Although hypertension is a major risk factor for SVD, the direct causal effects of increased blood pressure are unknown. The Hyperintense study is designed to examine cerebrovascular and structural abnormalities, possibly preceding SVD, in young adults with hypertension. These patients undergo a diagnostic work-up that requires patients to temporarily discontinue their antihypertensive agents, often leading to an increase in blood pressure followed by a decrease once effective medication is restarted. This allows examination of the effects of blood pressure increase and decrease on the cerebral small vessels.

Methods

Hyperintense is a prospective observational cohort study in 50 hypertensive adults (18-55 years) who will temporarily discontinue antihypertensive medication for diagnostic purposes. MRI and clinical data is collected at four timepoints: before medication withdrawal (baseline), once antihypertensives are largely or completely withdrawn (T=1), when patients have restarted medication (T=2) and reached target blood pressure and 1 year later (T=3). The 3T MRI protocol includes conventional structural sequences and advanced techniques to assess various aspects of microvascular integrity, including blood-brain barrier function using Dynamic Contrast Enhanced MRI, white matter integrity and micro perfusion. Clinical assessments include motor and cognitive examinations and blood sampling.

Discussion

The Hyperintense study will improve the understanding of the pathophysiological mechanisms following hypertension that may cause SVD. This knowledge can ultimately help to identify new targets for treatment of SVD, aimed at prevention or limiting disease progression.

Background

Cerebral small vessel disease (SVD) is an umbrella term covering a variety of pathologies that affect the small arteries, arterioles and capillaries in the brain[1]. SVD can be clinically covert, but is associated with cognitive decline, dementia and disturbances in gait and mood[1]. Moreover, most haemorrhagic strokes and a fourth of all ischemic strokes are caused by SVD[1].

Small vessel pathology cannot be visualized in vivo with standard 1.5 or 3T Magnetic Resonance Imaging (MRI). SVD is therefore usually defined by tissue alterations on MRI thought to be a consequence of small vessel pathology, such as white matter hyperintensities (WMH), lacunes and microbleeds[2]. However, these MRI markers are likely the result of long ongoing pathological processes and studying them provides limited insights into their (early) pathogenesis. Endothelial dysfunction is hypothesized to play a key role in the pathophysiology of SVD and may explain the various pathologies seen in SVD patients, including loss of integrity of the blood-brain barrier (BBB), vessel wall stiffening, impairments in vasodilation, reduced cerebral blood flow and increased inflammation[2]. The relation between these pathological processes, the order in which they occur and the role they play in the aetiology of SVD remains largely unknown.

To advance our understanding of SVD aetiology and progression, research in young- and middle-aged adults at risk or with early-stage SVD is crucial. Hypertension is the most established risk factor for SVD, but evidence is based on prospective and cross-sectional studies[3]. Higher blood pressure in midlife is shown to lead to higher SVD burden and smaller brain volumes at later age[4]. Moreover, the presence of cardiovascular risk factors, including hypertension, at a young age (18-40 years), is associated with changes in vessel morphology (i.e. vessel density and vessel calibre) and higher WMH volume[5]. This suggests that small vessel changes precede end-stage MRI markers and the accompanying clinical symptoms for decades, but the underlying mechanisms are unknown. In addition, the causal relation with hypertension is predominantly based on prospective cohort studies and trials that have shown slower SVD progression among those with lower blood pressure or active treatment with anti-hypertensives, but no studies have examined the actual effect of *increasing* blood pressure on MRI markers of SVD.

In this paper we describe the protocol of the Hyperintense study. We will apply an advanced MRI protocol to examine early functional and (micro)structural changes in young-and middle-aged adults with hypertension, the most important

cardiovascular risk factor of SVD. Specifically, we will examine the effects of blood pressure increases in patients with hypertension who undergo a routine diagnostic work up which includes temporary withdrawal of their antihypertensives, followed by subsequent decrease after reinstatement of therapy. The design of this study, i.e. temporal withdrawal of antihypertensive drugs in patients with refractory hypertension, allows assessment of the pathological mechanisms following blood pressure increase. At four time-points, advanced MRI sequences will be applied to probe potential changes in BBB integrity, microvascular perfusion, microstructural integrity and functional connectivity.

Methods

Study population and design

Patients with hypertension (n=50) will be recruited at the outpatient clinic of the Department of Internal Medicine of the Radboud University Medical Centre (Radboudumc), which is a national referral centre for patients with complex hypertension. Approximately 120 patients aged 18-55 years with hypertension are referred to the Radboudumc annually. These patients are often referred by general practitioners or other hospitals when blood pressure is not well-controlled (>140/90 mmHg) despite the use of three or more antihypertensive drugs or when there is clinical suspicion for secondary forms of hypertension. To determine if high blood pressure is caused by an overproduction of aldosterone in the adrenal gland (i.e. primary hyperaldosteronism), the plasma aldosterone/renin ratio (ARR) can be determined. Because many common hypertensive drugs interfere with this ratio, patients often have to discontinue antihypertensive drugs prior to screening or switch to drugs that are known not to affect ARR (i.e. doxazosin, verapamil, diltiazem, hydralazine) according to local protocols[6]. Medication has to be stopped for at least four weeks (for mineralocorticoid receptor antagonists) or two weeks (for diuretics, Angiotensin Converting Enzyme (ACE) inhibitors, Angiotensin Receptor Blockers (ARBs)). This often leads to a temporary increase in blood pressure. After diagnostics are completed, medication is adjusted accordingly, and blood pressure levels drop again. This diagnostic protocol with temporary withdrawal of antihypertensive medication has been proven to be safe [7].

Antihypertensive medication is discontinued in approximately 50% of patients referred to Radboudumc. We expect that at least 30% of these patients will be willing to participate in our study, making it feasible to include 50 patients within the planned 2.5 years. All participants will be asked to provide written

informed consent. This study has been approved by the medical ethics committee region Arnhem-Nijmegen.

Inclusion criteria

1. Age of 18-55 years
2. Antihypertensive drug treatment for hypertension
3. Temporary antihypertensive medication withdrawal as part of clinical diagnostic routine

Exclusion criteria

Subjects will be excluded if they meet any of the following criteria:

1. A history of an ischemic or haemorrhagic stroke or transient ischemic attack (TIA). This is based on self-report and subsequent verification of available medical documentation.
2. Conditions leading to similar appearance of MRI markers as SVD, including:
   a. Large-artery disease, defined as stenosis >50% in the internal carotid artery or vertebral artery based on ultrasound collected at baseline and medical records
   b. Cardio embolism, defined as atrial fibrillation or other high risk cardio-embolic conditions (based on medical history)
   c. Vasculitis
3. Major neurological/psychiatric diseases or other diseases that prevent long-term follow-up
4. Contraindications for 3T MRI
   a. Renal insufficiency (eGFR < 30 ml/min, insufficient kidney function to receive gadolinium-based contrast agent for DCE-MRI)
   b. Pregnancy
5. Inability to give informed consent.

Study visits

Participants will be requested to complete four study visits, combined with routine visits to the outpatient clinic when possible (Fig. 1). The baseline measurement is conducted just before antihypertensive medication is withdrawn (T=0). The second study visit (T=1) will take place approximately 2-6 weeks after antihypertensive medication withdrawal when blood pressure is increased. T=2 is scheduled within 2-4 months once patients have reached their target blood pressure and blood pressure is stable. The final study visit takes place approximately one year after T=2 (T=3). After the final study visit, long-term future incident clinical events will be

monitored by contacting the GP or treating physician of the participants. Table 1 shows the data that will be collected at each study visit.

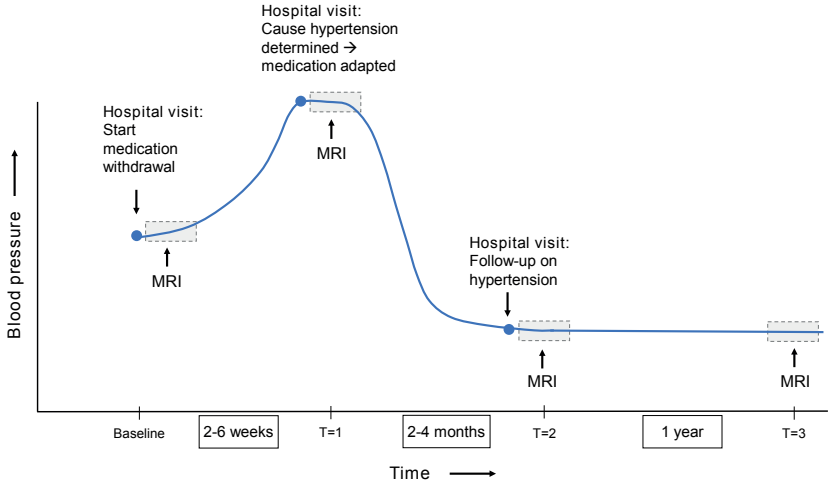

Figure 1: **Schematic presentation of the design of the Hyperintense study.**

Table 1: Schedule of all assessments in the Hyperintense study.

	Pre-visit	***Baseline***	***T=1***	***T=2***	***T=3***
Screening					
Ultrasound of carotid arteries	X				
MRI		X	X	X	X
Cognitive assessment					
Full cognitive assessment		X		X	X
Test of Attentional Performance		X	X	X	X
Motor assessment					
Timed Up & Go test		X	X	X	X
Six-meter walk test		X	X	X	X
Physical assessment					
Blood pressure		X	X	X	X
Weight, length, BMI		X			X
Questionnaires					
Educational level		X			
Medical history		X			
Medication use		X	X	X	X
Substance use		X	X	X	X
Lifestyle and behaviour		X	X	X	X
Blood sampling			X	X	

Imaging protocol

During each study visit, a brain MRI scan will be performed on a 3T MRI system (MAGNETOM PrismaFit, Siemens Healthcare, Erlangen, Germany) using a 20 channel head-neck coil. The total imaging protocol will take approximately 75 minutes and includes the following sequences: 3D T1-weighted MP2RAGE, Fluid Attenuated Inversion Recovery (FLAIR), susceptibility-weighted imaging (SWI), multi-shell Diffusion Weighted Imaging (DWI), resting-state functional MRI (rs-fMRI), Intravoxel Incoherent Motion (IVIM) and Dynamic Contrast Enhanced (DCE) MRI. In addition, a B0 field map and a diffusion-weighted sequence with reversed phase-encoding direction were acquired for distortion correction of DWI, IVIM and rs-fMRI scans. Acquisition parameters for all sequences are shown in Supplementary Table 1. DCE-MRI consists of successive slow, fast and slow T1-weighted saturation recovery spoiled GRE pulse sequences[8]. Gadobutrol contrast agent (0.1mmol/kg) will be injected at a 3 ml/s rate followed by a 20 ml saline flush during the fast sequence. This macrocyclic agent is shown to be more stable than other gadolinium-base contrast agents and can therefore safely be used for multiple MRI assessments[9].

MRI processing and analysis

Structural MRI measures

MRI markers of SVD

Conventional MRI markers of SVD, such as WMH, lacunes, microbleeds, enlarged perivascular spaces and recent infarcts will be examined following the STRIVE guidelines[10]. WMH volume will be calculated using an in-house developed and validated technique that has previously been described[11].

DWI

Diffusion-weighted images will be processed according to a previously described protocol[12]. In short, images will be visually inspected to exclude major artifacts. The following preprocessing steps will be applied: denoising, removal of Gibbs artefacts and correction for head motion, susceptibility-induced distortions and eddy current-induced distortions. To do this we will use the Functional Magnetic Resonance Imaging of the Brain (FMRIB) software library (FSL; v5.0, topup, eddy) and MRtrix3 (mrtrix.org/, dwidenoise, mrdegibbs)[13, 14]. BET (FSL) is used to extract brain tissue, after which the diffusion tensor metrics (FA and MD) will be calculated using DTIFIT (FSL).

Next to these common DTI-derived measures, the Peak Width of Skeletonized Mean Diffusivity (PSMD) will be calculated. PSMD is based on skeletonization

and histogram analysis and is calculated as the difference between the 95th and 5th percentiles of MD values within the masked skeleton[15]. This approach is suggested to improve detection of subtle diseases in the brain[15, 16]. PSMD will be calculated using a fully automated pipeline that was previously described[15].

Functional MRI measures

IVIM

IVIM is a DWI-technique that is used to study both microvascular perfusion and changes in microstructural tissue properties, which is especially useful in SVD research since both are impaired in this disease[17]. Processing of IVIM images has been previously described[17]. In short, preprocessing steps will include visual inspection of image quality, distortion corrections and head motion correction. To model the diffusion-attenuated signal, a 2-compartment diffusion model is used that describes vascular and nonvascular compartments[18]. Fitting of the model will be performed on a voxel-by-voxel basis using a previously described two-step method[19]. Measures derived from this model include: the perfusion volume fraction *f*, the diffusion coefficient of parenchymal water *D* and the pseudodiffusion coefficient of circulating blood *D**. Furthermore, *fD** is a blood perfusion related measure that will be calculated[20].

DCE-MRI

DCE-MRI images will be processed according to previously described protocol by performing pharmacokinetic modelling and histogram analyses[21]. Images are segmented into white and grey matter using the T1-weighted image and WMH are segmented on FLAIR images. After coregistration of T1-weighted images and FLAIR, Normal Appearing White Matter (NAWM), WMH, cortical grey matter and deep grey matter are selected as regions of interest (ROI).

To calculate the concentration of the contrast agent in tissue, the relative signal enhancement and T1 maps derived from the MP2RAGE sequence will be used[22]. The vascular input function used to calculate the contrast concentration in blood plasma will be derived from the superior sagittal sinus[23]. The slope and intercept of the graphical Patlak model will be used to calculate the leakage rate (transfer constant K_i)[24]. A histogram is created for the K_i values in each ROI in a voxel-wise manner. Two measures to quantify BBB leakage are derived from these histograms: the mean transfer constant K_i as a measure of leakage rate and the area under the histogram curve as a measure of tissue volume of leaking microvessels (V_i). These measures will be calculated for all ROIs. T1 values in these ROIs will be computed

at the individual level to evaluate the interaction between hypertension and T1, a marker of water mobility.

Resting-state fMRI

Processing of resting-state functional MRI (rs-fMRI) includes the following steps: removal of artifacts, correction for slice time and head motion, coregistration of functional and structural images, normalization of subject brain to Montreal Neurological Institute (MNI) space and spatial filtering. Graph theory will be used to examine functional brain networks using a previously described protocol[25]. In short, 264 functional areas will be used as network nodes in the cerebral cortex, subcortical structures and cerebellum[26]. We will define five functional networks: a global brain network, the default mode network, the fronto-parietal task control network, the somatosensory-motor network of the hand and the visual network[25]. For these networks, we will calculate the weighted global efficiency and the weighted clustering coefficient, since these measures have previously been shown to be sensitive to structural network abnormalities in SVD patients[27].

Primary outcomes

Primary outcomes of this study will be the changes in MRI outcomes after withdrawal of antihypertensive medication (highest blood pressure) and subsequent restart (lowest blood pressure). MRI outcomes include WMH volume, PSMD, BBB leakage rate and volume, IVIM outcomes (D, fD*), PSMD, network global efficiency and clustering coefficients and are described in more detail in the "Imaging Protocol" section.

Secondary study outcomes include:

- Effects of antihypertensive medication withdrawal and restart on cognitive and motor functioning
- Baseline associations between cardiovascular risk factors and MRI markers of cerebral structure and vascular functioning
- Association between circulating markers of inflammation, including cytokines and chemokines, measured at T=1 and blood pressure and brain MRI parameters
- Changes in MRI parameters of brain structure and vascular function at 1.5-year follow-up
- Number of future incident clinical events. This includes all-cause mortality, death due to vascular causes, non-fatal strokes (ischemic and haemorrhagic) and TIAs.

Cognitive assessments

At baseline, T=2 and T=3 patients will undergo a 60-min cognitive assessment covering six domains: processing speed, attention, executive functioning, verbal

memory, working memory and psychomotor functioning (Supplementary Table 2). All administered tests are validated and widely used. To minimize intra-individual variability, we use standardized tests with high test-retest reliability. Parallel versions will be used to take task-specific practice effects into account. Furthermore, participants will perform the Alertness subtask of the Test of Attentional Performance on a laptop during each study visit. The Alertness subtask is a sensitive test for processing speed and attention, during which participants have to press a button as quickly as possible then a target stimulus is shown[28].

Motor functioning

Gait speed (m/s) will be determined over a 6-m distance during each study visit to examine motor functioning. Gait and balance of participants will be assessed using the Timed Up & Go Test, measuring the time a participant needs to get up from a chair, walk three meters, turn around and sit back down[29].

Questionnaires

A structured questionnaire will be used at baseline to assess demographic data, medical history and lifestyle behaviour (including smoking, alcohol consumption and drug use). Educational level is determined using a seven-point Dutch rating scale, the Verhage scale[30]. Medical history includes age of hypertension onset and medication use. At each follow-up visit, changes in medication use and lifestyle behaviour, and incident clinical events are assessed.

Blood sampling

Blood will be collected at T=1 and T=2 to determine levels of circulating inflammatory markers, such as cytokines after overnight fasting. We will collect 45 ml of blood (20 ml serum, 10 ml EDTA plasma, 9 ml citrate plasma and 6 ml blood for DNA isolation. Samples will be stored in the Hyperintense BioBank at the Radboudumc for future analyses.

Physical examination

We will measure height and weight and calculate the Body Mass Index (BMI). Blood pressure will be measured 3 times while participant is in seated position.

Sample size calculation

Because the effects of blood pressure increase and decrease on MRI outcomes assessed here have never been examined, there is no information available about the size of these possible effects. A formal sample size calculation is therefore not feasible. Instead, we based our sample size on the number of patients referred to

the Radboudumc annually that are eligible for participation in this study within the limited time period of 2.5 years.

Statistical analysis

To analyse primary outcome measures, MRI outcomes at different timepoints will be compared. MRI outcomes are discussed in the imaging analysis section and include: WMH volume, PSMD, BBB leakage rate, BBB leakage volume, IVIM outcomes, FA, MD, network global efficiency and clustering coefficients. All outcome measures are continuous measures and data will be log-transformed in case of non-normality. We assume a linear (or parametric) relationship between blood pressure and MRI parameters and will therefore run linear mixed models, including all time points. These will be adjusted for age, sex, education, and other conventional MRI markers of SVD. We will not correct for multiple analysis since different MRI measures probe different aspects of the brain in terms of brain structure, physiology, and function. Missing data will be described in our scientific reports.

To examine the association between changes in blood pressure and cognitive and motor functioning, individual cognitive test scores will be adjusted to Z-scores using available normative data[31, 32], adjusting for age, sex and educational levels when possible. The association between blood pressure and incident clinical events will be investigated using Cox proportional hazard analyses, adjusted for age, sex, education and SVD MRI markers where appropriate.

Discussion

Little is known about the pathophysiological mechanisms underlying SVD. Research into SVD pathogenesis is hampered by difficulties with visualizing the smallest cerebral vessels with conventional MRI, allowing only the detection of end-stage disease cerebral lesions. Most of the knowledge about SVD is derived from studies conducted in individuals older than 60 years in whom SVD most likely has been present for decades. To advance the understanding of key mechanisms implicated in SVD, studies conducted in young- and middle-aged adults that are able to catch the first signs of SVD, before the occurrence of widespread irreversible tissue damage, are needed. The Hyperintense study is the first serial MRI study designed to examine the effects of changes in blood pressure on cerebral microvasculature in young- and middle-aged adults with hypertension, the strongest risk factor for SVD[1].

One of the main strengths of this study is the unique design that includes induced hypertension due to temporary withdrawal of antihypertensive medication, followed by blood pressure lowering due to medication restart as part of routine clinical practice, without the need for an intervention study. This design allows analysis of the effects of both increases as well as decreases in blood pressure. Previous studies have examined the effects of blood pressure lowering on SVD measures, but the effects of blood pressure increase have never been studied[33]. Furthermore, we combine several advanced MRI techniques to examine early microvascular changes. We use DCE-MRI to examine BBB integrity, which is associated with clinical and imaging features of SVD[34]. Since both microstructural integrity and cerebral perfusion are suggested to be diminished in SVD patients, we use multi-shell DWI and IVIM to examine this simultaneously[17]. We also use rs-fMRI to examine functional connectivity since (micro)structural damage in SVD patients can lead to disturbed connectivity both between and within brain networks[27]. These MRI techniques allow analysis of early pathological mechanisms and the order in which they occur before MRI markers of SVD become visible. Another strength of this study is the extensive amount of cognitive, motor and biobank data that is collected in a structured and standardized way. MRI outcomes can therefore be linked to cognitive functioning, measured by tests that are widely accepted and shown to be sensitive to SVD-related brain changes. Assessment of circulating inflammatory markers will help identify ongoing inflammatory responses that may play a central role in SVD pathogenesis[35]. We expect the external validity of this study to be high. Since the patients included in this study are highly likely to develop SVD later in life, abnormalities in vascular functioning observed here are presumably also present in other populations at risk of SVD. Findings of this study will therefore have implications for treatment optimization in patients at risk of cerebrovascular damage; reaching conventional target blood pressure may not be sufficient and additional treatment to further lower blood pressure or reduce inflammation and BBB leakage can have beneficial effects on clinical outcomes.

In conclusion, the Hyperintense study is a unique serial MRI project that has the potential to further unravel the association between early-life hypertension and SVD. Although hypertension is considered a main risk factor for developing SVD, it remains unknown how hypertension exerts an effect on brain structure and vascular function. This study will help to identify early-life pathological mechanisms of SVD caused by hypertension. Improved understanding of the pathological mechanisms driving SVD pathogenesis and progression will contribute to identification of new targets for treatment.

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Supplemental material

Supplementary Table 1: MRI acquisition parameters.

	FLAIR	T1w	B_0 field map	SWI	DWI	DWI - Reversed phase encoding
Sequence	SPACE	3D MP2RAGE	Dual-echo 2D SPGR	3D SPGR	Multi-band accelerated single-shot EPI	Multi-band DW single shot EPI
Voxel size (mm)	0.5×0.5×0.9	1.0×1.0×1.0	2.0×2.0×3.0	1.0×1.0×3.0	2.0×2.0×2.0	2.0×2.0×2.0
TR (ms)	5000	6000	531	27	4600	3500
TE (ms)	388	1.71	5.07, 7.53	20	80	66
TI (ms)	1800	900, 2200		-	-	-
Flip Angle (°)	Optimized T2var refocusing	6, 5	60	15	90, 180	90, 180
Number of slices	192	176	48	52	70	70
Acquisition time (mm:ss)	05:47	07:50	01:53	02:43	07:00	00:21
Other	R = 2 Turbo factor = 278	Also used for T1-mapping			MB = 2 R = 2 5 × b = 0 s/mm^2, 40 × b = 1000 s/mm^2, 40 × b = 2000 s/mm^2)	b = 0 s/mm^2 Phase encoding direction = P > A

Supplementary Table 1: Continued

	DCE			IVIM	rs-fMRI
Sequence	Pre-bolus slow T1w SR SPGR	Fast T1w SR SPGR	Post-bolus slow T1w SR SPGR	Multi-band DW single shot EPI	Multi-band accelerated single-shot EPI
Voxel size (mm)	1.0×1.0×2.0	1.0×1.0×3.0	1.0×1.0×2.0	2.0×2.0×2.0	2.0×2.0×3.0
TR (ms)	3.4	3.4	3.4	3500	1210
TE (ms)	1.56	1.56	1.56	66	30
TI (ms)	120	120	120	-	-
Flip Angle (°)	20	20	20	90, 180	60
Number of slices	60	10	60	70	48
Acquisition time (mm:ss)	01:47	02:00	24:52	05:32	08:13
Other	NM = 5 R = 3	NM = 32 R = 2 Injection of Gadobutrol	NM = 70 R = 3	b = 5, 10, 15, 20, 30, 40, 50, 60, 100, 200, 400, 600, 800, 1000 s/mm^2	MB = 3 NM = 400

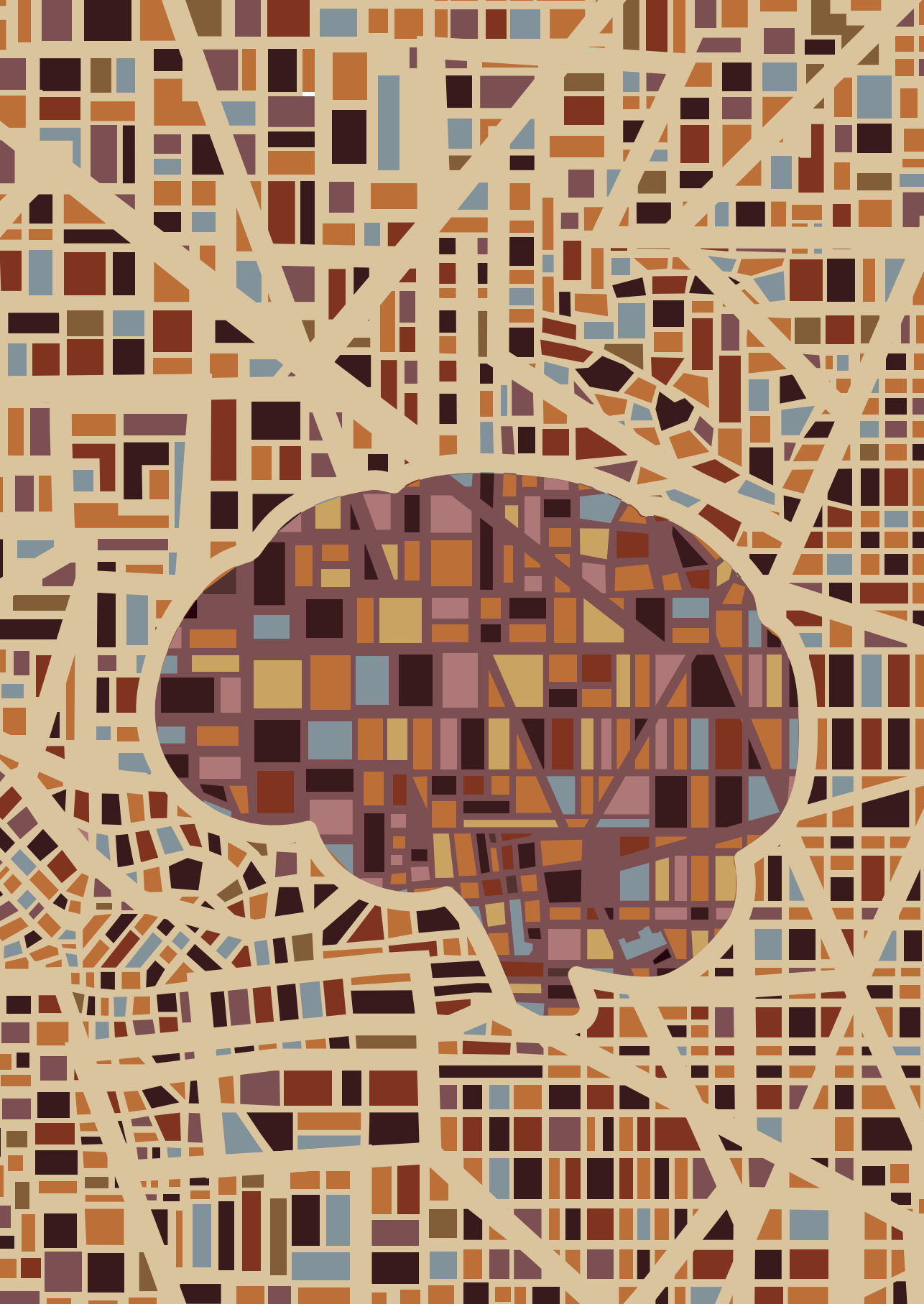
A =Anterior, DCE =Dynamic contrast-enhanced; DWI =Diffusion-weighted imaging; EPI =Echo Planar Imaging; FA =Flip Angle; FLAIR =Fluid-attenuated inversion recovery; IVIM =Intravoxel Incoherent Motion; MB =Multiband acceleration factor; MP2RAGE =Magnetization-prepared 2 rapid acquisition with gradient echo; NM =number of measurements; P = Posterior, R =parallel imaging acceleration factor; rs-fMRI =resting-state functional MRI; SPACE =Sampling perfection with application-optimized contrast using different flip-angle evolutions; SWI =Susceptibility weighted imaging; SPGR=Spoiled gradient recalled echo; SR = Saturation Recovery; TE =Echo time; TI =Inversion time; TR =Repetition time.

Supplementary Table 2: Cognitive test battery administered at baseline, T=2 and T=3.

Cognitive domain	Cognitive test
Global cognitive function	Mini Mental State Examination
Visuospatial episodic memory	Complex Figure Test [37]*
Verbal episodic memory	Rey Auditory Verbal Learning Test [38]*
Information processing speed	Trail Making Test Part A [39]
Executive function (mental flexibility)	Trail making Test Part B [39]
Executive function (semantic fluency)	Verbal fluency: animal and profession naming
Information processing speed	Symbol Digit Modalities Test (written version) [32]
Language (naming)	Boston Naming test (CERAD version) [40]
Working memory	Digit Span Test (forward & backward) [41]

* parallel versions of test used at each follow-up to avoid learning effects
Supplementary Table 1: Continued

Part II – MRI abnormalities in young patients with hypertension



Chapter 3: On the origin of cerebral small vessel disease: MRI markers of cSVD in young adults with hypertension

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Abstract

Background

Cerebral small vessel disease (cSVD) contributes to stroke and cognitive decline, with MRI markers of cSVD increasing with age. Hypertension is an important risk factor for cSVD in older adults but its impact in younger individuals remains less clear. This study investigates whether MRI markers of cSVD are more prevalent in young hypertensive individuals compared to normotensive controls.

Methods

In this cross-sectional study, 60 patients with hypertension and 21 controls aged 18-55 years underwent 3T MRI to assess cSVD markers: white matter hyperintensities (WMH), lacunes, and microbleeds. Group differences were assessed using t-tests, chi-square tests, or non-parametric methods. We examined associations between blood pressure and cSVD markers using multivariable regression models, including linear, logistic, ordinal logistic, and penalized logistic regression, adjusting for potential confounders.

Results

Patients with hypertension were older (median (IQR) 35.6 (29.6-41.4) years vs 29.2 (27.8-33.2 years), had a higher BMI, and lower education levels while proportion of females was similar. Deep WMH burden was significantly higher in hypertensive participants (median Fazekas score: 1 [IQR: 0-1] vs 0 [IQR: 0-0]; **$p < 0.001$**). Hypertension increased odds of deep WMH (OR 5.49, **$p = 0.011$**). Lacunes and microbleeds were rare and observed only in hypertensive participants. Duration since hypertension diagnosis was not significantly associated with WMH volume (β=7.27, $p = 0.111$) after adjusting for age.

Conclusion

WMH are more prevalent in young adults with hypertension, suggesting early microvascular brain changes. These findings underscore the importance of early detection and treatment of hypertension to potentially prevent long-term cerebrovascular changes.

Introduction

Cerebral small vessel disease (cSVD) is a condition affecting small brain blood vessels, resulting in white matter hyperintensities (WMH), microbleeds, and lacunes (so called MRI markers of cSVD)[1]. cSVD is an important cause of both ischemic and haemorrhagic stroke, cognitive decline, and vascular dementia[2]. MRI markers of cSVD are frequently seen in older adults (> 60 years) and the prevalence increases with age[2]. Around 5% of individuals in their 50s show signs of cSVD and by the age of 90, almost everyone is affected[2]. However, the exact aetiology of the disease remains unclear, as most studies focus on examining these end-stage MRI markers of cSVD in patients over 60 years[3], thereby lacking the opportunity to investigate cSVD onset.

Hypertension, which affected 1.28 billion adults globally in 2023, is the primary modifiable risk factor for cSVD[4]. Although hypertension prevalence increases with age, it also affects younger populations, with approximately 1 in 8 adults under 40 years diagnosed with hypertension[5, 6]. While the association between hypertension and cSVD is well-established in older adults, this has rarely been investigated in young individuals with hypertension, providing limited insight into the early stages of cSVD development[7-9]. Studying younger individuals at risk for cSVD, such as individuals with hypertension, provides the opportunity to study cSVD in a much earlier stage, before irreversible brain damage and neurological symptoms are present. In the general population, the frequency of MRI markers of cSVD is low in young individuals[10]. However, several studies have shown that changes in small vessel characteristics, such as density and calibre, and WMH could be observed in adults with cardiovascular risk factors[11-13]. As hypertension is the number one risk factor of cSVD, we however already expect to see first visible signs of MRI markers in these at-risk individuals. We aimed to examine the prevalence and location of MRI markers of cSVD in young individuals with hypertension (aged 18-55 years) compared to normotensive controls.

Methods

Study population and design

This study is a cross-sectional study conducted at the Radboud University Medical Centre in the Netherlands[14]. Patients with hypertension were recruited from the outpatient clinic of the Department of Internal Medicine at the Radboud University Medical Centre in Nijmegen and the Rijnstate Hospital in Arnhem, both in the

Netherlands. Recruitment of the normotensive controls involved the social circles of the researchers and the participants with hypertension. The study protocol was approved by the METC region Arnhem-Nijmegen and all participants provided written informed consent.

Inclusion and exclusion criteria

Patients aged 18-55 years who were referred to the department of Internal Medicine to analyse the cause of their hypertension were eligible for participation. Hypertension was defined as a systolic blood pressure of >140 mmHg/diastolic blood pressure of >90 mmHg within three months before study inclusion, regardless of antihypertensive medication use. Normotensive controls were defined as individuals aged 18-40 years with a blood pressure <120/85 mmHg without any history of antihypertensive treatment.

Participants were excluded if they had contraindications for MRI, such as pregnancy or metal implants. Individuals with a history of a clinically symptomatic ischemic or haemorrhagic stroke, or transient ischemic attack (TIA) were also excluded. In addition, participants with conditions that could result in MRI abnormalities mimicking cSVD were excluded. These included large artery disease (defined as >50% stenosis of the internal carotid or vertebral arteries, based on ultrasound collected at baseline), cardio-embolism, and vasculitis. Other exclusion criteria were the presence of major neurological or psychiatric disorders or any condition that would prevent long-term follow-up, and the inability to provide written informed consent. Information on possible exclusion criteria was obtained through self-report and medical documentation available in the hospital.

Study procedures

During the study visit, blood pressure was measured three times while participants were seated, with their feet flat on the floor, after five minutes of rest. Measurements were done in a quiet room with a researcher present using an automatic blood pressure monitor (Dinamap; GE Healthcare, Chicago, IL). In addition, standardized questionnaires were administered to collect information about demographic characteristics, cardiovascular risk factors, and relevant medical history. Educational level was assessed using the revised Verhage classification system[15], which is widely used in Dutch neuropsychological research.

MRI acquisition and analysis

All participants underwent brain MRI during the study visit using a 3T MRI system (MAGNETOM PrismaFit, Siemens Healthcare, Erlangen, Germany) with a 20-channel

head-neck coil. The standardized protocol included high-resolution structural sequences (T1-weighted Magnetization Prepared 2 Rapid Acquisition Gradient Echo (MP2RAGE), Fluid-Attenuated Inversion Recovery (FLAIR), and Susceptibility-Weighted Imaging (SWI). Acquisition parameters are described in the protocol of the Hyperintense Study[14].

WMH segmentation and probability map

We employed a deep learning–based segmentation tool, the MIAC Automated Region Segmentation for White Matter Hyperintensities (MARS-WMH) algorithm (available at https://github.com/miac-research/MARS-WMH) to automatically segment WMH using T1-weighted and FLAIR MRI scans[16, 17]. All resulting WMH masks were visually inspected for segmentation accuracy and manually corrected when necessary. WMH volume was calculated separately for periventricular WMH (defined as =< 10 mm of lateral ventricles) and deep WMH (defined as >10 mm distant to ventricles).

To create the WMH probability map, MRI scans of the patients with hypertension and controls were used separately. The binary WMH mask of each patient was registered to the 1 mm resolution Montreal Neurological Institute (MNI)-152 brain template[18]. To this end, the brain extracted FLAIR image was first coarsely aligned to brain extracted MNI-152 T2-template using linear registration with FSL's flirt tool[19]. Then, the registration was finetuned using the deformable registration tool SynthMorph[20]. All voxels that were not located in the white matter (using the MNI probabilistic white matter atlas, thresholded at 30%) were removed and the final result was visually inspected.

To create the probability map in MNI space, all coregistered WMH masks were summed and divided by the total number of patients with hypertension or controls, resulting in a map indicating the probability that each voxel contains WMH or not.

Ratings of cSVD markers

MRI markers of cSVD, including WMH, lacunes, and cerebral microbleeds, were visually assessed according to the STRIVE criteria[1]. WMH burden was scored separately for periventricular and deep regions using the Fazekas scale[21]. Lacunes were defined as round or ovoid lesions (3-15 mm in diameter) with CSF-like signal characteristics on T1. Although the STRIVE criteria also include perivascular spaces as a marker of cSVD, these were not assessed in this study since these are best visualized on a T2-weighted MRI sequence, which was not available in this study. Cerebral microbleeds were classified according to their location as follows: lobar (involving the cortex and/or deep white matter regions); basal ganglia/thalamus; and infratentorial (involving the brainstem and/or cerebellum).

The MRI data were independently assessed by two raters (E.J and M.S) to ensure reliability and reduce bias. After the assessments, they discussed the discrepancies and resolved any differences. The Intraclass Correlation Coefficient (ICC) was calculated to assess the level of agreement between two raters for the number of lacunes and microbleeds and the weighted kappa was calculated for the ordinal Fazekas scores. The ICC for lacunes was 1.00, indicating complete agreement between raters. For microbleeds, the ICC was 0.653 (95% CI: 0.509 – 0.762), indicating good interrater reliability. The weighted kappa for deep WMH was 0.796 ($p < 0.001$), indicating good to excellent reliability. For periventricular WMH, the weighted kappa was 0.447 ($p < 0.001$), which corresponds to moderate reliability.

Statistical analysis

We assessed the normality of continuous variables within each group using the Shapiro-Wilk test. To evaluate group differences between hypertensive and normotensive individuals, we used independent samples t-tests or Mann-Whitney U tests for continuous or ordinal variables, and Chi-square or Fisher's exact tests for categorical variables, depending on data distribution.

We conducted all regression analyses as multivariable models, adjusting for potential confounders including age, sex, BMI, and education level when data permitted. If variables contained only zero values in one or both groups, or lacked sufficient variability, we did not perform group comparisons or regression analyses and instead reported descriptive statistics.

We applied regression models to examine the association between systolic blood pressure and cSVD markers. For continuous outcomes such as WMH volume, we used linear regression models. To analyse count-based outcomes, including the number of lacunes and cerebral microbleeds, we initially used negative binomial regression models to account for overdispersion. However, the extremely low prevalence of these markers did not allow meaningful regression analyses, so we reported only descriptive statistics.

For markers measured on ordinal scales, such as Fazekas scores for WMH burden, we first applied ordinal logistic regression. Due to limited variability, particularly in the control group, we could not reliably estimate these models. Consequently, we dichotomized WMH scores and used binary logistic regression instead. Because all controls had a Fazekas score of 0 for periventricular WMH, we employed Firth's penalized logistic regression.

We examined the association between the duration since hypertension diagnosis (in years) and total WMH volume using linear regression, adjusting for age. We excluded one participant from this analysis due to a rare genetic form of hypertension and an unusually high WMH volume at a young age, which we considered an outlier.

We did not apply multiple comparisons correction, as the conducted analyses were pre-specified and limited to well-established SVD markers. Given the exploratory nature of the study and the small sample size, applying such correction would substantially reduce statistical power. However, not correcting for multiple comparisons may increase the risk of type I error and results should therefore be interpreted with caution.

We conducted all statistical analyses using R software (version 4.4.3) and considered p-values <0.05 statistically significant.

Results

Study population

The study included 60 patients with hypertension and 21 normotensive controls. Baseline characteristics are shown in Table 1. Patients with hypertension were older (35.9 years (SD 9.7)) than the normotensive controls (29.9 years (SD 4.9)). The proportion of females was similar between groups: 55% (n = 33) in the hypertensive group and 52% (n = 11) in the control group. Both mean systolic and diastolic blood pressure were higher in the hypertensive group (147 mmHg (SD 17.0) and 91 mmHg (SD 11.7)) compared to controls (120 mmHg (SD 10.9) and 75 mmHg (SD 8.3)).

Table 1: Baseline characteristics of patients with hypertension and controls.

	Hypertension (n = 60)	Control (n = 21)	p-value
Age (years, median (IQR))	35.6 (29.6 – 41.4)	29.2 (27.8 – 33.2)	<0.001
Female, n (%)	33 (55)	11 (52)	1.000
Systolic blood pressure (mmHg, mean (SD))	147 (17.0)	120 (10.9)	<0.001
Diastolic blood pressure (mmHg, mean (SD))	91 (11.7)	75 (8.3)	<0.001
BMI (mean (SD))	28.5 (5.9)	22.7 (2.8)	<0.001
Total Defined Daily Dose (DDD) (median (IQR))	1.5 (1.0 - 2.8)	0 (0 – 0)	<0.001
Antihypertensive medications, n (%) *	52 (86.7)	0 (0)	<0.001
Calcium-antagonist	39 (65)	0 (0)	
RAS-inhibitors **	28 (46.6)	0 (0)	
Diuretic	17 (28.3)	0 (0)	
Betablocker	7 (11.6)	0 (0)	
Alphablocker	6 (10)	0 (0)	
Cholesterol lowering medication, n (%)	6 (10)	0 (0)	0.331
Alcohol use ever, n (%)	50 (83.3)	21 (100)	0.103
Daily smoking, n (%)	5 (8.3)	2 (9.5)	1.000
Education level (Verhage classification) (median (IQR))	6.0 (5.0 - 6.3)	7.0 (7.0 - 7.0)	<0.001

*Patients may be counted in more than one category due to use of multiple antihypertensive medications. **Angiotensin Converting Enzyme (ACE)-inhibitor/Angiotensin Receptor Blocker (ARB)

MRI markers of cSVD

Deep white matter hyperintensities (WMH)

Patients with hypertension had significantly higher Fazekas scores compared to controls (p = <0.001) (table 2). The median (IQR) deep WMH volume in patients with hypertension was 10.9 mm^3 (1.0 - 43.5), compared to 1.07 mm^3 (0.0 - 12.4) in controls (p = 0.021) (table 2). Using Firth's penalised logistic regression, patients with hypertension had significantly higher odds of having deep WMH compared to normotensive controls after adjusting for age, sex, BMI and education level (OR = 5.49, 95% CI: 1.46 -0 25.5, p = 0.011).

Periventricular white matter hyperintensities (WMH)

Only patients with hypertension showed periventricular WMH with Fazekas scores of 1 or higher (Table 2), limiting statistical comparisons. The median (IQR) periventricular WMH volume in patients with hypertension was 12.0 mm^3 (1.4 - 66.6), compared to 3.2 mm^3 (0.0 - 24.9) in controls (p = 0.164) (Table 2). Firth's logistic regression showed a higher odds ratio for the hypertensive group (OR = 3.31, 95% CI: 0.24-474), but this was not statistically significant (p = 0.40).

Table 2: Prevalence of cSVD markers in patients with hypertension and controls.

	Hypertension (n = 60)	Control (n = 21)	p-value
Deep WMH Fazekas score (0/1/2/3), n (%)			
Score 0	25 (41.7)	18 (85.7)	
Score 1	34 (56.7)	3 (14.3)	
Score 2	1 (1.7)	0 (0)	
Score 3	0 (0)	0 (0)	<0.001
Periventricular WMH Fazekas score (0/1/2/3), n (%)			
Score 0	49 (81.7)	21 (100)	
Score 1	9 (15.0)	0 (0)	
Score 2	2 (3.3)	0 (0)	
Score 3	0 (0)	0 (0)	NA
WMH volume (mm^3, median (IQR))			
Deep WMH	10.9 (1.0 - 43.5)	1.07 (0.0 – 12.4)	0.021
Periventricular WMH	12.0 (1.4 - 66.6)	3.2 (0.0 - 24.9)	0.164
Total WMH	44.3 (9.4 – 120.0)	12.4 (1.93 – 35.8)	0.018
Microbleeds present, n (%)	3 (5)	0 (0)	NA
Lacunes present, n (%)	1 (1.7)	0 (0)	NA

WMH were assessed using the Fazekas score separately for deep and periventricular WMH and as a volumetric measure. Lacunes and microbleeds are shown as number of individuals with lacunes or microbleeds present. Differences between groups were assessed using binary logistic regression for deep Fazekas score and Mann-Whitney U tests for WMH volumes.

Spatial distribution of WMH

Figure 1 shows the WMH probability map for the patients with hypertension and controls. Only a small proportion of the white matter was affected by WMH and the probability was low in most regions. The most commonly affected area is the periventricular area, both frontal and parietal. Furthermore, WMH were observed in the centrum semiovale as small punctate lesions.

Microbleeds

A total of 10 microbleeds were observed in 3 patients: one patient had a single infratentorial microbleed; a second patient had 3 microbleeds, all were located lobar; and the third patient had 6 microbleeds, of which 5 were lobar and 1 was in the basal ganglia/thalamus (see Figure 2). No microbleeds were observed in the control group (Table 2).

Lacunes

Only one patient in the hypertensive group had a lacune, located in the temporal lobe, as shown in Figure 3. No lacunes were observed in the control group (Table 2).

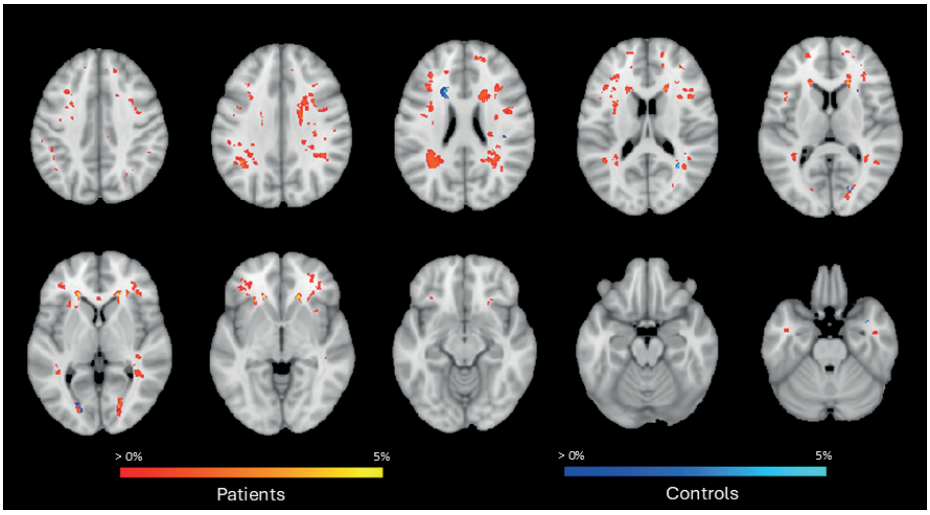

Figure 1: WMH probability map for patients with hypertension and controls. This figure shows the spatial probability of WMH displayed on the MNI-152 template at the voxel level. Red/yellow colours indicate the percentage of patients with hypertension who have a WMH in that voxel, blue the percentage of normotensive controls

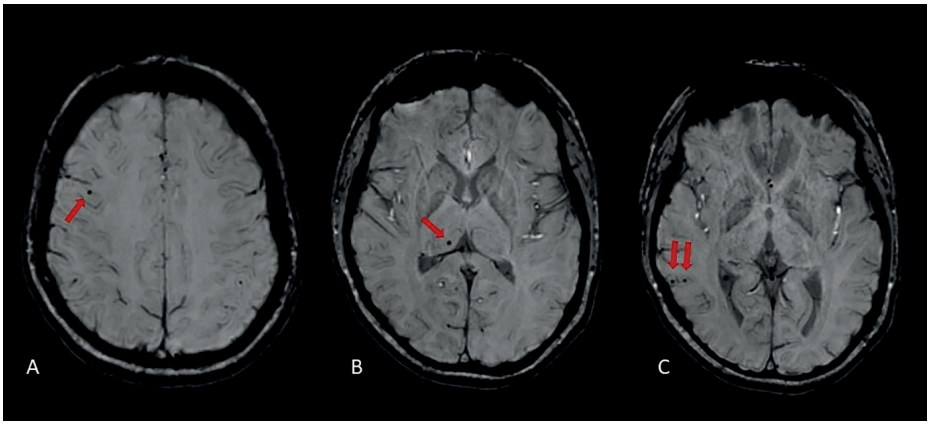

Figure 2: examples of microbleeds in one patient with hypertension in lobar regions (A, C) and the basal ganglia (B).

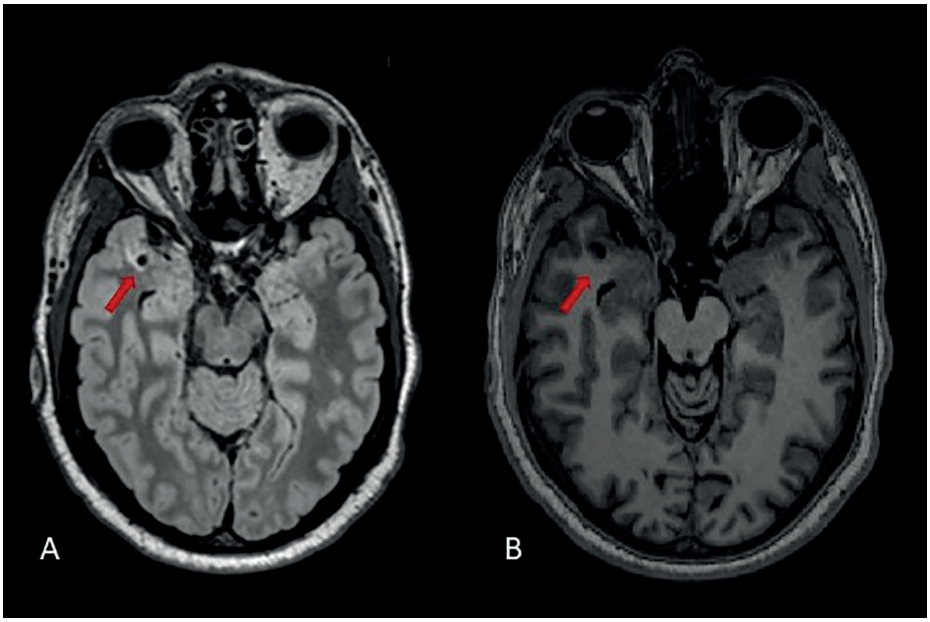

Figure 3: Lacune visible in the temporal lobe in one patient with hypertension, shown on FLAIR (A) and T1 (B).

Blood pressure and WMH volume

There was no association between systolic blood pressure and WMH volume (β = 5.84, p =0.164). In the same model, age at inclusion (β =-13.08, p =0.139, sex (β =158.64, p = 0.139), and BMI (β = 11.26, p = 0.429) were not significantly associated with total WMH volume.

Duration since hypertension diagnosis and WMH

Duration since hypertension diagnosis was positively associated with WMH volume after adjusting for age (β = 7.27, p = 0.111), where β represents the estimated increase in WMH volume (in mm^3) per additional year of hypertension. However, this association was not statistically significant.

Discussion

This study investigated the prevalence of cSVD markers in young patients with hypertension compared to normotensive controls. Patients with hypertension had more WMH, especially in the deep WMH, demonstrating that conventional MRI markers of cSVD are already visible in young at-risk patients, offering an interesting population to study early disease mechanisms of cSVD[22]. No significant associations

were found between blood pressure or duration since hypertension diagnosis and WMH volume.

These findings are in line with previous research showing an association between hypertension and WMH in older individuals[7-9]. Other studies, like the Framingham Heart Study, have found that higher systolic blood pressure is related to lower white matter integrity in young adults[23, 24]. The low prevalence of lacunes and microbleeds we found is also in line with existing literature, which shows that these markers are uncommon before the age of 50[2]. This indicates that WMH could be the earliest detectable MRI marker of cSVD. However, larger studies are necessary to validate these results. In our cohort, BMI was higher in the individuals with hypertension, making it difficult to separate the independent effect of these variables. Data among older patients with hypertension demonstrated a strong effect of hypertension duration on WMH volume and white matter microstructure[25]. This highlights the cumulative effect of high blood pressure on cerebral microvasculature over time, but our population is likely too young to observe this. Furthermore, the recorded duration may include periods of well-controlled blood pressure due to medication use which we do not account for in this analysis. The actual risk of microvascular damage likely depends on the overall period with hypertension, but also on the quality of blood pressure management during that time.

We observed significant differences in deep WMH but not in periventricular WMH when comparing the Fazekas scores. This is likely due to the strict threshold we applied for rating deep WMH, where the presence of more than one deep WMH lesion was classified as Fazekas 1 according to the original Fazekas classification. Furthermore, the moderate interrater reliability identified for periventricular WMH likely reflects the mild overall WMH burden within this group, resulting in several cases that were challenging to categorise at the lower end of the Fazekas scale. This highlights the limitations of using a categorical rating scale, as it does not fully capture the true WMH burden. The low sensitivity of categorical ratings such as the Fazekas scale likely explains the incongruency between results obtained with visual scores and those obtained quantitatively. In contrast, volumetric measures provide a more accurate reflection of the overall WMH load. When looking at the probability map, WMH in patients with hypertension were mostly located in periventricular areas in parietal and frontal white matter while the overall burden of WMH was relatively low. In normotensive controls, WMH volume was very low and mostly observed in the periventricular WM. This is in line with other studies, describing the periventricular areas as the most commonly affected in cSVD in older individuals, thus supporting the notion that young individuals with hypertension may be a

good model to examine early manifestations of cSVD[26, 27]. Disruption of the anterior thalamic radiation and forceps minor in this area was most strongly associated with cognitive functioning in SVD patients, emphasising the clinical significance of white matter disruption in specific tracts[28].

The low prevalence of cSVD markers such as lacunes and microbleeds, especially in the control group, limited the use of multivariable regression models. However, the absolute differences observed in WMH between patients and controls strongly suggest a higher SVD burden in patients with hypertension. To improve the reliability of the estimates despite the low prevalence of some MRI markers, we used Firth's penalised logistic regression for the analysis of deep and periventricular WMH. This method reduces bias in maximum likelihood estimates and provides more stable odds ratios when complete separation occurs.

Clinical implications

The clinical significance of early lesions in the brain of young individuals was not studied, however, similar lesions are linked to cognitive decline and an increased risk of stroke in older adults[2]. Early detection of WMH could be important for risk stratification and preventing neurological complications in young hypertensive patients. Our results highlight that early detection and treatment through routine monitoring of blood pressure in young individuals is important. Recent evidence suggests that maintaining blood pressure below the traditional threshold of 140/90 mmHg in midlife is important for optimal brain health[29].

Strengths and limitations

An important strength of this study is the use of a young and well-defined population, with strict inclusion and exclusion criteria and standardised MRI acquisition. The reliability of the MRI assessment was good to excellent for most of the cSVD markers. However, the moderate interrater agreement for periventricular WMH suggests that evaluation of this specific marker may be more subjective and less reproducible. Therefore, WMH volumes were also analysed, as these are more objective and thus minimise observer bias.

A limitation is the cross-sectional design, which makes it difficult to draw conclusions about causality and to assess the progression of cSVD over time. Furthermore, perivascular spaces, another important cSVD marker, were not evaluated because T2-weighted imaging was unavailable. In addition, socioeconomic status and genetic predisposition were not studied in this study, which may influence cSVD burden. It should be noted that patients with hypertension included in this study

are a selective group of patients with hypertension, as most young individuals with hypertension do not go to the hospital. Finally, selecting normotensive controls from the social circles of researchers and patients may have introduced selection bias, which limits the generalizability of the results.

Future directions

Future studies should include more advanced imaging techniques such as diffusion tensor imaging (DTI) to assess white matter microstructural integrity or dynamic contrast-enhanced (DCE) MRI to assess blood-brain barrier leakage to provide more information about early pathological processes. Longitudinal studies are needed to determine if early WMH in young individuals with hypertension contribute to more cerebrovascular damage and cognitive decline later in life. Furthermore, further investigating modifiable risk factors, such as diet and physical activity, and how these influence the development of cSVD in young hypertensive individuals could provide important insights into potential targeted preventive strategies for this group.

Conclusion

In conclusion, this study shows that cSVD is more common in young patients with hypertension than in normotensive controls. This highlights the need for early detection and management of hypertension and other modifiable risk factors to prevent brain damage later in life. Future research should focus on the long-term effects of hypertension on brain health and develop targeted strategies to reduce these effects in younger populations.

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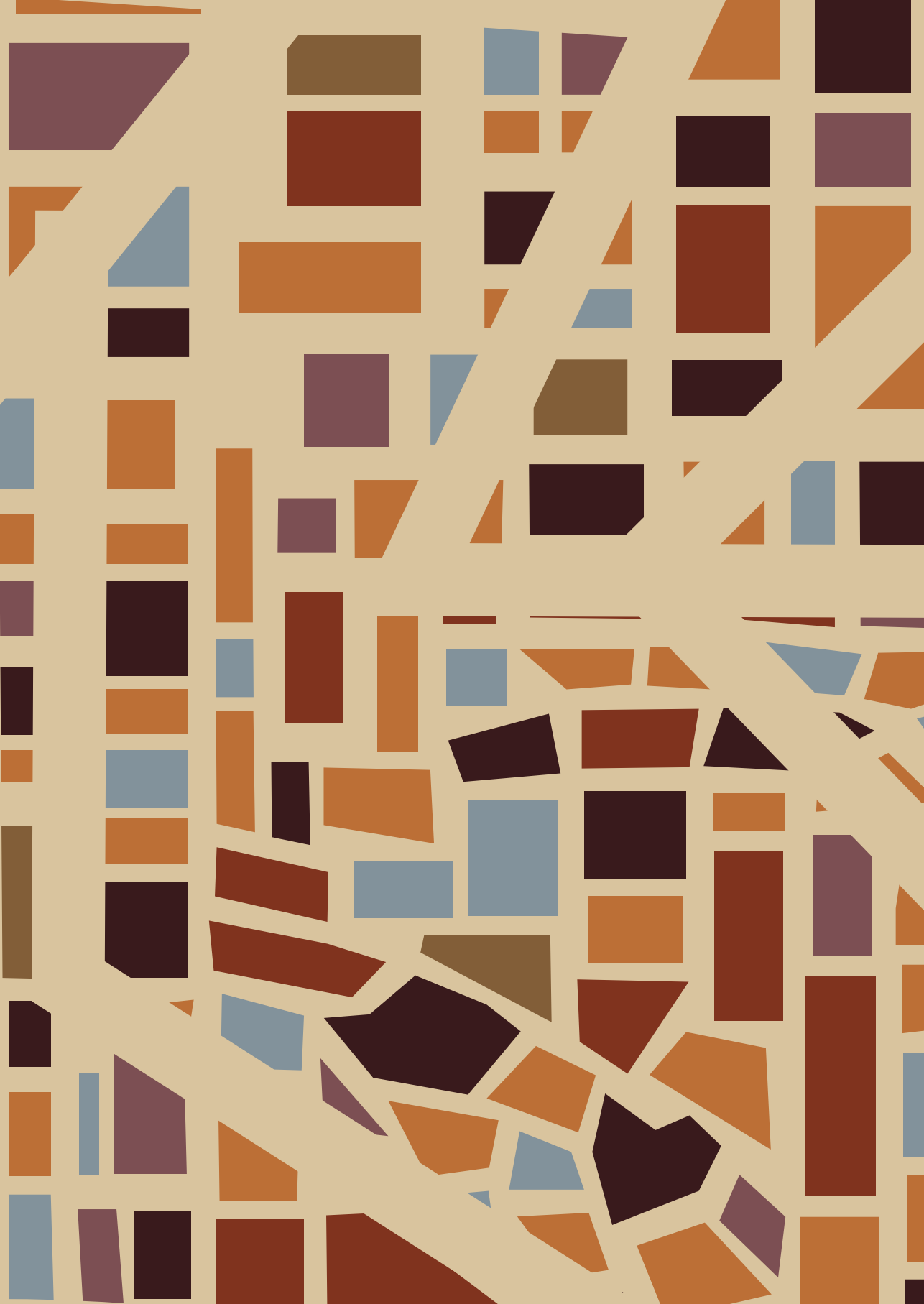

Chapter 4: Higher blood-brain barrier permeability in middle-aged adults with hypertension

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Hypertension, 2025, 7.3: 331-338.

Abstract

Background

Hypertension is a major risk factor for cerebral small vessel disease (SVD), but the underlying mechanisms remain incompletely understood. Blood-brain barrier (BBB) leakage is hypothesized to be involved in SVD pathogenesis, but this was mainly demonstrated in older patients with established SVD on magnetic resonance imaging (MRI). We investigated BBB leakage in young patients with hypertension, at risk for SVD, compared to controls.

Methods

BBB leakage of gadolinium contrast agent was measured with 3Tesla MRI in 59 patients with hypertension (mean age 35.8 ± 9.7 years; 54% female, mean blood pressure 146/92 mmHg) and 21 healthy controls (mean age 29.9 ± 4.8 years; 52% female; mean blood pressure 120/75 mmHg). Dynamic Contrast-Enhanced (DCE)-MRI was used to measure BBB leakage (leakage rate, *Ki* [min-1] and blood plasma volume fraction *Vp*). Linear regression adjusted for age and sex was used to compare BBB leakage between patients and controls. We additionally adjusted for amended SVD score and white matter hyperintensity volume. We also compared patients with primary aldosteronism (PA) to those with hypertension due to other causes.

Results

Patients with hypertension had 17% higher BBB leakage (*Ki*) in the white matter (**p=0.049**) and 15% in the grey matter (p=0.070) compared with controls, independent of SVD burden. No differences were found in *Vp* or between patients with PA and other causes.

Conclusion

Young patients with hypertension show BBB disruption independent of MRI-detectable SVD. This suggests a possible role of BBB dysfunction in SVD pathogenesis. Restoration of BBB integrity may be a relevant target for interventions in SVD, but longitudinal and intervention studies are needed to confirm causality.

Introduction

Cerebral small vessel disease (SVD) is the term for pathological processes that affect the penetrating small vessels of the brain that can cause ischemic and haemorrhagic stroke, vascular dementia, gait dysfunction and neurobehavioral symptoms[1]. Despite the major public health burden that SVD poses, surprisingly little is known about the pathological mechanisms causing SVD and possible therapeutical targets to prevent progression[2]. This is partly because risk factors and causes of SVD are often studied in individuals aged 60 years or over, when SVD most likely has been present for decades and brain damage has already occurred[3, 4]. These studies have shown that hypertension is the key risk factor for SVD, but we lack understanding of early changes in the brain caused by hypertension that possibly lead to cognitive impairment and SVD.

The blood-brain barrier (BBB) is a protective barrier that surrounds the cerebral microvasculature and separates the blood circulation from the brain tissue[5]. The BBB is essential for maintaining homeostasis in the brain as it regulates transport of small molecules including nutrients, that are needed for functioning of the brain, but also prevents passage of potentially harmful substances[5]. If the BBB is not functioning properly or shows unintended increased permeability, these harmful plasma components may enter the brain and potentially lead to brain damage[5]. In elderly individuals (>60 years) with SVD, there is increasing evidence that hypertension propels the progression of SVD, among others, by affecting the integrity of the BBB[6-8]. However, due to the cross-sectional design of most of the aforementioned studies, it remains unknown if BBB breakdown precedes the development of SVD magnetic resonance imaging (MRI) markers. If so, restoration of BBB integrity could be a promising therapeutic target for treatment of SVD.

Hypertension has no evident underlying cause in most cases. However, in 5-15% of patients, hypertension is caused by an adrenal overproduction of aldosterone in a condition known as primary aldosteronism (PA)[9, 10]. Patients with PA have an increased risk of cerebrovascular and cardiovascular events beyond mean blood pressure, suggesting that aldosterone directly affects cardiovascular health through other mechanisms[9]. It is unclear whether PA affects BBB integrity differently than other forms of hypertension.

We investigated the relation between dynamic contrast-enhanced (DCE) MRI-detected BBB disruption and hypertension in patients at risk for SVD. This study included young and middle-aged individuals (18-55 years) with hypertension, the

major risk factor for SVD, but without evident MRI markers of SVD. We aimed to assess the integrity of the BBB in young patients with hypertension compared to normotensive participants. Additionally, we explored variations in BBB leakage between treated and untreated patients as well as between patients with PA and other causes of hypertension.

Methods

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study population

Between July 2021 and December 2024, 60 patients with hypertension and 21 normotensive controls were included. We included patients with hypertension that were referred to the outpatient clinic of the Department of Internal Medicine of the Radboud University Medical Centre (Radboudumc) and Rijnstate hospital. Inclusion criteria were: age 18-55 years, diagnosis of hypertension, office-based blood pressure measurement (OBPM) ≥140 mmHg systolic and/or ≥90 mmHg diastolic measured within three months prior to inclusion. Healthy, normotensive controls were recruited via social networks of hypertensive participants and the involved researchers, including family members and friends. Inclusion criteria for controls were: age 18-40 years and no history of hypertension or use of antihypertensive medication for other purposes. Patients and controls were excluded if they had pre-existing symptomatic cerebrovascular disease (ischemic/haemorrhagic stroke or transient ischemic attack (TIA)) based on self-report and verification of available medical documentation. Furthermore, we excluded patients with other major risk factors for stroke according to the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification due to potential similar appearance of MRI markers as SVD[11]. This included large-artery disease, defined as stenosis >50% in the internal carotid artery or vertebral artery (based on ultrasound collected at baseline and medical records) and cardioembolism. Subjects were also excluded if there were contraindications for 3T contrast-enhanced MRI (i.e. renal insufficiency of estimated glomerular filtration rate (eGFR) < 30 ml/min, pregnancy) or if they had other major (neurological) diseases that may prevent long-term follow-up[12].

Study procedures

All participants were asked to fill in an online questionnaire about their medical history, medication use, profession, educational level and lifestyle habits such as smoking and alcohol use. Antihypertensive medication use and type of medication was verified using medical records.

Blood pressure measurements

All participants underwent automated blood pressure measurement (Dinamap; GE Healthcare, Chicago, IL) on the same day as the MRI scan. Measurements were done in a quiet room while participants were in a seated position with both feet on the floor, after five minutes of rest. Blood pressure was measured three times after which the average of these measurements was used in the data analysis. The examiner was present during the measurements.

MRI protocol

Images were obtained using a 3T MRI system (MAGNETOM PrismaFit, Siemens Healthcare, Erlangen, Germany) with a 20 channel head-neck coil. To detect BBB leakage, a DCE-MRI protocol consisting of a successive slow, fast and slow T1-weighted saturation recovery spoiled Gradient Echo (GRE) pulse sequences was performed. During the fast sequence, 0.1 mmol/kg of gadobutrol contrast agent (Gadovist, Bayer, Germany) was injected at a rate of 3 ml/s followed by a saline flush. 3D T1-weighted Magnetization Prepared 2 Rapid Acquisition Gradient Echoes (MP2RAGE) sequence was acquired prior to DCE-MRI for structural segmentation and to calculate quantitative T1 relaxation time maps in order to calculate contrast agent concentration following changes in signal intensity[13]. Furthermore, a pre-contrast fluid-attenuated inversion recovery sequence (FLAIR) was acquired to improve segmentation and to rate the extent of white matter hyperintensities (WMH). We acquired a susceptibility-weighted imaging sequence (SWI) to assess cerebral microbleeds. Details about the scan parameters are shown in table S1.

MRI preprocessing

Quantitative T1 maps were calculated from the MP2RAGE sequence using a previously described image processing protocol[13]. The UNI images resulting from the MP2RAGE sequence were denoised[14] and used for segmentation of the brain tissue into total grey matter (GM) consisting of cortical and deep GM, and white matter (WM) using the cross-sectional Sequence Adaptive Multimodal SEGmentation (SAMSEG) pipeline (Freesurfer, version 7.2)[15]. Robustness of the segmentation was inspected visually.

We used a deep learning-based segmentation tool to automatically segment WMH using T1 and FLAIR images (MIAC Automated Region Segmentation (MARS-WMH), available on https://github.com/miac-research/MARS-WMH)[16]. We visually inspected all the WMH masks for correct segmentation and corrected them manually where necessary. WMH volumes were calculated separately for periventricular WMH (=<10 mm of lateral ventricles) and deep WMH (>10 mm distant to ventricles).

To correct for head movement during DCE sequences, all images were coregistered to a reference image, i.e. the averaged pre-contrast slow image (FLIRT, FMRIB version 6.0.1; Functional MRI of the Brain Group, Oxford, UK).

Quantification of BBB leakage

To calculate the concentration of contrast agent in the blood, we derived the vascular input function from the superior sagittal sinus[17]. The BBB leakage rate (transfer constant Ki [min -1]) and the fractional blood plasma volume (*Vp* [-]) were calculated using voxel-wise pharmacokinetic modelling with the graphical Patlak approach[18]. We calculated the mean *Ki* and *Vp* of all voxels within each ROI (total, cortical and deep GM, total WM).

SVD rating

To assess SVD burden, WMH load, lacunes and cerebral microbleeds were visually rated by a trained neuroscientist (E.J.) under the supervision of an experienced neurologist (F-E.D.L) according to the Standards for Reporting Vascular Changes on Neuroimaging-2 (STRIVE-2) criteria[19]. We used these ratings to calculate the amended SVD score described previously, with a score ranging from 0-7[20]. We used this score because it does not require rating of perivascular spaces. Since perivascular spaces are best visualized on T2 MRI, which was not included in our MRI protocol, we did not assess them in our participants. WMH were graded from 0 to 3 using the Fazekas scale[21], cerebral microbleeds were graded as absent (0) or present (1) and number of lacunes were graded from 0 to 3 (none = 0, 1-2 = 1, 3-5 = 2, >5 = 3). Since our participants have a low SVD burden, the SVD score may not capture subtle between-subject variations. We therefore also included a volumetric approach described above to assess WMH volume, as a reflection of total SVD burden.

Statistical analysis

First, we examined differences in BBB leakage (*Ki* and *Vp*) between patients with hypertension and controls using multivariable linear regression analyses adjusted for age and sex separately for every ROI (i.e. total GM, cortical GM, deep

GM, total WM). Second, we examined the association between systolic/diastolic blood pressure and *Ki* and *Vp* in the ROIs using multivariable linear regression adjusted for age and sex. Within these models, we also assessed whether age was independently associated with BBB leakage. We repeated these analyses with additional adjustment for cardiovascular risk factors that differed between patients and controls (body mass index (BMI) and alcohol use) in separate models. To examine whether the association between blood pressure and BBB leakage is influenced by BMI, we tested for an interaction between hypertension and BMI in a separate model by including an interaction term (participant group*BMI). Finally, we repeated these analyses adjusting for total WMH volume/amended SVD score in separate models to examine the influence of presence of SVD.

We performed several post-hoc analyses. First, we repeated the main analyses after excluding one patient with hypertension who unexpectedly had a normal blood pressure (133/87 mmHg) on the day of the MRI scan while not taking antihypertensive medication and two healthy controls who unexpectedly had high blood pressure (142/93 and 144/90 mmHg), although they were never diagnosed with hypertension and did not have hypertension when measured at home. Second, we examined the effect of antihypertensive treatment on BBB leakage. We explored the differences in BBB leakage between patients with controlled hypertension while using antihypertensive medication (treated controlled, n=16), patients with uncontrolled hypertension who were not using any antihypertensive medication (untreated uncontrolled, n=7) and patients with uncontrolled hypertension despite using antihypertensive medication (treated uncontrolled, n=35). One participant with hypertension had a normal blood pressure on the day of the MRI scan without antihypertensive medication and was defined as "other" in this analysis. Differences between treatment groups in *Ki* and *Vp* were examined using multivariable linear regression analysis adjusted for age and sex. Third, we examined differences in BBB leakage in patients with primary aldosteronism (PA) and patients with hypertension due to other causes using multivariable linear regression analysis adjusted for age and sex. Finally, we examined whether duration of hypertension (operationalized as the number of years since first diagnosis) is associated with BBB leakage in a multivariable linear regression analysis adjusted for age and sex.

We calculated Cook distance and deleted studentized residuals to identify influential outliers (defined as Cook distance >1 and deleted studentized residual >3). Although no influential outliers were detected, visual inspection of Figure 2 and 3 suggested the presence of a potential outlier. We therefore repeated the analyses after excluding this participant.

For all statistical analyses, $p<0.05$ was considered statistically significant. All statistical analyses were conducted using RStudio (version 4.3.1).

Results

We included 59 patients with hypertension (mean age of 35.8 years (SD 9.7), 54% women) and 21 normotensive controls (mean age of 29.9 years (SD 4.9), 52% women) in the final analysis (Table 1). The post-contrast DCE-MRI scan of 1 patient with hypertension was incomplete due to unknown reasons and therefore excluded from the analyses. No participants were identified as outliers in leakage rate.

Both systolic and diastolic blood pressure were significantly higher in patients with hypertension compared to controls, while 86% of patients with hypertension were taking antihypertensive medication during the study. The patients not taking antihypertensive medication were often recently diagnosed and had therefore not started pharmacological treatment.

Differences in BBB leakage between groups

The leakage rate (*Ki*) in patients with hypertension was 16.7% higher in the WM (**p=0.049**) compared to controls, after adjusting for age and sex (Table 2). Although not significant, *Ki* was 14.7% higher in the GM (p=0.070), specifically the cortical GM (p=0.066). Representative examples of *Ki* maps of one hypertensive patient and one normotensive control are shown in Fig. 1. Age was not associated with *Ki* (WM: p=0.96; GM: p=0.78; deep GM: p=0.80; cortex: p=0.77) or *Vp* (WM: p=0.84; GM: p=0.79; deep GM: p=0.54; cortex: p=0.80). There was no difference in *Vp* in any ROI between patients with hypertension and controls.

Table 1: Characteristics of patients with hypertension and normotensive control participants.

Patient characteristics	Patients with hypertension (n=59)	Healthy controls (n=21)	P value
Age, y, mean (SD)	35.8 (9.7)	29.9 (4.9)	**<0.001**
Sex (female), %	32 (54.2)	11 (52.4)	1.000
Systolic blood pressure, mmHg, mean (SD)	146.4 (17.3)	119.9 (11.0)	**<0.001**
Diastolic blood pressure, mmHg, mean (SD)	91.7 (11.6)	74.8 (8.3)	**<0.001**
Duration of hypertension, y, median [IQR]	4.2 [1.5 – 11.5]	NA	NA
Blood pressure medication			
Antihypertensive medication use, n (%)	51 (86.4)	0 (0)	NA
Calcium antagonists, n (%)	39 (66.1)	0 (0)	NA
ACE inhibitors and ARBs, n (%)	28 (47.5)	0 (0)	NA
Diuretics, n (%)	16 (27.1)	0 (0)	NA
α-blocker, n (%)	6 (10.2)	0 (0)	NA
β-Blockers, n (%)	6 (10.2)	0 (0)	NA
Cardiovascular risk factors			
Body mass index, kg/m^2, mean (SD)	28.6 (6.0)	22.7 (2.8)	**<0.001**
Body mass index > 25, n, (%)	41 (69.4)	5 (23.8)	**<0.001**
Current smoking, n (%)	5 (8.5)	2 (9.5)	1.000
History of smoking, n (%)	22 (37.3)	11 (52.4)	0.303
Alcohol, units/wk (SD)	2.8 (4.1)	5.2 (3.3)	**0.021**
SVD MRI markers			
WMH volume, mm^3, median [IQR]	44.2 [9.1 – 120.4]	12.4 [1.9 – 35.8]	**0.018**
WMH volume, %ICV x 10^{-3}, median [IQR]	2.7 [0.6 – 7.2]	1.6 [0.2 – 3.5]	0.120
Fazekas score, 0/1/2/3, n (%)	49 (83.1) / 9 (15.3) / 1 (1.7) / 0 (0)	21 (100) / 0 (0) /0 (0) / 0 (0)	**0.046**
Presence of lacunes, n (%)	1 (1.7)	0 (100)	1.000
Presence of cerebral microbleeds, n, (%)	3 (5.1)	0 (100)	0.701
Amended SVD score, n (%)			
0	44 (74.6)	21 (100)	NA
1	14 (23.7)	0 (0)	NA
2	0 (0)	0 (0)	NA
3	1 (1.7)	0 (0)	NA
4	0 (0)	0 (0)	NA
5	0 (0)	0 (0)	NA
6	0 (0)	0 (0)	NA
7	0 (0)	0 (0)	NA

Differences in continuous variables between groups were examined using independent t-tests or Mann-Whitney U tests. Differences in categorical variables were examined using Pearson $\chi 2$ or Fisher exact tests where appropriate**.** ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blocker; NA, not applicable; SVD, small vessel disease; WMH, white matter hyperintensity.

Table 2: Differences in *Ki* and *Vp* between patients with hypertension and normotensive control participants, adjusted for age and sex.

Brain region	Patients with hypertension (n=59)	Normotensive controls (n=21)	P-value
BBB leakage rate (Ki, unit 10^{-4} min^{-1})			
Total WM	2.85 (1.03)	2.37 (0.50)	**0.049**
Total GM	3.91 (1.11)	3.38 (0.75)	0.070
Deep GM	4.31 (1.33)	3.80 (0.92)	0.132
Cortical GM	3.85 (1.10)	3.31 (0.73)	0.066
Blood plasma volume fraction (Vp, 10^{-2})			
Total WM	0.80 (0.26)	0.84 (0.52)	0.619
Total GM	1.32 (0.33)	1.35 (0.53)	0.657
Deep GM	1.30 (0.37)	1.43 (0.57)	0.194
Cortical GM	1.32 (0.33)	1.34 (0.53)	0.721

Data is presented as mean (SD). GM indicates grey matter; WM, white matter.

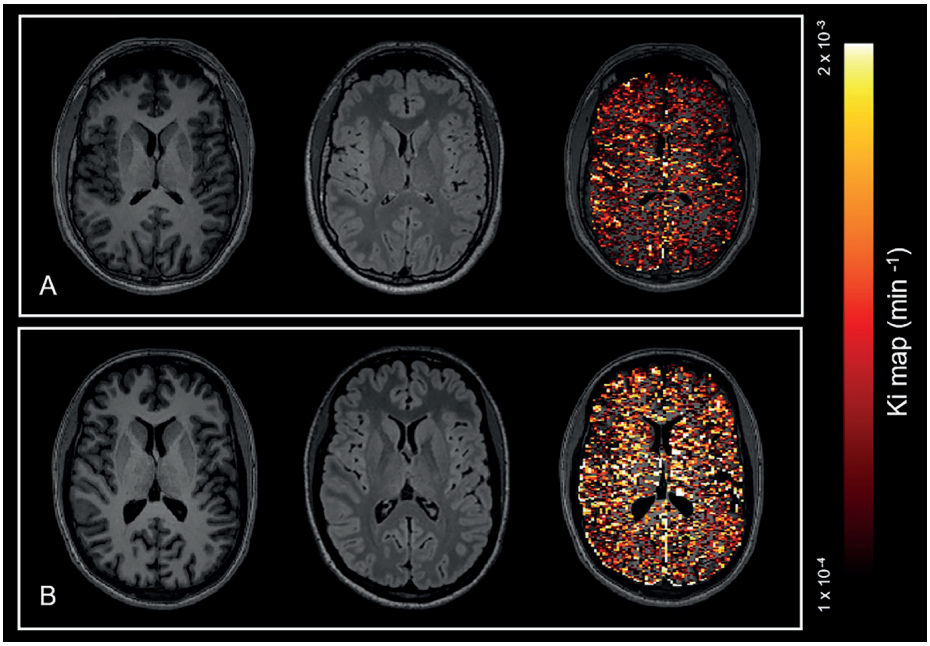

Figure 1: Color-coded gadolinium leakage in healthy control (A) and age-matched participant with hypertension (B). T1, FLAIR and leakage map overlayed on T1 images of 26-year-old control with blood pressure 125/79 mmHg (A) and 26-year-old participant with hypertension with blood pressure 145/94 mmHg (B). Lighter colours reflect a higher leakage rate value.

Association between blood pressure and BBB permeability

There was a positive relation between systolic blood pressure and BBB permeability (*Ki)* in the WM although this did not reach statistical significance (standardized β = 0.201, p=0.080), shown in Table 3/Fig. 2 (GM shown in Figure S1). There were no significant associations between systolic or diastolic blood pressure and *Vp* in any ROIs.(Table 3).

Table 3: Results of multivariable linear regression between blood-brain barrier leakage rate, blood plasma volume fraction and blood pressure measures, adjusted for age and sex.

Brain region	Systolic blood pressure		Diastolic blood pressure	
	BBB leakage rate (Ki, unit 10^{-4} min -1)			
	Standardized β	*P-value*	*Standardized β*	*P-value*
Total WM	0.201	0.080	0.145	0.204
Total GM	0.164	0.176	0.144	0.215
Deep GM	0.143	0.215	0.117	0.307
Cortical GM	0.166	0.170	0.150	0.198
	Blood plasma volume fraction (Vp, 10^{-2})			
	Standardized β	*P-value*	*Standardized β*	*P-value*
Total WM	-0.054	0.649	-0.041	0.726
Total GM	-0.124	0.286	-0.066	0.568
Deep GM	-0.154	0.191	-0.122	0.296
Cortical GM	-0.116	0.320	-0.052	0.653

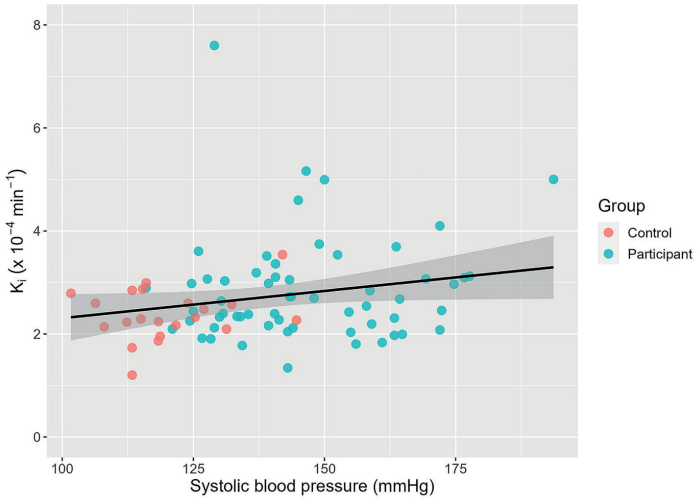

Figure 2: scatterplot of the BBB leakage rate in the white matter versus the systolic blood pressure. Pink dots indicate control participants and blue dots indicate patients with hypertension. The regression line is for visualization purposes only and is not corrected for age and sex.

Cardiovascular risk factors and small vessel disease

In the model with additional adjustment for the amended SVD score, the observed difference in *Ki* between the groups in the WM remained statistically significant (p=**0.042**) but not in the GM (total GM: p=0.064; deep GM: p=0.137; cortex: p=0.061). Adjusting for WMH volume instead of SVD score yielded similar results (WM: **p=0.039**; total GM: p=0.065; deep GM: p=0.123; cortex: p=0.062). There were no differences in *Vp* between the groups in any ROIs.

The difference in *Ki* in the WM between individuals with hypertension and controls remained significant after adjusting for alcohol use. However, the group difference in *Ki* was no longer significant after adjusting for BMI (total WM: p=0.175; total GM: p=0.180; deep GM: p=0.318; cortex: p=0.176). There was no significant interaction between BMI and participant group in any ROI.

Sensitivity analyses

After excluding participants with opposite blood pressure according to the groups assigned on the day of the MRI (2 controls, 1 hypertensive patient), the group differences in *Ki* between patients with hypertension and controls remained significant and became more pronounced (WM: **p=0.035**; total GM: p=0.055; deep GM: p=0.086; cortex: p=0.054). The *Vp* did not differ significantly between the two groups in any of the ROIs.

Ki was highest in treated uncontrolled participants (WM: 3.0 ± 0.9 x 10^{-4} min^{-1}; GM: 4.1 ± 1.1 x 10^{-4} min^{-1}), see Fig. 3). However, post-hoc analysis of subgroups based on treatment did not reveal any statistically significant differences in *Ki* or *Vp* between subgroups in any of the ROIs. Furthermore, there were no significant differences in BBB leakage between patients with hypertension caused by PA (n=16) and other causes in any of the ROIs (total WM: p=0.998; total GM: p=0.791; deep GM: p=0.968; cortex: p=0.745). Duration of hypertension was not associated with *Ki* (total WM: p=0.957; total GM: p=0.922, deep GM: p=0.960; cortex: p=0.933) or *Vp* (total WM: p=0.865; total GM: p=0.934; deep GM: p=0.684; cortex: 0.917) in any of the ROIs.

Although there were no significant outliers detected as described above, visual inspection of Figure 2 and 3 suggested the presence of a potential outlier. We therefore repeated the analyses after excluding this participant. This slightly attenuated statistical outcomes but the overall findings were unchanged. Group differences in *Ki* between controls and participants were no longer statistically significant (WM: p=0.085; total GM: p=0.124; deep GM: p=0.240; cortex: p=0.118).

However, there was a strong association between BBB leakage *Ki* and systolic blood pressure in the WM (**p=0.014**) which was not significant in the total GM (p=0.083), deep GM (p=0.091) and cortex (p=0.082).

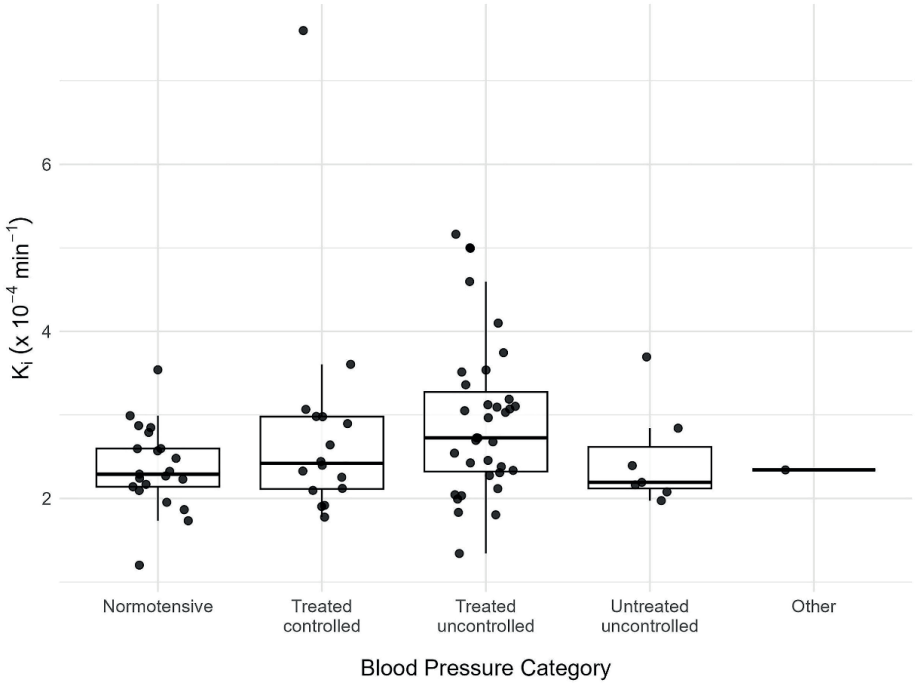

Figure 3: Blood-brain barrier leakage in the white matter for hypertension treatment status groups.

Discussion

This study examined the relation between early-life hypertension and BBB integrity. We demonstrated that young and middle-aged patients with hypertension with the earliest manifestations of SVD already have significantly stronger BBB leakage in the WM compared to normotensive controls. There was a positive association between systolic blood pressure and leakage rate in the WM although not statistically significant.

BBB leakage is considered to be one of the early pathophysiological mechanisms in SVD[22]. Whether BBB leakage is a primary cause or secondary consequence remains uncertain. Most previous studies were conducted in adults aged over 65 years who had likely been developing SVD for decades, often with cognitive impairment or dementia[23]. This is the first study to demonstrate that hypertension is already

associated with BBB disruption at a young age, even before evident MRI markers of SVD were present. This is supported by a recent study that also examined BBB leakage in patients with hypertension, at an average age of 66 years, while we demonstrated that increased BBB leakage already occurred at a much younger age (mean age of 36 years)[24]. Interestingly, lower BBB integrity in the current study is more profound in the WM, the predilection site for SVD, whereas others only observed this association in the GM[24]. Other studies reported mixed results by demonstrating increased BBB leakage in both the normal appearing white matter (NAWM), WMH, cortex and GM[7, 25, 26]. These findings suggest that BBB disruption occurs throughout the whole brain. This is supported by our results as we also observe a trend of higher BBB leakage in the GM, although not statistically significant. While other studies report increasing BBB leakage with increasing age in healthy older adults[27], we do not observe this association in our younger population. This may be due to the younger age of our participants and a limited age range. Additionally, the presence of hypertension likely exerts a stronger effect on BBB leakage, masking the more subtle age-related effects.

When the Patlak model is applied, different outcomes of the model reflect the microvasculature. *Ki* represents the BBB leakage rate and *Vp* is a measure of the fraction of tissue volume occupied by plasma[23]. While we observe higher *Ki* in patients, there are no differences in blood plasma volume fraction *Vp* between groups. Since *Vp* highly depends on vascularization and cerebral blood flow, reduced *Vp* suggests poorer perfusion. This has been described in SVD patients[25] whereas conversely, increased *Vp* has been reported in patients with hypertension[24]. Because we measured higher leakage rate, but no changes in blood plasma volume, this suggests that the vessels may have become leakier, though neither cerebral perfusion nor vascular density seems (yet) affected in these young and middle-aged patients with hypertension. However, as we did not assess cerebral blood flow explicitly, we cannot objectively verify this.

Our results remain statistically significant after additional adjustments for SVD score and WMH volume, indicating that BBB leakage is higher in patients with hypertension independent of pre-existing SVD markers. Importantly, this suggests that subtle BBB leakage occurs before structural MRI markers of SVD become visible, however this needs to be confirmed in prospective studies[8]. As expected, we saw a small, but yet detectable number of MRI markers of SVD already in these young and middle-aged patients, which confirms that this study captured the earliest manifestations of SVD. The lack of a significant interaction between blood pressure and BMI suggests that BMI does not modify the effect of hypertension on

BBB leakage. While adjusting for BMI changed the observed group differences, this is likely because patients with hypertension had a significantly higher BMI in this study, a finding consistent with previous research[28].

We did not observe a significant effect of hypertension treatment status on BBB leakage, in contrast to a previous study[24]. However, in our study, this analysis was subject to confounders because the untreated participants were younger (mean age of 26.1 vs 38.3 years) and had often been diagnosed with hypertension more recently, meaning they had not yet started treatment. Assuming that hypertension causes progressive damage to the cerebral blood vessels, it is possible we did not observe a significant difference because the treated group had a longer duration of hypertension, making it difficult to detect a restorative effect of antihypertensive medication. Additionally, our sample size was small (n=7 in the untreated group) and we had no information on medication adherence, which could further influence the results.

This study included patients with different causes of hypertension, including PA. The overproduction of aldosterone in PA patients is suggested to have direct effects beyond blood pressure levels[9]. In line with this, it was reported that patients with hypertension due to PA have a higher arterial wall inflammation, measured with fluorodeoxyglucose-positron emission tomography, compared to patients with similar blood pressures due to primary hypertension[29]. We did not observe any differences in BBB leakage rate between the groups which suggests that the disruption in BBB integrity is affected by blood pressure rather than aldosterone levels. But again, these results should be interpreted with caution since the sample size of this subgroup is small (n=16 patients with PA).

Interestingly, we do not see an association between the duration of hypertension and BBB leakage. Although it could be argued that hypertension has a cumulative effect on the brain over time, we did not observe this for BBB leakage. This suggests that BBB leakage measured with DCE-MRI is more a reflection of the current level of blood pressure, rather than long-term alterations in the small vessels. It is also possible that we do not observe this effect because our population is relatively young, with a rather short duration of hypertension (median: 4.2 years). Furthermore, the duration of hypertension recorded here may include periods of well-controlled blood pressure with antihypertensive medication use, but we do not have information about this. Our study has several strengths. The design of our study enabled us to examine early changes in BBB leakage prior to the appearance of conventional MRI markers of SVD and cognitive problems. Because we recruited

patients through hospital outpatient clinics, detailed information about their medication use and history and diagnosis of hypertension was available. However, some methodological limitations should be discussed. First, we used OBPM, which has a risk of "white-coat effects" and measurement error[30]. Although we measured blood pressure three times during each visit to minimize this error and compared measurements to values available in medical files, a standardized 30-minute automated protocol or ideally 24-hour ambulatory blood pressure monitoring would have provided greater accuracy and allowed for the assessment of additional blood pressure characteristics such as nocturnal dipping and short-term blood pressure variability. Second, our healthy control population was slightly younger than the patients with hypertension; however, all our analyses were adjusted for age to account for this difference. Third, it is challenging to disentangle specific effects of hypertension since these patients also had a higher BMI. This reflects real-world conditions, where hypertension often coexists with overweight or obesity and related metabolic disturbances such as hyperlipidaemia and hyperglycaemia in SVD pathogenesis. Fourth, results are not corrected for multiple comparisons since this study was exploratory and the sample size was small. Consequently, the findings should be considered preliminary and interpreted with caution. Finally, the DCE-MRI protocol used in this study to detect BBB leakage is relatively demanding due to the long scan duration (29 minutes) and injection of contrast agent. While this is currently the preferred imaging technique and the long protocol allowed us to quantify very subtle leakage, this stresses the need for employing non-invasive techniques such as arterial spin labelling or blood biomarkers[31]. Furthermore, the injected contrast agent has a small molecular weight (0.60 kDA) and can likely cross the BBB more easily; it is therefore unknown if our reported findings also apply to larger plasma proteins. Another limitation relates to the possibility of partial volume effects in the cortex in our study. We observed a border of elevated *Ki* around the cortex, which we believe reflects leakage from blood vessels within or between the (lepto)meninges. These vessels lack a BBB and may contribute to signal in adjacent cortical voxels. Although eroding of the cortical GM mask did not alter our results when comparing patients with controls, future studies could consider accounting more carefully for potential signal from adjacent meningeal vessels, especially when evaluating subtle cortical leakage.

The mechanisms through which hypertension can damage the brain are complex and diverse. Hypertension induces changes in mechanical, cellular and molecular pathways that affect the vascular cells comprising the neurovascular unit[32, 33]. Specifically, endothelial dysfunction plays a key role in loss of BBB integrity[6, 7]. When the BBB loses integrity, this can cause leakage of plasma proteins and harmful

substances from the blood vessels, causing inflammation, oxidative stress and edema[6]. Some studies have demonstrated that BBB leakage precedes structural damage on MRI and cognitive decline, which is supported by our study[34-36]. This emphasizes the potential of targeting endothelial dysfunction and accompanying BBB disruption with therapeutic interventions in order to delay progression or onset of SVD[37]. Our study is particularly relevant in the context of the increased risk of late-life cognitive impairment in individuals with early-life hypertension[38, 39]. This association is thought to be driven by a cumulative injury process, in which prolonged exposure to subtle vascular damage during adulthood is critical [40]. Our findings suggest that increased BBB leakage is one of the earliest pathological changes in this process, potentially mediating the link between hypertension and cognitive decline, although follow-up of our cohort is required to demonstrate the association between BBB permeability and progression of SVD. Sustained loss of BBB integrity may contribute to irreversible brain damage. Next to SVD, hypertension is one of the strongest risk factors for Alzheimer's Disease (AD)[41]. It is suggested that breakdown of the BBB contributes to AD by reducing clearance and increasing accumulation of amyloid-β[41]. This highlights the importance of early and intensive blood pressure treatment to protect the brain from dementia, possibly more rigorous than current treatment guidelines suggest[40]. Our findings should be replicated in a larger sample. This would allow the investigation of a class-effect of antihypertensive medication on BBB leakage, which has been suggested previously in SVD and dementia[42, 43].

Perspectives

In summary, this study examined BBB permeability in young and middle-aged patients with hypertension. compared to normotensive controls. BBB leakage is observed in the WM of patients with hypertension, independent of pre-existing SVD. This suggests that loss of BBB integrity is an early pathophysiological change in patients with hypertension at risk for SVD and, as such, is a relevant target for therapeutic interventions aimed at stopping development or progression of SVD.

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Supplemental material

Table S1: acquisition details MRI sequences.

	DCE			FLAIR	T1w	SWI
Sequence	Pre-bolus slow T1w SR SPGR	Fast T1w SR SPGR	Post-bolus slow T1w SR SPGR	SPACE	3D MP2RAGE	3D SPGR
Voxel size (mm)	1.0×1.0×2.0	1.0×1.0×3.0	1.0×1.0×2.0	0.5×0.5×0.9	1.0×1.0×1.0	1.0×1.0×3.0
TR (ms)	3.4	3.4	3.4	5000	6000	27
TE (ms)	1.56	1.56	1.56	388	1.71	20
TI (ms)	120	120	120	1800	900, 2200	-
Flip Angle (°)	20	20	20	Optimized T2var refocusing	6, 5	15
Number of slices	60	10	60	192	176	52
Acquisition time (mm:ss)	01:47	02:00	24:52	05:47	07:50	02:43
Other	NM = 5 R = 3	NM = 32 R = 2 Injection of Gadobutrol	NM = 70 R = 3	R = 2 Turbo factor = 278	Also used for T1-mapping	

DCE =Dynamic contrast-enhanced; FLAIR =Fluid-attenuated inversion recovery; MP2RAGE =Magnetization-prepared 2 rapid acquisition with gradient echo; NM =number of measurements R =parallel imaging acceleration factor; SPACE =Sampling perfection with application-optimized contrast using different flip-angle evolutions; SWI =Susceptibility weighted imaging; SPGR=Spoiled gradient recalled echo; SR = Saturation Recovery; TE =Echo time; TI =Inversion time; TR =Repetition time.

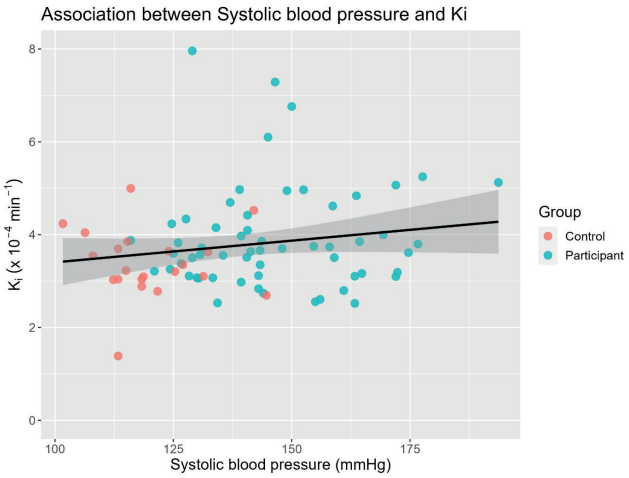
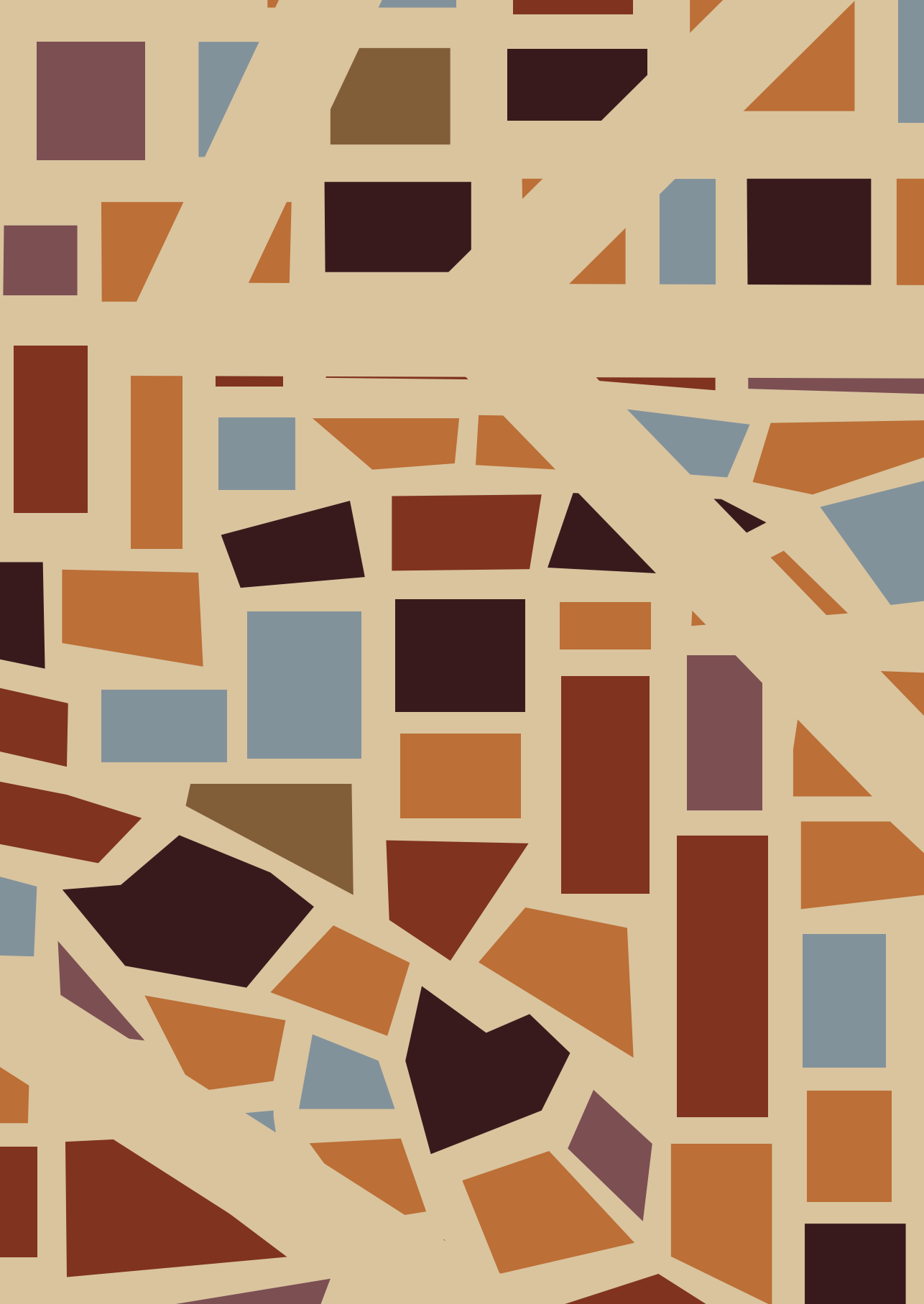


Figure S1: scatterplot of the BBB leakage rate in the grey matter versus the systolic blood pressure. Pink dots indicate control participants and blue dots indicate patients with hypertension. The regression line is for visualization purposes only and is not corrected for age and sex.



Chapter 5: Blood-brain barrier disruption in response to blood pressure changes in adults with hypertension: a serial MRI study

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Abstract

Background

Loss of blood-brain barrier (BBB) integrity is observed in patients with hypertension and is hypothesized to contribute to cerebral small vessel disease (SVD) pathogenesis. Blood pressure increase and decrease within individuals and the effects on BBB integrity have never been examined. We aimed to examine BBB leakage in middle-aged patients with hypertension following subsequent blood pressure increase and decrease.

Methods

The Hyperintense study included hypertensive adults who temporarily discontinued antihypertensive medication for diagnostic purposes. MRI was done at three timepoints: before medication withdrawal (visit 1), during withdrawal (visit 2) and after medication restart (visit 3). BBB integrity was measured with 3T Dynamic Contrast-Enhanced (DCE)-MRI. We assessed leakage rate *Ki* [min-1] and blood plasma volume *Vp*. Linear regression was used to examine association between changes in blood pressure and BBB leakage.

Results

Fourteen patients with hypertension (mean age 48.2 ± 4.5 years; 36% female) were included. After medication withdrawal, blood pressure increased in 11/14 participants (79%). Increase in blood pressure between visit 1-visit 2 was associated with increased *Ki* in white (WM) (β=0.683, **p=0.007**) and grey matter (GM) (β=0.587, **p=0.027**) and *Vp* in the GM (β=0.666, **p=0.009**). Following medication restart, blood pressure decreased in seven participants, with *Ki* decreasing in four of them. There was no association between decrease in blood pressure and *Ki* or *Vp* between visit 2-visit 3.

Conclusions

Blood pressure increase was associated with increased BBB leakage. Reduction in BBB leakage following medication restart was observed in most, but not all participants. This suggests that BBB leakage is dynamic and influenced by blood pressure.

Introduction

Cerebral small vessel disease (SVD) is a vascular disorder that affects the smallest perforating vessels of the brain[1]. Its magnetic resonance imaging (MRI) markers include white matter hyperintensities (WMH), lacunes, microbleeds and perivascular spaces, as well as loss of microstructural integrity[2]. It is estimated that SVD causes up to a quarter of ischemic strokes, most haemorrhagic strokes and almost half of dementias worldwide[1]. Despite the major public health burden that SVD poses, there are currently no proven therapeutic treatments to stop progression of SVD apart from preventative measures such as intensive blood pressure control[3]. Hypertension has been identified as a major risk factor for SVD. However, this is based on intervention studies that assessed the effects of blood pressure lowering on SVD and results from population studies that relate differences in blood pressure levels and SVD between individuals rather than within individuals. Hypertension is known to affect the condition of the blood vessels, including endothelial dysfunction, promotion of inflammation, plaque formation and remodelling of the vessel wall[4]. The effects of blood pressure increase on BBB integrity and SVD have not yet been examined[5]

Gadolinium-enhanced MRI is increasingly used to detect BBB leakage in SVD, both in WMH and NAWM[6-8]. We have recently demonstrated that BBB leakage is higher in young patients with hypertension, likely prior to the development of structural MRI markers of SVD, suggesting that loss of BBB integrity precedes structural tissue damage[9]. If BBB breakdown plays a causal role in SVD pathogenesis, targeting this mechanism may be a relevant therapeutical strategy. However, longitudinal studies measuring changes in BBB integrity within individuals over time are lacking. In addition, gadolinium-enhanced MRI provides information on cerebral blood perfusion, which may alter in response to short-term blood pressure changes.

We examined the effects of blood pressure increase and subsequent decrease in patients with hypertension, who temporarily withdraw and restart antihypertensive medication for diagnostic purposes. This temporary increase and subsequent decrease in blood pressure provides a unique opportunity to assess the impact thereof on the smallest brain vessels. We use dynamic contrast-enhanced (DCE) MRI to measure changes in BBB leakage rate (*Ki*) and blood plasma volume fraction (*Vp*) as a measure of perfusion at three time points.

Methods

Study population and design

The Hyperintense study is a longitudinal observational cohort study conducted in young and middle-aged patients with hypertension[10]. Participants (18-55 years) were recruited at the outpatient clinics of the Department of Internal Medicine of the Radboud University Medical Centre (Nijmegen) and Rijnstate Hospital (Arnhem) in the Netherlands.

In most patients with high blood pressure, there is no identifiable underlying cause, but in 5-10% a potentially reversible cause can be identified[11]. Primary aldosteronism (PA) is the most common cause of therapy-resistant hypertension, where hypertension is caused by an overproduction of aldosterone[11]. To diagnose this, the plasma aldosterone/renin ratio is measured in patients. Prior to this, patients have to temporarily discontinue antihypertensive drugs or switch to medication that does not affect this ratio (i.e. doxazosin, verapamil, diltiazem, hydralazine)[12]. The decision to stop all antihypertensive drugs, or to switch them to these non-interfering drugs in dependent mainly on blood pressure levels and potential co-morbidities. A washout period of at least 2 weeks (for diuretics, Angiotensin Converting Enzyme (ACE) inhibitors, Angiotensin Receptor Blockers (ARBs)) or 4 weeks (for mineralocorticoid receptor antagonists) is required before measurement of renin/aldosterone[12]. This washout often causes a transient rise in blood pressure. Note that this is a well-established and safe diagnostic protocol, after which medication is often adjusted accordingly and restarted[13].

The in- and exclusion criteria were described in detail before[10]. In short, we included patients aged 18-55 years with hypertension who temporarily withdrew antihypertensive medication or switched to non-interfering medication for 2-4 weeks as part of their diagnostic routine to examine potential reversible causes of hypertension. Participants were excluded if they had a history of imaging-proven symptomatic ischemic or haemorrhagic stroke or transient ischemic attack (TIA), based on self-report and verification of medical documentation. We excluded patients with large-artery disease, defined as more than 50% stenosis of the internal carotid or vertebral artery, as assessed through ultrasound at baseline. Furthermore, we excluded patients with a high risk of thromboembolic conditions and atrial fibrillation, based on medical history and self-report. Exclusion criteria also included contraindications for gadolinium enhanced 3T MRI (renal insufficiency, defined as eGFR <30 ml/min or pregnancy) and other major (neurological) diseases that could compromise long-term follow-up. This study was approved by the medical ethics committee region Arnhem-Nijmegen and all participants provided written informed consent.

Study visits

Visit 1 was scheduled before antihypertensive medication was withdrawn. Visit 2 took place 2-6 weeks after participants discontinued their antihypertensive medication and their blood pressure was increased. The third visit was scheduled 2-4 months later, when the diagnostic trajectory was completed and participants reached a stable target blood pressure.

Blood pressure measurements

We measured blood pressure using an automated device (Dinamap; GE Healthcare, Chicago, IL) at the day of the MRI. Measurements were done in a quiet room, with participants seated, feet flat on the floor, and after a five-minute rest period. Blood pressure was the mean of three consecutive measurements for analysis. The examiner remained present throughout the procedure.

Lifestyle and clinical parameters

Participants completed an online questionnaire covering their medical history, current medication use, occupation, level of education, and lifestyle factors such as smoking and alcohol consumption. Use of antihypertensive drugs and all other medication was verified through medical records at every timepoint.

MRI protocol

At each study visit, we performed a 3T brain MRI scan (MAGNETOM PrismaFit, Siemens Healthcare, Erlangen, Germany) with a 20-channel head-neck coil. Details about the imaging protocol and acquisition parameters were published previously[10]. In short, the protocol included several sequences to assess structural integrity of the brain, including 3D T1-weighted magnetization prepared 2 rapid acquisition gradient echoes (MP2RAGE), fluid attenuated inversion recovery (FLAIR) and susceptibility-weighted imaging (SWI). All structural sequences were acquired prior to contrast agent injection. To assess BBB integrity, DCE MRI was performed. This scan consisted of consecutive slow, fast and slow T1-weighted saturation recovery spoiled GRE sequences to follow the contrast agent distribution over time. Gadovist contrast agent was injected during the fast sequence, immediately followed by a saline flush.

Image processing

Quantitative T1 relaxation time maps were obtained from the MP2RAGE sequence following an established image processing protocol[14]. Brain tissue was segmented into total grey matter (GM) (consisting of deep and cortical grey matter) and white matter (WM) from the denoised UNI images produced by the MP2RAGE sequence,

using the Sequence Adaptive Multimodal SEGmentation (SAMSEG) method within Freesurfer (version 7.2)[15, 16]. The quality of the segmentation was verified through visual inspection.

For the identification of white matter hyperintensities (WMH), we applied a deep learning-based segmentation approach that utilizes both T1-weighted and FLAIR images: the MIAC Automated Region Segmentation (MARS) tool, available at https://github.com/miac-research/MARS-WMH[17, 18]. Each WMH mask was visually inspected, and manual corrections were made when required. Volumes for WMHs were measured separately for periventricular regions (≤10 mm from the lateral ventricles) and for deep WM regions (>10 mm from the ventricles).

Quantification of BBB leakage and Vp

Processing of DCE-MRI images was described previously[9]. To correct for head motion during the DCE sequences, all dynamic images were spatially aligned to the averaged pre-contrast slow image using the FLIRT registration tool (FMRIB Software Library, version 6.0.1; FMRIB, Oxford, UK). Per voxel, the graphical Patlak approach was used to calculate the BBB leakage rate (transfer constant *Ki* [min -1]) and the fractional blood plasma volume (*Vp* [-]), which is a measure of the volume of blood plasma in the tissue[19]. The Patlak model has been recommended to measure subtle contrast leakage in SVD[20]. The vascular input function (VIF) was calculated by selecting a region of approximately 10-15 voxels in the middle of the superior sagittal sinus. For every ROI (total WM, total GM, cortical and deep GM) we calculated the mean *Ki* and *Vp*.

Statistical analysis

First, we examined differences in systolic and diastolic blood pressure, *Ki* and *Vp* between time-points using paired t-tests to examine the effect of medication change. Next, we used linear regression to examine the association between increase in blood pressure and changes in BBB leakage between visit 1 and visit 2 separately for every ROI. We performed these analyses separately for systolic and diastolic blood pressure change, as well as for changes of *Ki* (transfer constant) and *Vp* (plasma volume). As a sensitivity analysis, we repeated this analysis after excluding participants that did not exhibit the expected blood pressure increase between visit 1 and 2.

We repeated these analyses for changes between visit 2 and visit 3 to examine the effect of blood pressure decrease on BBB leakage.

For all statistical analyses, we considered $p<0.05$ statistically significant. All statistical analyses were conducted using RStudio (version 2025.05.1).

Results

16 participants were included in this study between July 2021-October 2024. The post-contrast MRI scan of 1 participant at visit 1 was incomplete and was therefore excluded from the analysis and one participant only completed the first study visit. The final dataset for analyses therefore included 14 participants, of whom baseline characteristics are presented in Table 1.

Table 1: baseline characteristics.

	Participants (n=14)
Age (years), mean (SD)	48.2 (4.5)
Sex (female, %)	5 (36)
Baseline systolic blood pressure (mmHg, mean (SD))	148 (18.1)
Baseline diastolic blood pressure (mmHg, mean (SD))	90 (9.2)
Antihypertensive medication use, n (%)	14 (100)
Number of different antihypertensive medications, 1/2/3/4, n (%)	4 (29) / 4 (29) / 3 (21) / 3 (21)
Antihypertensive medication type at baseline	
Calcium antagonists, n (%)	11 (79)
ACE inhibitors and ARBs, n (%)	10 (71)
Diuretics, n (%)	8 (57)
α-Blockers, n (%)	1 (7)
β-Blockers, n (%)	2 (12)
Body mass index (kg/m^2, mean (SD))	29.5 (4.4)
Current smoking, n (%)	3 (21)
History of smoking, n (%)	6 (43)
SVD characteristics	
Total WMH volume (mm^3, median [IQR])	68.9 [32.0 – 129.0]
Fazekas score, 0/1/2/3, n (%)	12 (86) / 2 (14) / 0 (0) / 0 (0)
Lacunar infarcts, n (%)	1 (6)
Cerebral microbleeds, n, (%)	0 (0)

Abbreviations: ACE: Angiotensin-Converting Enzyme; ARBs: Angiotensin II Receptor Blockers; SVD: Small Vessel Disease; WMH: White Matter Hyperintensity.

Change in blood pressure, BBB integrity and plasma volume over time

Median time between visit 1 and visit 2 was 55.5 days (range: 21-228 days) and between visit 2 and visit 3 203.0 days (range: 133-800 days). In Table 2, mean *Ki* and *Vp* for every visit are shown. Between visit 1 and 2, both mean *Ki* and *Vp* increased after medication discontinuation in all ROIs. Between visit 2 and 3, *Ki* decreased when treatment was reinstalled, while *Vp* remained increased. In Figure 1, representative examples of *Ki* maps at three visits are shown for two participants.

Table 2: mean (SD) systolic and diastolic blood pressure, *Ki* and *Vp* per ROI at three time-points and mean (SD) changes in variables between time-points.

	Visit 1 (n=14)	Visit 2 after discontinuation (n=14)	Visit 3 after restart (n=9)	Δ visit 1-2	Δ visit 2-3	Δ visit 1-3
Systolic blood pressure, mmHg	147 (18)	158 (17)	137 (15)	11 (13)*	-20 (22)*	-7 (21)
Diastolic blood pressure, mmHg	89 (10)	94 (9)	83 (10)	4 (11)	-10 (11)*	-7 (13)
BBB leakage rate (Ki, 10^{-4} min^{-1})						
WM	2.84 (0.68)	3.30 (1.20)	2.96 (1.07)	0.47 (1.17)	-0.43 (1.65)	-0.02 (1.40)
Total GM	3.84 (0.72)	4.65 (1.92)	3.72 (1.06)	0.81 (1.88)	-0.80 (1.99)	-0.27 (1.43)
Deep GM	4.29 (0.98)	5.12 (2.10)	4.01 (1.14)	0.83 (2.10)	-1.1 (2.35)	-0.37 (1.45)
Cortex	3.79 (0.71)	4.56 (1.89)	3.66 (1.05)	0.78 (1.86)	-0.75 (1.92)	-0.28 (1.42)
Blood plasma volume fraction (Vp, 10^{-2})						
WM	0.83 (0.25)	0.93 (0.34)	1.1 (0.35)	0.09 (0.37)	0.08 (0.54)	0.27 (0.52)
Total GM	1.35 (0.31)	1.59 (0.56)	1.52 (0.43)	0.24 (0.57)	-0.02 (0.64)	0.25 (0.58)
Deep GM	1.39 (0.45)	1.55 (0.59)	1.50 (0.43)	0.17 (0.67)	-0.02 (0.77)	0.25 (0.61)
Cortex	1.35 (0.30)	1.58 (0.56)	1.51 (0.43)	0.23 (0.57)	-0.02 (0.63)	0.24 (0.59)

*indicates a significant difference between time-points ($p<0.05$).

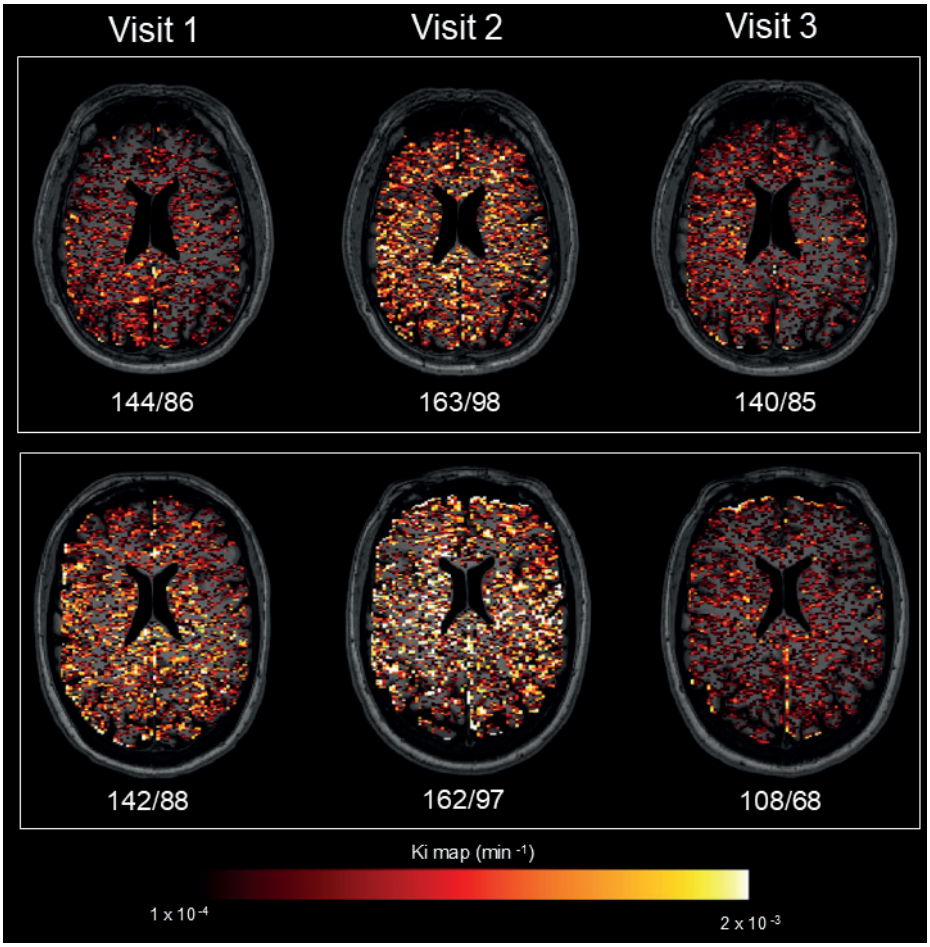

Figure 1: Color-coded gadolinium leakage in two patients with hypertension at three visits. Leakage map (*Ki*) is overlayed on T1 images. Blood pressure at each visit is shown (in mmHg). Lighter colours reflect a higher BBB leakage rate.

Increase in blood pressure at visit 2 (after medication switch)

Between visit 1 and visit 2, all 14 participants switched or discontinued antihypertensive medication for diagnostic purposes. Most participants switched to one calcium-channel blocker (CCB) (n=6), one alpha blocker (n=3), two CCBs (n=1) or a CCB and alpha blocker combined (n=1). Two participants discontinued all antihypertensive medications. One participant was mistakenly taking a CCB with a diuretic. Among these 14 participants, blood pressure increased in 11 participants. Mean changes in blood pressure are shown in Table 2.

In Figure 2, the association between the increase in systolic blood pressure and BBB leakage after medication discontinuation (between visit 1 and 2) in the WM is

shown. In absolute values, increase in *Ki* in the WM ranged from 0.08 – 3.2 x 10^{-4} min^{-1} which corresponds to a percentual increase of 3.2 - 107%. In the three participants where blood pressure was lower at follow-up 1, *Ki* in the WM decreased with -0.1 – 1.2 x 10^{-4} min^{-1}. Change in systolic blood pressure was associated with change in *Ki* in all ROIs: WM (β=6.1 x 10^{-6}, **p=0.007**), GM (β=8.4 x 10^{-6}, **p=0.027**), deep GM (β=10.5 x 10^{-6}, **p=0.010**) and cortex (β=8.1 x 10^{-6}, **p=0.032**), see Figure 2/Table 2. Similar associations are observed between Δ diastolic blood pressure and Δ *Ki* (Supplementary Table 1). After excluding the participants that did not exhibit an increase in blood pressure after medication withdrawal (n=3), the magnitude of the association between increase in systolic blood pressure and *Ki* increased in all ROIs (WM: (β=13.4 x 10^{-6}, **p=0.007**), GM (β=23.6 x 10^{-6}, **p=0.027**), deep GM (β=22.2 x 10^{-6}, **p=0.010**) and cortex (β=23.6 x 10^{-6}, **p=0.032**), and similarly for diastolic blood pressure.

Increase in systolic blood pressure was associated with increase in *Vp* in the deep GM (β=3.4 x 10^{-4}, **p=0.009**) and in the WM (β=1.5 x 10^{-4}, p=0.050). No such associations were observed for the total GM and cortex. A similar pattern was observed for diastolic blood pressure (Supplementary Table 1).

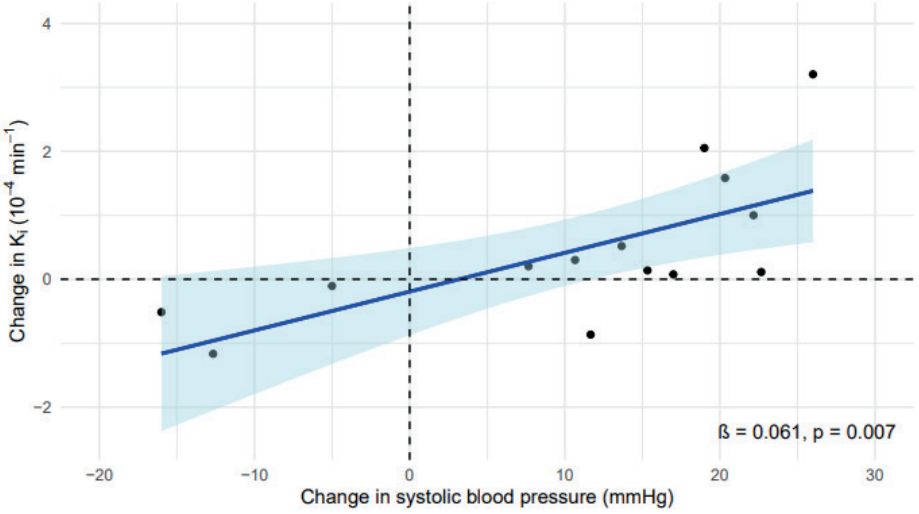

Figure 2: plot of change in systolic blood pressure vs change in *Ki* in the white matter between visit 1 and 2. β is x 10^{-4}.

Decrease in blood pressure after restoration anti-hypertensives at visit 3

In seven out of nine participants who participated in visit 3, blood pressure decreased after treatment reinstalment. In four of these seven participants, leakage rate *Ki* also decreased. In three participants, *Ki* was restored to baseline levels or lower, in one participant *Ki* was still higher than at visit 1 (Supplementary Figure 1). There was no association between a decrease in systolic and *Ki* in any of the ROIs; the WM (β=2.7 x 10^{-6}, p=0.343), the total GM (β=3.2 x 10^{-6}, p=0.348), the deep GM (β=4.6 x 10^{-6}, p=0.251) and the cortex (β=3.0 x 10^{-6}, p=0.369) (Table 3). Similar results are found between diastolic blood pressure and *Ki* (Supplementary Table 1). *Vp* decreased in only three of seven participants and there was no association between a decrease in systolic nor diastolic blood pressure and *Vp* in any of the ROIs.

Table 3: Association between Δ systolic blood pressure and Δ *Ki* and *Vp* between visit 1-visit 2 and visit 2-visit 3 per ROI.

	Visit 1 – visit 2 (n=14)		Visit 2 – visit 3 (n=9)	
	BBB leakage rate (Ki, unit 10^{-6} min^{-1})			
WM	6.1	**0.007**	2.7	0.343
GM	8.4	**0.027**	3.2	0.348
Deep GM	10.5	**0.010**	4.6	0.251
Cortex	8.1	**0.032**	3.0	0.369
	Blood plasma volume fraction (Vp, 10^{-4})			
WM	1.5	0.050	-12.3	0.898
GM	1.8	0.147	9.9	0.931
Deep GM	3.4	**0.009**	52.5	0.700
Cortex	1.7	0.176	4.1	0.971

Shown are betas and p-values.

Discussion

This exploratory longitudinal study examined changes in BBB leakage following subsequent increase and decrease in blood pressure in middle-aged patients with hypertension who withdrew or switched antihypertensive medication as part of routine diagnostic protocols for secondary hypertension. Increase in blood pressure after temporary withdrawal or switch of antihypertensives was associated with increased BBB leakage and perfusion as reflected by an increase in *Ki* and *Vp* in the WM and GM. This association between blood pressure and BBB leakage was less pronounced when blood pressure was lowered following medication reinstatement.

Previous studies assessing BBB leakage in patients with SVD performed cross-sectional studies or examined the effects of BBB leakage at baseline on (progression of) SVD imaging markers at a later time-point[21, 22]. We and others have demonstrated that higher blood pressure is associated with BBB leakage in young and middle-aged patients with hypertension at the cross-sectional level[9]. Now we extend this knowledge by showing that short-term increases in blood pressure are associated with higher BBB leakage, supporting the idea that the BBB can be modified in a short period of time. Assessing BBB leakage at multiple time-points can provide more information about dynamic changes in BBB integrity and potential treatment targets thereof. We noticed the strongest association between blood pressure increase and increased BBB leakage, while a decrease in BBB leakage following blood pressure lowering was only observed in four of seven participants.

Several mechanisms may explain the increased leakage of contrast agent observed with higher blood pressure. Higher systemic blood pressure may increase (intracranial) arterial pressure and drive blood plasma with small molecules, such as gadobutrol with an approximate size in the order of 1 nm, across the BBB more easily, especially if the BBB is already disrupted[23]. However, the brain is normally well protected from fluctuations in blood pressure through autoregulation of cerebral blood flow. It is generally thought that gradual increases in blood pressure, such as those occurring over a few weeks in this study, can be effectively compensated by autoregulatory mechanisms, thus preventing excessive increase in intracranial pressure[24]. Unfortunately we have not measured cerebral blood flow or intracranial arterial pressure. Another explanation might be that higher blood pressure leads to damage of the BBB structure such as loss or disruption of tight-junction molecules[25]. For example, activation of angiotensin I and II in hypertension can damage tight junctions[6]. Tight junctions are essential for BBB function and changes in tight junctions can lead to increased BBB leakage. Furthermore, the surface area of the microvessels increased due to higher blood pressure or vessel wall relaxation, parallel to the increase in microvascular blood volume. This facilitates the influx of gadolinium into the parenchyma as *Ki* is the product of intrinsic vascular permeability and surface area, leading to increased *Ki*.

We did not observe an immediate reduction in BBB leakage after blood pressure lowering in all participants. If hypertension affects the structure of the BBB, BBB repair is likely not instantaneous since it may take a longer period to restore tight junction integrity and reverse structural damage. Our imaging may have taken place too early to detect this recovery. Furthermore, hypertension can lead to more persistent alterations in the small vessels in the brain, such as vascular remodelling,

arteriosclerosis, ischemia, chronic low-grade inflammation, endothelial dysfunction, and oxidative stress[6]. Another possible explanation for the less evident association with blood pressure reduction is the longer interval between scans (55.5 days between visit 1-visit 2 vs 203 days between visit 2-visit 3), during which external factors such as infections may affect BBB integrity[26]. It is important to note that this observation is based on a small subset of participants (n=7) with a reduction in blood pressure after medication restart, and these findings should be replicated in a larger cohort.

We observed an increase in *Vp* following blood pressure increase between visit 1 and 2, especially in the GM, that persisted after blood pressure was lowered again after visit 3. Higher *Vp* in patients with hypertension was described before[27]. A possible explanation is that elevated arterial pressure increases microvascular plasma volume within a short time, particularly when blood pressure is higher than the upper limit of autoregulation[28]. The relative increase in *Vp* between visit 1 and 3 corresponds with a general working mechanism of blood pressure lowering agents, being relaxation of the (arterial) vessel wall which leads to vasodilation with an attendant increase in blood plasma per unit of tissue volume.

Although the clinical consequences of gradual BBB disruption in SVD are poorly understood since most studies lack long-term follow-up, we know (acute) BBB degradation causes severe damage to brain tissue from other diseases, such as Multiple Sclerosis (MS), stroke and infections[29, 30]. Plasma proteins, inflammatory molecules and harmful substances may enter the brain and accumulate, causing demyelination, axonal damage and eventually imaging markers like WMH[31]. This hypothesis is supported by studies reporting that higher baseline BBB leakage was associated with more microstructural damage, but also with a more rapid cognitive decline and poor functional outcome at a later time-point[21, 22, 32]. Improving BBB integrity may therefore serve as a promising target for treatment. Not many trials have examined BBB leakage as a (surrogate) outcome for SVD, with the exception of the MINERVA trial, that evaluated the effect of minocycline on BBB leakage or inflammation. The investigators found no effect although the sample size was small (n=23)[33]. The reduction in BBB leakage observed in a subset of our participants after blood pressure lowering is suggestive of the potential for BBB modification through treatment, but replication in future studies is essential.

Several limitations of this study are worth mentioning. First, our study was an explorative study with a small sample and our results should therefore be interpreted with caution. The small sample size also limited our statistical power

for more comprehensive analyses. For instance, subgroup analyses regarding differential effects of antihypertensive medication classes were not feasible given the limited number of participants. While we initially aimed to recruit 50 patients with hypertension, recruitment rate was lower than expected, likely due to the high burden of the study protocol. Second, in three of 14 participants, blood pressure did not increase after medication withdrawal. Potential explanations for this are low medication adherence or the presence of underlying causes of hypertension, such as PA, which are more resistant to conventional antihypertensives. However, excluding these participants did not alter the results. Third, this study may be subject to selection bias. The patient population consisted of individuals with hypertension with treatment-resistant high blood pressure or suspicion of a secondary cause. This population may differ from the general hypertensive population seen in primary care, where blood pressure is often well controlled with one or two antihypertensive agents. Therefore, the findings of this study may not be generalizable to the broader population with hypertension. Fourth, we used office-based blood pressure measurements to determine blood pressure on the day of the MRI scan, but this has a risk of measurement errors and "white-coat effects" [34]. We tried to minimize measurement errors by taking three readings per visit and verifying them against medical records. Similarly, while moderate-to-excellent short- to long term reproducibility has been reported for measurement of BBB leakage using DCE MRI[26, 35], measuring changes in subtle BBB leakage over a prolonged time period is challenging as both measurement variability as well as biological variability will influence the outcome.

Nonetheless, this study has a unique design that enabled us to examine the effect of blood pressure *increase* and subsequent *decrease* on BBB leakage over time, which has not been done before. Since we recruited adults without or with only very subtle MRI markers of SVD, we could examine early changes in BBB leakage in a population at risk of SVD. Furthermore, patients had several hospital visits as part of their diagnostic workup throughout their study participation, which allowed us to collect detailed clinical information without imposing additional burden on the participants.

In conclusion, we examined fluctuations in BBB integrity in patients with hypertension following blood pressure increase and decrease. While we observed a strong association between blood pressure increase and BBB leakage after medication withdrawal, this relationship was less evident for blood pressure changes following reinstalment of anti-hypertensive treatment. This suggests that BBB function is dynamic and blood pressure may directly influence BBB integrity. This

study emphasizes the importance of intensive blood pressure control in adults with hypertension, since blood pressure increase is directly associated with increased BBB leakage, a mechanism implicated in SVD and cognitive decline. Moreover, our findings suggest that the BBB is dynamic and may be therapeutically modifiable.

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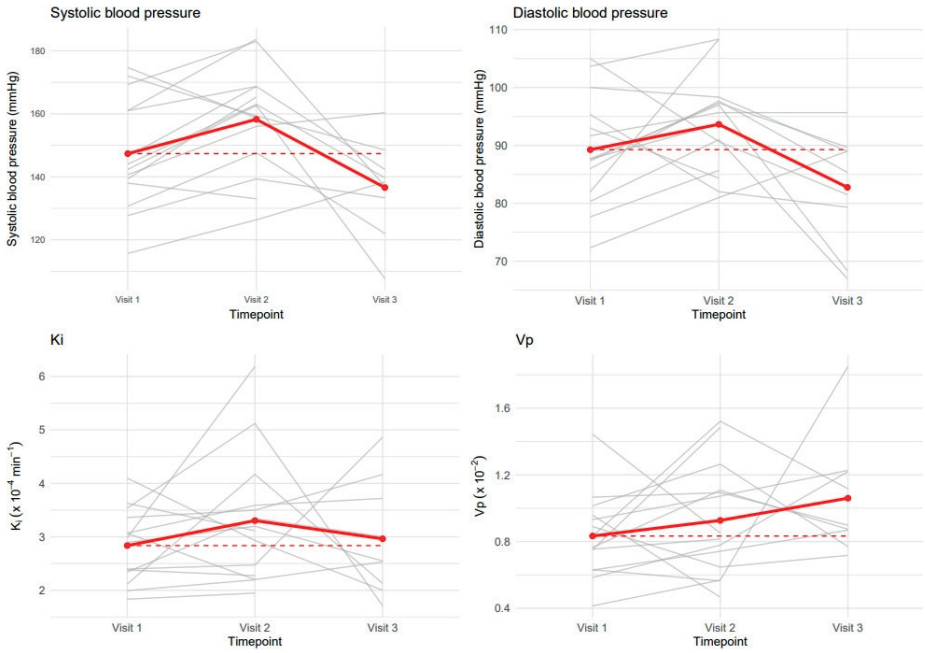
Supplemental material

Supplementary Table 1: Association between Δ diastolic blood pressure and Δ *Ki* and *Vp* between all follow-ups per region of interest.

	Visit 1 – visit 2 (n=14)		Visit 2 – visit 3 (n=9)	
	BBB leakage rate (Ki, unit 10^{-6} min^{-1})			
	β	P-value	β	P-value
WM	6.8	**0.016**	3.2	0.576
GM	9.6	**0.041**	2.7	0.694
Deep GM	11.6	**0.024**	3.9	0.625
Cortex	9.3	**0.045**	2.4	0.716
	Blood plasma volume fraction (Vp, 10^{-4})			
WM	1.7	0.079	-0.6	0.764
GM	2.1	0.157	-1.1	0.624
Deep GM	3.6	**0.031**	-0.8	0.777
Cortex	2.0	0.174	-1.1	0.599



Supplementary figure 1: **Systolic and diastolic blood pressure, *Ki* and *Vp* in the white matter over the visits.** Red line represents the mean of all participants, grey lines show individual trajectories over time

Chapter 6: Blood-brain barrier leakage and white matter microstructural integrity in young patients with hypertension

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Abstract

Background

Hypertension is a major risk factor for conventional MRI markers of cerebral small vessel disease (SVD) such as white matter hyperintensities (WMH), potentially through disruption of the blood-brain barrier (BBB). Whether BBB leakage is linked to early, microstructural white matter (WM) changes remains unknown. We therefore examined the association between BBB integrity and Peak Width of Skeletonized Mean Diffusivity (PSMD), a marker of WM damage, in young patients with hypertension and normotensive controls.

Methods

We included 59 patients with hypertension (median [IQR] age: 35.4 [29.2-41.1] years; mean blood pressure 146/92 mmHg) and 21 healthy controls (median [IQR] age: 29.2 [27.8-33.2] years; mean blood pressure 120/75 mmHg). Dynamic contrast-enhanced (DCE)-MRI with gadolinium contrast was used to measure BBB leakage (leakage rate *Ki* [min-1]). Multi-shell diffusion tensor imaging was used to calculate PSMD. Linear regression adjusted for age and sex was used to examine the association between BBB leakage in the WM and grey matter (GM) and PSMD.

Results

Patients with hypertension had higher PSMD compared to controls (β=0.269, **p=0.019**). BBB leakage rate (*Ki*) in the WM was associated with higher PSMD (β=0.221, **p=0.050**).

Conclusion

BBB leakage was associated with lower WM integrity in young patients with hypertension. This supports the hypothesis that BBB dysfunction is an early feature of SVD and highlights the importance of early prevention and intervention in hypertension.

Introduction

Hypertension affects more than 1 billion individuals worldwide[1]. The brain is one of the organs that is particularly susceptible to high blood pressure. Not only is hypertension the largest risk factor for ischemic and haemorrhagic stroke, it is also a leading risk factor for cognitive impairment and dementia. This is for a major part mediated through cerebral small vessel disease (SVD)[2-4].

SVD is an umbrella term for pathologies affecting the small arteries and capillaries of the brain, that is characterized by MRI lesions, including lacunes, white matter hyperintensities (WMH) and microbleeds[5]. These imaging markers represent an advanced stage of the disease, possibly beyond the window where these pathological processes can be reversed[4]. Unfortunately, our current understanding of SVD is largely based on studies conducted in older patients where these end-stage lesions are already present, leaving the early pathological changes and origin of these SVD markers largely unknown.

These early stages of SVD can be assessed with Diffusion tensor imaging (DTI)[6]. For example, the Peak Width of Skeletonized Mean Diffusivity (PSMD) derived from DTI is a sensitive marker for white matter damage[7]. DTI MRI studies have shown that high blood pressure is associated with microstructural damage to the white matter, even when structural SVD markers are not (yet) visible[8, 9]. It has been suggested that blood-brain barrier (BBB) dysfunction precedes these structural changes and mediates the association between hypertension and MRI markers of SVD[10], however this has mainly been assessed in individuals with end-stage SVD. When the BBB loses its integrity, harmful substances may leak into the brain, causing oedema, inflammation and eventually neuronal damage[11]. We have demonstrated previously that this barrier is indeed more permeable in young patients with hypertension compared to controls, while they also have more conventional markers of SVD[12, 13]. This supports the hypothesis that BBB disruption is one of the early changes in SVD and as such, may be a relevant target for therapeutical intervention. However, it is still unclear whether loss of BBB integrity is related to early manifestations of SVD, as indicated by alterations in white matter integrity. We therefore examined the association between BBB integrity and white matter damage, quantified by PSMD derived from DTI, in young patients with hypertension and normotensive controls.

Methods

Study population

We included patients (aged 18-55 years) with hypertension and normotensive controls between July 2021 and December 2024[14]. Patients with hypertension were recruited through the outpatient clinic of the Department of Internal Medicine of the Radboud University Medical Centre (Radboudumc) and Rijnstate hospital. Patients were referred to these centres with treatment-resistant hypertension or suspicion of secondary causes of hypertension. Inclusion criteria were: age 18-55 years, diagnosis of hypertension (according to the European Society of Hypertension (ESH) guideline[15]) and office-based blood pressure measurement (OBPM) ≥140 mmHg systolic and/or ≥90 mmHg diastolic measured within three months prior to study inclusion. Normotensive controls were recruited through the social circles of participants and the research team. We included healthy individuals aged 18-40 years, without a history of hypertension or use of antihypertensive medication for other purposes. Exclusion criteria for both groups comprised: pre-existing symptomatic SVD (defined as ischemic/haemorrhagic stroke or transient ischemic attack (TIA), based on self-report and medical documentation), other causes of stroke according to the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification (large artery disease, defined as stenosis >50% in the internal carotid artery or vertebral artery based on ultrasound, and cardio-embolism. Other exclusion criteria were contraindications for 3T contrast-enhanced MRI (i.e. renal insufficiency <30 ml/min or pregnancy) and other major (neurological) diseases.

The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study and all participants gave written informed consent.

Study procedures

Participants completed an online questionnaire about their medical history, medication use, professional life, education and lifestyle habits such as smoking. Educational level was assessed using the revised Verhage classification system, which is commonly used to classify the Dutch educational system[16]. Antihypertensive medication use and type of medication were verified using medical records. Height and weight were measured using standard procedures.

Blood pressure measurements

Blood pressure was measured using an automated blood pressure cuff (Dinamap Carescape V100, GE Healthcare, Finland). Three consecutive measurements were taken while the participants were in a sitting position, with both feet flat on the

ground after five minutes of rest. The average of these measurements was used in this analysis. The researcher was present during the measurements.

MRI protocol

MRI scans were performed using a 3Tesla system (MAGNETOM PrismaFit, Siemens Healthcare, Erlangen, Germany) equipped with a 20-channel head-neck coil. Full MRI protocol and scanning parameters were described previously[14]. In short, the MRI protocol included 3D T1-weighted MP2RAGE (Magnetization Prepared 2 Rapid Acquisition Gradient Echoes), fluid-attenuated inversion recovery (FLAIR), susceptibility-weighted imaging (SWI) and multi-shell DTI. To assess blood–brain barrier (BBB) integrity, a DCE-MRI protocol was employed, comprising consecutive slow–fast–slow T1-weighted saturation recovery spoiled Gradient Echo (GRE) sequences. During the fast acquisition phase, 0.1 mmol/kg of gadobutrol (Gadovist, Bayer, Germany) was administered intravenously at 3 ml/s, followed by a saline flush.

Structural MRI preprocessing

We calculated quantitative T1 maps from the MP2RAGE sequence with a previously described protocol[17]. Brain tissue was segmented from the denoised UNI MP2RAGE images into total grey matter (GM), consisting of cortical and deep GM, and WM using the cross-sectional Sequence Adaptive Multimodal SEGmentation (SAMSEG) pipeline (Freesurfer, version 7.2)[18]. Furthermore, we segmented WMH on T1 and FLAIR images using a deep-learning based segmentation tool (https://github.com/miac-research/MARS-WMH)[19, 20]. All WMH masks were visually checked and corrected manually when necessary.

DTI preprocessing

Preprocessing steps included visual quality control, Gibbs artefact removal, eddy-current induced distortion (top-up) and head-motion correction and was done using Functional Magnetic Resonance Imaging of the Brain Software Library (FSL) software[21] and MRtrix 3.0 software[22]. Peak width of skeletonized mean diffusivity (PSMD) was calculated using a fully automated pipeline (available at: http://www.psmd-marker.com/)[23].

Quantification of blood-brain barrier leakage with DCE-MRI

Quantification of BBB leakage has been described earlier[12]. In short, all images were first coregistered to the averaged pre-contrast slow image to correct for head movement during the DCE scan using FLIRT (FSL 6.0). The vascular input function was derived from the superior sagittal sinus[24].We applied the graphical Patlak pharmacokinetic modelling approach to calculate the BBB leakage rate

(transfer constant Ki [min -1]). *Ki* was averaged across all voxels within each ROI (total, cortical GM, deep GM, and total WM). The post-contrast DCE-MRI scan of one patient with hypertension could not be analysed due to faulty acquisition and was therefore excluded from the analysis.

Statistical analysis

We first assessed differences in baseline characteristics between patients with hypertension and normotensive controls using independent t-tests/Mann-Whitney U tests for continuous variables and using Pearson $\chi 2$/Fisher exact tests for categorical variables. We used linear regression adjusted for age and sex to assess differences in PSMD between groups. Across all subjects, we conducted multivariable linear regression analyses to assess the association between BBB leakage and PSMD. Separate models were specified for *Ki* in WM and GM, adjusting for age and sex. All analyses were repeated with additional adjustment for WMH volume, since patients with hypertension had more WMH and this can affect both PSMD and BBB leakage.

We calculated Cook distance to identify potential outliers (defined as datapoints with a Cook distance > $4/n$). Five participants were considered outliers using this cut-off and we repeated the analyses above after excluding these participants. A level of significance of 0.05 was used in all tests, and all statistics were performed using RStudio.

Results

We included 59 patients with hypertension and 21 normotensive controls in this analysis. Patients with hypertension were older than controls (median [IQR] age: 35.4 [29.2-41.1] vs 29.2 [27.8-33.2] years) with no difference in sex distribution between the groups (55% vs 52% female). Both systolic and diastolic blood pressure was higher in patients with hypertension and 86% of patients was taking antihypertensive medication during the study visit (see Table 1). BMI was also significantly higher in patients with hypertension compared to controls (mean (SD): 28.6 (5.9) vs 22.7 (2.8)).

Table 1: Baseline characteristics of patients with hypertension and normotensive controls.

	Hypertension (n = 59)	Control (n = 21)	p-value
Age (years, median [IQR])	35.4 [29.2-41.1]	29.2 [27.8-33.2]	**<0.001**
Female, n (%)	32 (54)	11 (52)	1.000
Systolic blood pressure (mmHg, mean (SD))	146 (17)	120 (11)	**<0.001**
Diastolic blood pressure (mmHg, mean (SD))	92 (12)	75 (8)	**<0.001**
Duration of hypertension (years, median [IQR])	4.2 [1.5 – 11.5]	NA	NA
Blood pressure medication			
Antihypertensive medication use, n (%)	51 (86)	0 (0)	**<0.001**
Calcium antagonists, n (%)	39 (66)	0 (0)	NA
ACE inhibitors or ARBs, n (%)	28 (48)	0 (0)	NA
Diuretics, n (%)	16 (27)	0 (0)	NA
α-blocker, n (%)	6 (10)	0 (0)	NA
β-Blockers, n (%)	6 (10)	0 (0)	NA
Cardiovascular risk factors			
Body mass index (kg/m2, mean (SD))	28.6 (6.0)	22.7 (2.8)	**<0.001**
Current smoking, n (%)	5 (9)	2 (10)	1.000
Cholesterol lowering medication, n (%)	6 (10)	0 (0)	0.331
SVD MRI markers			
WMH volume (mm^3, median [IQR])	44.2 [9.1 – 120.4]	12.4 [1.9 – 35.8]	**0.018**
WMH volume (%ICV x 10^{-3}, median [IQR])	2.7 [0.6 – 7.2]	0.8 [0.1 – 2.2]	**0.020**
Fazekas score, 0/1/2/3, n (%)	49 (83) / 9 (15) / 1 (2) / 0 (0)	21 (100) / 0 (0) /0 (0) / 0 (0)	**0.046**
Presence of lacunes, n (%)	1 (2)	0 (100)	1.000
Presence of cerebral microbleeds, n, (%)	3 (5)	0 (100)	0.701

Association between BBB leakage and PSMD

PSMD was significantly higher in patients with hypertension compared to controls after adjusting for age and sex (β=0.269, **p=0.019**). Stronger BBB leakage (*Ki*) in the WM was associated with higher PSMD after correcting for age and sex (β=0.221, p=0.050), but not in the GM (β=0.176, p=0.117) (Figure 1). Additional adjustment for WMH volume did not alter our results. BBB leakage rate in the WM was significantly associated with PSMD (β=0.234, **p=0.035**) but not in the GM (β=0.178, p=0.107). Five participants were identified as outliers in this model and we repeated these analysis after excluding them. The association between BBB leakage and PSMD was no longer significant in the WM (β=0.182, p=0.124) nor in the GM (β=0.105, p=0.366) and also not with additional adjustment for WMH (WM: β=0.179, p=0.135, GM: β=0.103, p=0.379).

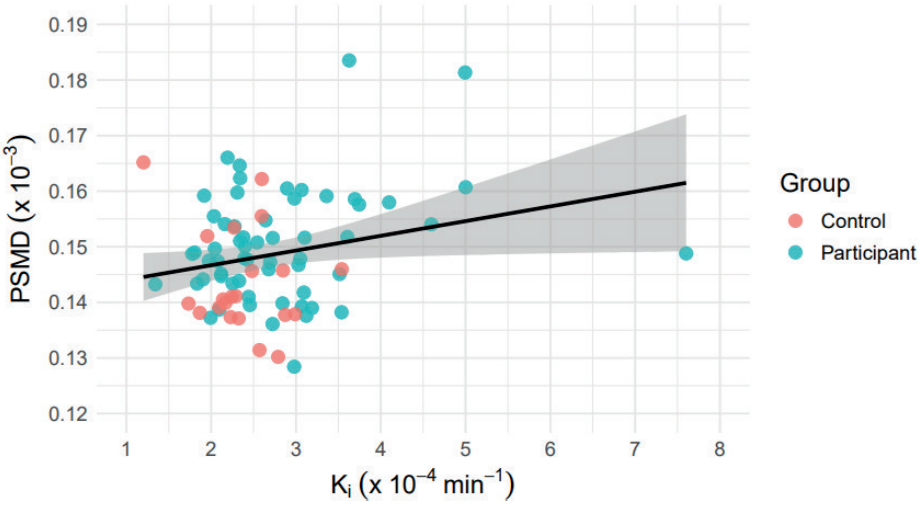

Figure 1: association between *Ki* (BBB leakage rate) in the white matter and PSMD.

Discussion

In this study, we examined the relation between BBB leakage and microstructural integrity by combining DCE-MRI with DTI, in young patients with hypertension. Higher BBB leakage was associated with higher PSMD, supporting the idea that BBB leakage contributes to hypertension-induced microstructural WM damage.

Higher BBB leakage in the WM and WMH has been demonstrated before in patients with hypertension and SVD, but the relation with microstructural integrity has rarely been examined[12, 25, 26]. One study has demonstrated an association between BBB leakage and PSMD in a mixed population with Alzheimer's Disease and SVD[10]. We extend these findings by demonstrating that BBB leakage is associated with WM integrity in a much younger population of patients with hypertension, who are at risk of future SVD and/or other cerebrovascular disorders. When the BBB loses its integrity, plasma components can leak from blood vessels, causing oedema and neuroinflammation and eventually damage to the WM[27]. This is supported by studies demonstrating that BBB permeability is linked to increased microstructural damage, but also faster cognitive decline and poorer functional outcomes at follow-up[28-30].

We have previously shown higher BBB leakage in these young patients compared with controls[12] and now show that this is associated with PSMD. Traditionally, DTI alterations are interpreted as microstructural tissue damage, such as loss of

fibre organization and axonal degeneration[31]. However, increased extracellular free water appears to be the main driver of DTI alterations[32]. This suggests that increased extracellular fluid caused by loss of BBB integrity may underlie the observed increase in PSMD in this study and to a smaller degree degeneration of white matter fibres.

Our results emphasize the importance of high blood pressure as a risk factor of early markers of SVD. Proper blood pressure control at relatively young age may prevent or postpone the first initiating steps in the etiology of SVD. Unfortunately, high blood pressure often has little symptoms young adults are thus unlikely to visit their GP[33]. Since the reversibility of WM damage is highly debatable, especially in more advanced stages, prevention through screening and appropriate blood pressure control, particularly in these young individuals, is key. Moreover, improving the BBB's integrity may be a promising approach to diminish or slow down SVD progression and cognitive decline. The MINERVA trial aimed to do so by reducing BBB leakage by an 8-week minocycline treatment but did not observe any effect on BBB leakage [34] However, the number of trials targeting this is small, as is the number of patients included.

The strengths of our study lie in the combination of state-of-the-art MRI techniques to assess microstructure and vascular function simultaneously, in a unique population at risk of SVD. This approach enables us to quantify the extent of cerebral damage and gain insight into the early pathological changes that occur before conventional SVD markers become apparent. However, some methodological limitations should be considered. First, the sample size of this study is relatively small, which reduces statistical power for whole-brain analysis. After excluding five potential outliers, our results were no longer statistically significant even though the direction of the effect remained unchanged. This suggests that a true underlying effect may exist, but our study may lack sufficient power once influential data points are excluded. Second, we cannot rule out selection bias. Our patient cohort consisted of patients with hypertension referred to the hospital for treatment-resistant hypertension or suspicion of secondary hypertension. Such patients are likely different from those commonly treated in primary care, where hypertension is often controlled with one or two antihypertensive drugs and modification of lifestyle factors is more important. This also limits generalizability to a broader hypertensive population. Finally, due to the cross-sectional design of this analysis, we cannot determine whether BBB leakage indeed precedes WM damage. Long-term follow-up with repeated MRI scans would help clarify the direction of the observed association. Moreover, examining BBB leakage in specific WM regions

that are more vulnerable to the effects of hypertension such as the corpus callosum and corona radiata could help disentangling whether BBB disruption precedes, coincides or results from microstructural damage[9].

In conclusion, our results show that BBB leakage is associated with loss of WM integrity in young patients with hypertension. This supports the hypothesis that BBB dysfunction may be an early feature of SVD and highlights the importance of early prevention and intervention in hypertension.

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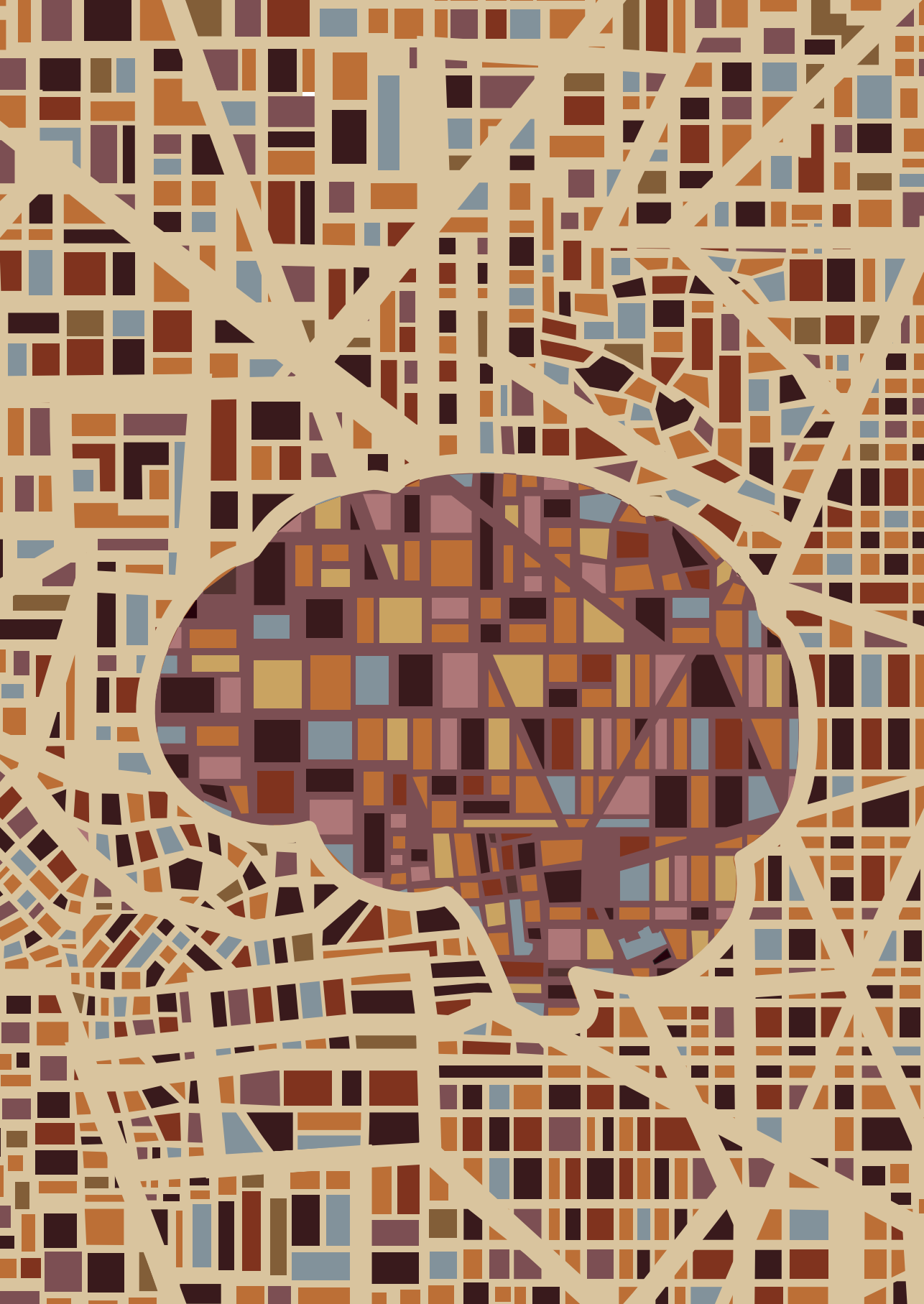
Part III – Blood pressure variability



Chapter 7: Visit-to-visit blood pressure variability and progression of white matter hyperintensities over 14 years

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Blood Pressure, 33(1), 2314498.

Abstract

Introduction

There is evidence that blood pressure variability (BPV) is associated with cerebral small vessel disease (SVD) and may therefore increase the risk of stroke and dementia. It remains unclear if BPV is associated with SVD progression over years. We examined whether visit-to-visit BPV is associated with white matter hyperintensity (WMH) progression over 14 years and MRI markers after 14 years.

Methods

We included participants with SVD from the Radboud University Nijmegen Diffusion tensor Magnetic resonance imaging Cohort (RUNDMC) who underwent baseline assessment in 2006 and follow-up in 2011, 2015 and 2020. BPV was calculated as coefficient of variation of BP at all visits. Association between WMH progression rates over 14 years and BPV was examined using linear-mixed effects model. Regression models were used to examine association between BPV and MRI markers at final visit in participants.

Results

A total of 199 participants (60.5 SD 6.6 years) who underwent four MRI scans and blood pressure measurements were included, with mean follow-up of 13.7 (SD 0.5) years. Systolic BPV was associated with higher progression of WMH ($\beta = 0.013$, 95% CI 0.005 – 0.022) and higher risk of incident lacunes (OR: 1.10, 95% CI 1.01-1.21). There was no association between systolic BPV and grey and white matter volumes, Peak Skeleton of Mean Diffusivity (PSMD) or microbleed count after 13.7 years.

Conclusion

Visit-to-visit systolic BPV is associated with increased progression of WMH volumes and higher risk of incident lacunes over 14 years in participants with SVD. Future studies are needed to examine causality of this association.

Introduction

Damage to the small arteries and arterioles of the brain is commonly referred to as cerebral small vessel disease (SVD).[1] SVD is the most important vascular contributor to dementia, causes the majority of intracerebral haemorrhage and about a quarter of all ischemic strokes, but its pathophysiology remains poorly understood[1, 2]. As pathology of these arterioles cannot be visualized in vivo with conventional 3T Magnetic Resonance Imaging (MRI), MRI markers of SVD including white matter hyperintensities (WMH) of presumed vascular origin, lacunes and microbleeds, as well as reductions in white matter microstructural integrity, are considered to reflect the underlying pathology[3, 4].

Although hypertension is the most established risk factor for SVD, studies examining the effects of lowering blood pressure (BP) showed mixed results[5]. The SPRINT-MIND trial has demonstrated that intensive BP reduction can reduce WMH progression, but this effect seems modest[6]. Moreover, trials of BP lowering drugs do not show a consistent reduction in risk of incident dementia or stroke[7, 8]. Emerging evidence suggests that other BP measures, such as blood pressure variability (BPV) over a period of hours, days or years hold additional prognostic value[9]. Higher BPV independent of mean BP was found to be associated with increased mortality,[10] higher dementia risk[11] and increased risk of stroke and other cardiovascular events[12].

In patients with SVD, higher systolic short- and long-term BPV is associated with higher burden of WMH and progression of WMH[13, 14]. Most of the previous studies only assess SVD burden at one time-point. However, it is crucial to determine if BPV is merely a consequence of SVD or a causal factor by studying incident SVD or progression of SVD. To date, studies that assessed progression of SVD markers only measure progression over a short time interval, which is unlikely to capture the long-term temporal dynamics of SVD progression[15, 16]. If BPV is associated with progression of MRI markers of SVD over a longer period of time beyond mean BP levels, a next step would be to investigate if reducing BPV results in prevention or mitigation of SVD in patients at risk. This study aims to examine the effects of visit-to-visit BPV on progression of WMH volumes over 14 years and SVD burden after 14 years.

Materials and methods

Study population

This study is part of the Radboud University Nijmegen Diffusion tensor Magnetic resonance imaging Cohort (RUN DMC) study, a single-centre prospective cohort study of 503 individuals with SVD, described in detail previously[17]. In short, this study aims to examine the risk factors, progression and clinical consequences of SVD[17]. Patients aged 50-85 years whom were referred to Radboud University Medical Centre Neurology outpatient clinic between October 2002 and November 2006 were screened for inclusion. SVD diagnosis was based on MRI and included presence of WMH and/or lacunes. Exclusion criteria were presence of dementia and/or parkinsonism, other (psychiatric) diseases interfering with testing or follow-up, SVD mimics (i.e. multiple sclerosis), a life expectancy <6 months or contraindications for MRI. The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study and all participants gave written informed consent.

Of the 503 participants at visit 1, 361 had an MRI at visit 2 (mean interval of 5.4 years), 296 at visit 3 (mean interval of 3.4 years) and 231 at visit 4 (mean interval of 5.0 years). Patients had more BP measurements available than MRI scans, due to MRI contraindications in the elderly population (Figure 1). Because missing BP measurements would lead to large intervals between BP measurements, participants were only included in this analysis if BP measurements and MRI data were available at all four visits (n=199). A flowchart of the study population over time is shown in Figure 1.

Blood pressure assessments and calculation of BP variability

At every study visit, BP was recorded as the mean of three measurements in supine position after five minutes of rest, using an automated oscillometric device (CARESCAPE V100, GE Dinamap, USA). BP was measured at four visits over 13.7 years in all participants included in this analysis. BPV was defined as the Coefficient of Variation (CV) of the blood pressure over all visits, calculated as the standard deviation divided by the mean BP, multiplied by 100%. This measure was used because it accounts for average BP levels. Both systolic and diastolic BPV were calculated separately. Information about current antihypertensive medication use (drug class) was obtained at each visit. Details about dosage and duration of treatment were not routinely collected.

MRI protocol

Images were acquired on 1.5T MRI at first three visits (in 2006: Siemens, Magnetom Sonata, in 2011 and 2015: Siemens, Magnetom Avanto) and 3T at visit 4 (Siemens, Magnetom Prisma). In short, brain scans included the following sequences: 3D T1 Magnetization Prepared Rapid Gradient Echo (MPRAGE), 3D fluid-attenuated inversion recovery (FLAIR) and a transversal T2*-weighted gradient echo. At visit 4, a Susceptibility-Weighted Image (SWI) was derived from magnitude and phase data from the multi-echo gradient sequence. The acquisition details have been published previously[18]. During visit 4 in 2020, the protocol included diffusion weighted imaging (DWI) consisted of diffusion-encoding directions: 10 x b=0 s/mm^2, 30 x b=1000 s/mm^2 and 60 x b=3000 s/mm^2.

MRI processing and brain volumetry

At visit 1, 2 and 3, WMH volumes were calculated using a semi-automated detection method using FLAIR and T1 sequences that was described previously[19]. At visit 4, MP2RAGE data were processed to obtain T1-weighted images using code freely available online (https://github.com/JosePMarques/MP2RAGE-related-scripts). Registered and bias-corrected T1 and FLAIR images were used to segment WMH using a 3D U-net deep learning algorithm[20]. All WMH segmentations were checked for and cleaned from artifacts using a custom 3D editing tool in Matlab (R2018a, The Mathworks, Natick, MA). The derived WMH volumes were highly comparable between the two segmentation algorithms (Intraclass Correlation Coefficient = 0.98) [18]. Moreover, follow-up FLAIR images were aligned to baseline FLAIR images using FMRIB's Linear Image Registration Tool (FLIRT) so all FLAIR scans have the same voxel size and slice thickness. This was done to minimize the effects of changes in FLAIR acquisition parameters during follow-ups. WMH volumes during follow-ups were corrected to baseline Intracranial Volume (ICV) to correct for interscan differences and normalized to baseline ICV.

Brain volumes, including grey matter volume (GM), white matter volume (WM) and cerebrospinal fluid volume (CSF) were calculated using SPM12 on the T1 MPRAGE images that were corrected for WMH. ICV was calculated by summing up GM, WM and CSF volumes. We corrected for differences in ICV between baseline and follow-up to account for MRI scanner changes.

MRI markers of SVD

SVD markers (i.e. WMH volume, lacune count and microbleed count) were rated using Standards for ReportIng Vascular changes on nEuroimaging (STRIVE) criteria[3]. Two trained raters manually rated lacunes on T1 and FLAIR scans and microbleeds

on SWI scans, followed by a consensus meeting. To improve identification of incident lacunes during follow-ups, difference images for T1 and FLAIR sequences were used[21]. First, the Brain Extraction Tool (BET) in FSL was used to create skull stripped images and image intensity was normalized to the 95% percentile. After this, follow-up images were registered to the baseline MRI scans. These baseline images were then subtracted from the registered and intensity-normalized follow-up images to create difference images. Incident lacunes are visible as hypointense voxel clusters on a uniform background, making it easier to identify them. Lacune count during follow-ups was calculated as the sum of the baseline lacune count and the number of incident lacunes for each subject.

Diffusion Tensor Imaging (DTI) analysis

To examine microstructural integrity, we used the DTI scans at visit 4 to calculate the Peak width of Skeletonized Mean Diffusivity (PSMD). This is a robust imaging marker to capture global SVD-related microstructural damage in main white matter tracts[22]. This was done using a fully automated pipeline (available at https://github.com/miac-research/psmd)[22].

Statistical analysis

Differences in baseline characteristics between participants included and not included in this analysis were examined using Mann-Whitney test or chi-square test where appropriate. We used multivariable linear regression to assess association between BPV and continuous outcomes at final visit (GM/WM volume, PSMD, all log-transformed due to non-normal distribution). Models were adjusted for baseline age, sex, mean BP (either systolic in models assessing association with systolic BPV or diastolic in models examining association with diastolic BPV) and whether participants were using antihypertensive medication at baseline. We assessed these markers only cross-sectional and not longitudinal, since changes in scanner system likely had a profound effect on volumetric measures and DTI measures (GM/WM volumes and PSMD). Separate models were used to examine effects of systolic and diastolic BPV. To examine if BPV is associated with microbleed count at visit 4, we used negative binomial regression. This can be used to model overdispersed count outcomes with excess zeroes, such as microbleed count. Model was adjusted for baseline age, sex, mean BP (either systolic in models assessing association with systolic BPV or diastolic in models examining association with diastolic BPV) and use of antihypertensive medication. Negative binomial regression was also used to examine the relation between BPV and incident lacune count, adjusting for age, gender, mean BP (systolic or diastolic, depending on model), use of antihypertensive medication and baseline lacune count.

Furthermore, linear-mixed effects (LME) models were used to examine WMH progression. Since LME models allow modelling of subject-specific response trajectories over time, this can be used to examine changes in continuous outcomes in the entire population while accounting for individual differences in trajectories. To calculate individual progression rates per participant, we used a base model with follow-up time and baseline age as fixed effects and follow-up time as the random effect for each participant (model A). We then extracted the random slopes of follow-up time for each participant to examine the individual WMH progression rates.

Separate models were used to assess the effects of systolic and diastolic BPV. In the LME models used to examine the effect of BPV on WMH progression, we included the following fixed effects: systolic or diastolic BPV, baseline age, sex, baseline WMH volume, mean BP (systolic or diastolic, depending on model), use of antihypertensive medication at baseline, smoking status, BMI, hypercholesterolemia, MRI scanner system and an interaction term between time and BPV (model B). We examined the effects of BPV using both continuous and quartile-based categorical scales in separate models. We evaluated change in Akaike information criterion (AIC) by one-way ANOVA to compare model fit with and without random intercepts and slopes per subject. Moreover, we compared model fit between the full model and the full model with a quadratic term to evaluate non-linear progression of WMH volume. As a sensitivity analysis, we repeated this analysis with a larger sample including the participants who only participated in the first three follow-ups (n=271).

One of the assumptions of LME models is (approximately) normal distribution of residuals. In models where this assumption was not met, non-parametric bootstrap method with 1000 samples was performed to calculate 95% confidence intervals (CIs) and to infer p-values. Statistical analyses were performed with R version 4.1.0.

Results

Baseline characteristics

Of the 503 participants in the RUNDMC study, 199 were included in this study (Figure 1). Mean follow-up time until visit 2 was 5.3 (SD 0.2) years, 8.7 (SD 0.2) years until visit 3 and 13.7 (SD 0.5) years until visit 4. Baseline characteristics of the included and excluded participants are shown in Table 1. Participants included in the present study were younger at baseline than nonparticipants (60.5 SD 6.6 vs 69.0 SD 8.5) and had less vascular risk factors (i.e. diabetes, hypercholesterolemia,

hypertension). Moreover, included participants had lower WMH volumes and less lacunes at baseline. The mean systolic and diastolic BP levels at baseline were 133.9 (SD 17.5) mmHg and 78.1 (SD 9.1) mmHg. In the included participants, mean systolic BPV was 9.9 (SD 4.9) and mean diastolic BPV was 9.6 (SD 3.9).

Table 1. Baseline characteristics of participants included and not included in analysis.

	Participants (n=199)	**Nonparticipants (n=304)**	**P value**
Age, years (mean (SD))	60.5 (6.6)	69.0 (8.5)	**<0.01**
Gender (male, %)	115 (57.8)	169 (55.6)	0.70
Vascular risk factors			
Diabetes, n (%)	12 (6.0)	53 (17.4)	**<0.01**
Hypercholesterolemia, n (%)	80 (40.2)	157 (51.6)	**0.02**
Hypertension, n (%)	126 (63.3)	242 (79.6)	**<0.01**
Use of antihypertensive medication	87 (43.7)	184 (60.5)	**<0.01**
Ever smoking, n (%)	142 (71.4)	212 (69.7)	0.90
Atrial fibrillation, n (%)	24 (12.1)	44 (15.4)	0.37
BMI, kg/m^2 (mean (SD))	26.8 (3.9)	27.4 (4.3)	0.13
SVD imaging characteristics			
WMH volume (ml; IQR)	1.7 [0.76 – 4.43]	6.2 [2.2 – 14.8]	**<0.01**
Presence of microbleeds, n (%)	26 (13.2)	57 (18.9)	0.12
Presence of lacunes, n (%)	36 (18.1)	98 (32.3)	**<0.01**
WMV (mean (SD))	475.0 (58.9)	441.5 (64.4)	**<0.01**
GMV (mean (SD))	634.6 (57.9)	585.2 (62.4)	**<0.01**
BP metrics			
Systolic BP, mmHg (mean (SD))	133.9 (17.5)	145.2 (21.3)	**<0.01**
Diastolic BP, mmHg (mean(SD))	78.1 (9.1)	78.2 (9.7)	0.86
Systolic BPV, CV (mean(SD))	9.9 (4.9)	N.A.	N.A.
Diastolic BPV, CV (mean(SD))	9.6 (3.9)	N.A.	N.A.

Data represent means (SD) and numbers (%). BMI: Body Mass Index, BP: Blood Pressure, BPV: Blood Pressure Variability, CV: Coefficient of Variation, GMV: Grey Matter Volume, WMV: White Matter Volume, WMH: White Matter Hyperintensity.

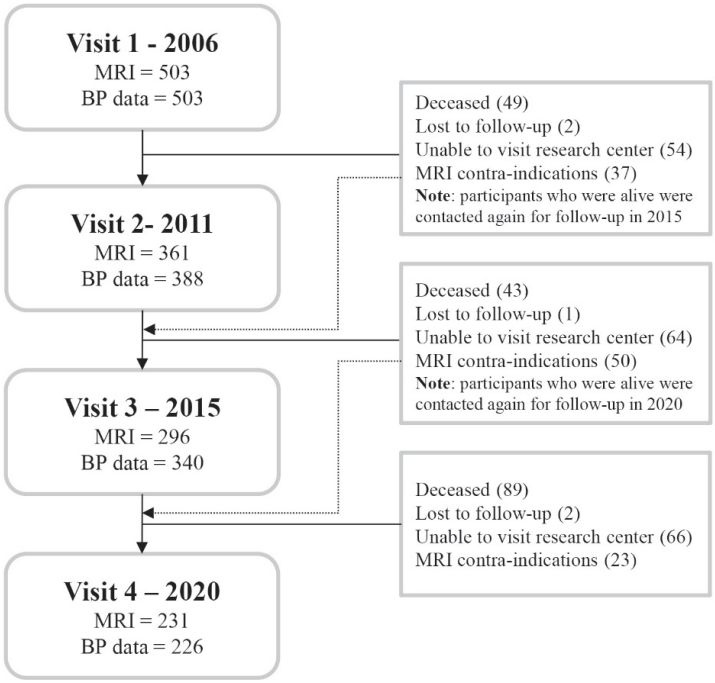

Figure 1: Flowchart of included participants at all study visits. Participants who were unable to visit research centre and were alive between 2011-2015 and 2015-2020 were contacted again for next follow-up (as shown by dotted line).

Association between BPV and WMH

Median WMH volume of all participants was 1.7 ml (Interquartile Range(IQR) 0.8 – 4.4) at baseline and 4.5 ml (IQR 1.7 – 11.3) at visit 4. Systolic BPV over all visits is associated with higher WMH volumes at baseline (standardized β = 0.18, p = 0.01), especially in the participants in the highest systolic BPV quartile (standardized β = 0.27, **p = 0.001**). When dividing the participants in quartiles based on systolic BPV, median WMH volume at visit 4 was 2.5, 4.5, 4.6, 7.2 ml for systolic BPV Q1, Q2, Q3 and Q4. BPV was only associated with WMH volume at 13.7 years in the participants in the highest systolic BPV quartile (standardized β = 0.21, **p = 0.01**) but not in the whole study population (standardized β = 0.12, p = 0.07) when adjusting for sex, age, mean systolic BP and use of antihypertensive medication. After additional adjustment for baseline WMH volume, this association was no longer significant in Q4 (standardized β = 0.12, p = 0.07).

The median annualized progression rate per quartile, estimated using LME, was 0.11, 0.21, 0.18, 0.29 mL/year for systolic BPV Q1, Q2, Q3 and Q4 (Model A; Figure 2). Both random intercept and random slope led to the best model fit for the mixed-

effects model, while including a quadratic term did not significantly improve model fit. Therefore, the model without quadratic terms was used as the final model. The mixed-effects model of WMH change over time showed an overall interaction between follow-up duration and systolic BPV on WMH volume (β = 0.013, CI: 0.006 – 0.022), meaning that one point increase in CV of systolic BP was significantly associated with 0.013 ml/y [95% CI 0.006 – 0.022] WMH progression (Model B). The rate of WMH change was significantly higher in participants with high systolic BPV (Q4) compared to participants with low systolic BPV (Q1) (β = 0.239, CI: 0.127 – 0.398) (Figure 2/Table 2), meaning that in participants with high BPV, WMH volume increases with 0.239 ml/y more than in participants with low BPV. Diastolic BPV was not associated with WMH progression (Table S1/Figure S1). Results were similar in the sensitivity analysis including all participants who joined for at least three follow-up visits (Table S3).

Table 2. Association between systolic BPV and progression of WMH and incident lacunes.

	Systolic BPV by quartile							**Continuous systolic BPV**	
	Q1	Q2		Q3		Q4			
		Estimate (95% CI)		*Estimate (95% CI)*		*Estimate (95% CI)*		*Estimate (95% CI)*	
WMH (ml/year)*	Ref	0.021 (-0.067 – 0.160)		0.026 (-0.071 – 0.142)		**0.239 (0.127 – 0.398)**		**0.013 (0.006 – 0.022)**	
		IRR (95% CI)	*P value*	*IRR (95% CI)*	*P value*	*IRR (95% CI)*	*P value*	*IRR (95% CI)*	*P value*
Incident lacune at any follow-up	1.00	2.23 (0.43-16.69)	0.368	5.76 (1.40-39.32)	**0.034**	5.29 (1.26-36.59)	**0.046**	1.09 (1.00-1.19)	**0.040**

Data represent β estimates (95% CI), IRR values (95% CI) or p-values. Model was adjusted for age, baseline WMH volume, use of antihypertensive medication at baseline, mean systolic BP, smoking status, BMI, hypercholesterolemia and MRI scanner system. *Bootstrapped 95% CIs. BPV: Blood Pressure Variability, IRR: Incidence Rate Ratio, WMH: White Matter Hyperintensity.

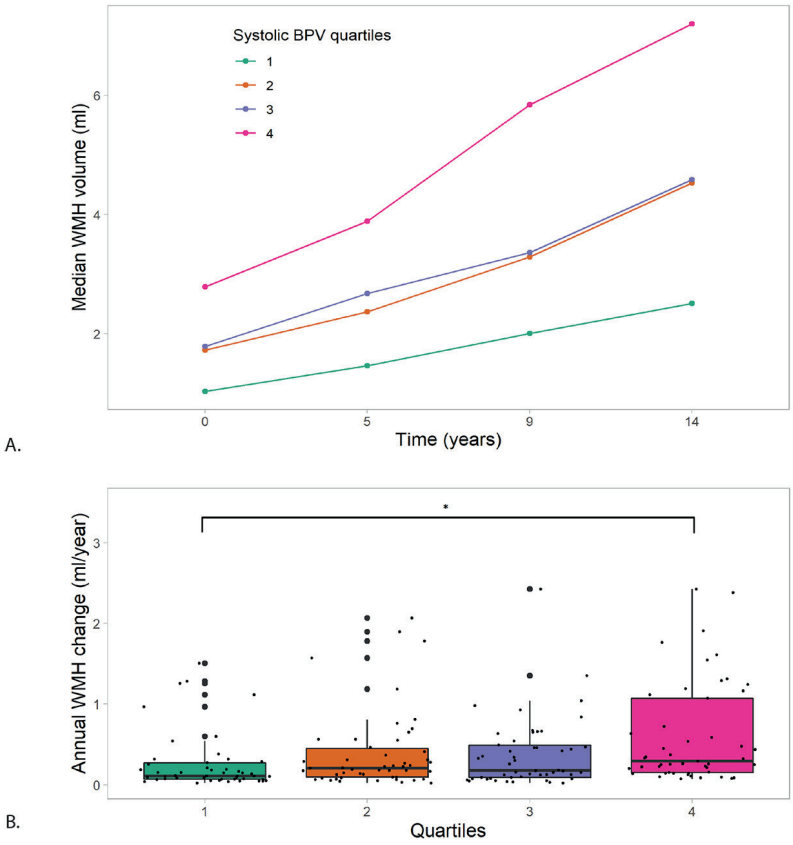

Figure 2. WMH volume changes over 14 years per systolic BPV quartile. A) Median WMH volume (ml) per systolic BPV quartile at each visit . B) Rate of WMH change (ml/year) per systolic BPV quartile, estimated using mixed-effects modelling (Model A). The boxes map to the median, 25th and 75th quartiles. * $p < 0.05$. BPV = Blood Pressure Variability, WMH = White Matter Hyperintensity.

BPV and risk of incident lacunes

Incident lacunes were observed in 30 of 199 participants (15.5%). Higher systolic BPV was associated with higher risk of incident lacunes after adjustment for confounders (Incidence Rate Ratio (IRR): 1.09, CI 1.00-1.19, **p = 0.040**). Risk of incident lacune at any timepoint was significantly higher in systolic BPV Q3 (IRR: 5.76, CI 1.40 – 39.32, **p = 0.034**) and Q4 (IRR: 5.29, CI 1.26 – 36.59, **p = 0.046**) compared with Q1 (Table 2). Diastolic BPV was not associated with risk of incident lacunes (IRR: 1.07, CI: 0.97 – 1.18, p = 0.15) (Table S1).

BPV and MRI markers after 14 years

There was no association between systolic BPV and GM volumes, WM volumes or PSMD at visit 4 (Table 3). Moreover, systolic BPV was not associated with increased

Table 3. Association between systolic BPV and MRI markers of SVD at visit 4.

	Systolic BPV by quartile							Continuous systolic BPV	
	Q1	Q2		Q3		Q4			
		Standardized β (95% CI)	*P value*	*Standardized β (95% CI)*	*P value*	*Standardized β (95% CI)*	*P value*	*Standardized β (95% CI)*	*P value*
GMV*	Ref	-0.02 (-0.43 – 0.30)	0.90	0.01 (-0.32 – 0.34)	0.96	-0.12 (-0.46 – 0.22)	0.50	-0.04 (-0.16-0.07)	0.47
WMV*	Ref	0.28 (-0.11-0.67)	0.16	0.31 (-0.08 – 0.7)	0.12	0.25 (-0.15 – 0.66)	0.22	0.03 (-0.12-0.17)	0.70
PSMD*	Ref	0.09 (-0.25 – 0.44)	0.60	0.16 (-0.19 – 0.51)	0.38	0.34 (-0.02 – 0.70)	0.07	0.08 (-0.05 – 0.20)	0.24
		IRR (95% CI)	*P value*	*IRR (95% CI)*	*P value*	*IRR (95% CI)*	*P value*	*IRR (95% CI)*	*P value*
Microbleed count	1.00	0.74 (0.32-1.68)	0.47	0.83 (0.37-1.86)	0.66	2.01 (0.87-4.65)	0.09	1.03 (0.97 – 1.10)	0.33

Data represent β estimates (95% CI), IRR values (95% CI) or p-values. *Log transformed. GMV: Grey Matter Volume, IRR: Incidence Rate Ratio, PSMD: Peak Skeleton of Mean Diffusivity, WMV: White Matter Volume, WMH: White Matter Hyperintensity.

risk of microbleeds at visit 4 (Table 3). Diastolic BPV was associated with lower WM volumes in participants in Q2, but no other associations with GM and WM volumes, PSMD and microbleed count were observed (Table S2).

Discussion

We found that higher systolic visit-to-visit BPV was associated with increased progression of WMH volume and higher risk of incident lacunes over 14 years in patients with SVD.

The cross-sectional association between long-term BPV and SVD markers has recently been evaluated in two meta-analyses. Both studies concluded that higher systolic short- and long-term BPV is associated with higher odds for SVD, while data for diastolic BPV were lacking. Evidence on the longitudinal association between BPV and SVD progression is scarce. Our findings further support the link between systolic BPV and progression of WMH but over a period of 14 years[15, 16]. This association was especially present in the participants in the highest BPV quartile and remained significant after adjusting for baseline WMH volume, one of the strongest predictors for WMH progression[23]. Moreover, we found an increased risk of incident lacunes over 14 years in patients with higher BPV, which has not been described previously. This may be explained by variability in method used to assess incident lacunes. Previous investigators compared measurements from independent readers instead of side-by-side rating of repeated MRI scans[16]. In our study, we made use of difference images, which reduced the risk of measurement errors. This method therefore offered more reliable assessment of incident lacunes than manual rating. There was no clear association between diastolic BPV and SVD burden or progression in this study. Most studies examining cerebrovascular damage and BPV mainly report associations with systolic BPV and the prognostic value of diastolic BPV remains to be determined[13, 24]. The different outcomes reported for systolic and diastolic BPV suggest that different pathophysiological mechanisms may be at play. It can be hypothesized that systolic BPV is a reflection of aging and vascular stiffness, while diastolic BPV reflects autonomic dysfunction[25].

The effects of BPV on white matter microstructural integrity are less commonly investigated and the results are inconsistent across studies[26]. We calculated PSMD, a DTI marker that is suggested to be more sensitive to SVD-related brain injury than other common SVD MRI markers and other DTI parameters (i.e. Fractional Anisotropy (FA) and Mean Diffusivity (MD))[22, 27]. The lack of an association between

BPV and PSMD in this study may be due to attrition bias, because the participants with the highest BPV likely had a higher SVD burden and may not have completed all follow-ups. Larger studies are needed to assess whether increased BPV is associated with lower microstructural integrity.

The mechanisms that link BPV and small vessel damage remain poorly understood. Animal studies suggest that large BPV may cause endothelial dysfunction by inhibiting nitric oxide production, which leads to an imbalance in vascular homeostasis[28, 29]. Furthermore, larger variations in BP cause mechanical stress on vessel walls, which triggers several structural changes that may contribute to greater arterial stiffness[22]. Increased arterial stiffness is suggested to cause increased pulsation of blood flow and less dampening of blood flow, and these processes may cause microstructural damage[30]. The involvement of arterial stiffening is confirmed by a recent post-mortem study, where greater BPV was associated with a higher burden of atherosclerosis of the circle of Willis and arteriolosclerosis[31]. It is important to note that the link between BPV and SVD could also reflect reverse causality, as pre-existing vascular damage may disturb central autonomic autoregulation, resulting in larger BPV[32]. In this study, systolic BPV was already associated with WMH volumes at baseline, supporting the hypothesis of reverse causality. However, this must be interpreted with caution, since we cannot rule out that BPV was already elevated prior to study participation. This finding emphasizes the importance of correcting for pre-existing SVD when examining the association between BPV and SVD.

It could be argued that BPV is higher in hypertensive patients and mean BP levels are therefore a confounder in the observed association between BPV and cerebral damage. We adjusted all our analyses for mean BP to reduce the effect of BP levels. Our findings further support the recent meta-analysis in which it was also concluded that BPV is associated with SVD burden independent of mean BP[24]. Also in the SPRINT-MIND trial, higher BPV was associated with higher risk of dementia despite excellent BP control[33]. This growing body of evidence highlights the clinical significance of BPV.

This is the first study that examines the effects of BPV on SVD progression over a 14-year time-period in a large cohort of participants with SVD. Because we only included participants with four extensive imaging assessments, we were able to accurately model progression of WMH over time. We used difference images to reliably assess incident lacunes and semi-automatic quantification to examine WMH volumes, which is more reliable to assess progression over different scans. BP was measured three times at each visit, which reduces risk of measurement error. Nevertheless, some limitations need to be addressed. First of all, it is important to

note that the interval between BP measurements used in this study is longer than in other studies assessing BPV and SVD (3.4-5.4 years). Long-term BPV might be affected by other factors, such as changes in antihypertensive treatment over time, seasonal BP changes, medication adherence and inaccuracy of BP measurements[34]. However, visit-to-visit BPV over years has still been shown to have prognostic value[35]. It is possible that diurnal fluctuations had an effect on measured BPV because BP measurements were not performed at the exact same time during each visit. We believe this effect is minimal since the largest BP fluctuation is observed between day and night and we conducted all our BP measurements during the day, not directly after awakening[36]. Another limitation are the changes in scanner and protocol during the follow-ups, possibly leading to measurement error of (progression of) WMH volume. This is almost inevitable in long follow-up studies due to technical advances over the years and we accounted for this by including MRI system as a term in our models. Furthermore, nonparticipants were older, had more vascular risk factors and had a higher SVD burden at baseline in this study. Since age and WMH burden are strong predictors for WMH progression, loss-to-follow-up of these participants could have led to an underestimation of the true WMH progression in our study population[23].

Since progression of WMHs is significantly associated with greater cognitive decline over time, strategies aimed at reducing WMH progression emerge as crucial targets to alleviate cognitive decline[37]. If a causal relationship between BPV and SVD is established, lowering BPV may be such a relevant target. It has been suggested that calcium channel blockers and non-loop diuretics reduce visit-to-visit BPV in addition to lowering mean BP levels[38]. Moreover, a healthy lifestyle was associated with lower BPV in young and healthy adults, emphasizing the importance of lifestyle modification in patients at risk of cerebrovascular disease[39]. However, this evidence is limited and randomized controlled trials are needed to examine if pharmacological or lifestyle modifications can be used to target elevated BPV and related vascular damage. Unfortunately we did not have sufficient details about the type and dosage of antihypertensive medication that participants in this study used to examine the effects of antihypertensive drugs on BPV.

In conclusion, this longitudinal observational study suggests that higher BPV is associated with more WMH progression and more incident lacunes over 14 years in SVD patients. If this association is causal, reducing BPV could be a target to slow down SVD progression and reduce risk of dementia and stroke. Future studies are needed to confirm our findings and examine the temporal order of the observed association.

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Supplemental material

Table S1. Association between diastolic BPV and progression of WMH and incident lacunes.

	Diastolic BPV by quartile							Continuous diastolic BPV	
	Q1	Q2		Q3		Q4			
		Estimate(95% CI)		*Estimate(95% CI)*		*Estimate (95% CI)*		*Estimate (95% CI)*	
WMH (ml/year)*	Ref	-0.120 (-0.218 - -0.019)		-0.057 (-0.1768 – 0.054)		0.100 (-0.049 – 0.228)		0.017 (-0.001 – 0.030)	
		IRR (95% CI)	*P value*	*IRR (95% CI)*	*P value*	*IRR (95% CI)*	*P value*	*IRR (95% CI)*	*P value*
Incident lacune at any follow-up	1.00	0.33 (0.08 – 1.16)	0.10	0.83 (0.28 – 2.45)	0.73	1.53 (0.58 – 4.13)	0.39	1.07 (0.97 - 1.18)	0.15

Data represent β estimates (95% CI), IRR values (95% CI) or p-values. Model was adjusted for age, baseline WMH volume, use of antihypertensive medication at baseline, mean systolic BP, smoking status, BMI, hypercholesterolemia and MRI scanner system.*Bootstrapped 95% CIs. BPV: Blood Pressure Variability, IRR: Incidence Rate Ratio, WMH: White Matter Hyperintensity.

Table S2. Association between diastolic BPV and MRI markers of SVD at visit 4.

	Diastolic BPV by quartile							Continuous diastolic BPV	
	Q1	Q2		Q3		Q4			
		Standardized β(95% CI)	*P value*	*Standardized β (95% CI)*	*P value*	*Standardized β (95% CI)*	*P value*	*Standardized β (95% CI)*	*P value*
GMV*	Ref	0.22 (-0.10 – 0.54)	0.18	-0.14 (-0.46 – 0.18)	0.39	0.07 (-0.25 – 0.39)	0.67	0.00 (-0.11 – 0.12)	0.98
WMV*	Ref	**-0.42 (-0.81 - -0.04)**	**0.031**	-0.05 (-0.43 – 0.33)	0.79	-0.06 (-0.44 – 0.33)	0.77	-0.02 (-0.16 – 0.11)	0.73
PSMD*	Ref	-0.35 (-0.69 – 0.00)	0.05	-0.20 (-0.54 – 0.15)	0.27	-0.17 (-0.52 – 0.18)	0.34	-0.02 (-0.14 – 0.10)	0.74
Microbleed count		*IRR(95% CI)*	*P value*	*IRR(95% CI)*	*P value*	*IRR(95% CI)*	*P value*	*IRR(95% CI)*	*P value*
	1.00	0.64 (0.27 – 1.50)	0.29	0.96 (0.43 – 2.14)	0.93	1.38 (0.63 – 3.05)	0.41	1.05 (0.98 – 1.12)	0.21

Data represent β estimates (95% CI), IRR values (95% CI) or p-values. *Log transformed. GMV: Grey Matter Volume, IRR: Incidence Rate Ratio, PSMD: Peak Skeleton of Mean Diffusivity, WMV: White Matter Volume, WMH: White Matter Hyperintensity

Table S3. Sensitivity analysis: association between systolic BPV and progression of WMH and incident lacunes in all participants with at least 3 follow-ups (n=271).

	Systolic BPV by quartile							Continuous systolic BPV	
	Q1	Q2		Q3		Q4			
		Estimate(95% CI)		*Estimate(95% CI)*		*Estimate (95% CI)*		*Estimate (95% CI)*	
WMH (ml/year)*	Ref	-0.100 (-0.231 – 0.028)		-0.061 (-0.182 – 0.067)		**0.161 (0.008 – 0.338)**		0.012 (0.002 – 0.024)	
		IRR (95% CI)	*P value*	*IRR (95% CI)*	*P value*	*IRR (95% CI)*	*P value*	*IRR (95% CI)*	*P value*
Incident lacune at any follow-up	1.00	2.05 (0.61 – 7.68)	0.252	2.84 (0.90 – 10.24)	0.081	3.13 (1.03 – 10.98)	**0.049**	1.08 (1.00 – 1.17)	**0.024**

Data represent β estimates (95% CI), IRR values (95% CI) or p-values. Model was adjusted for age, baseline WMH volume, use of antihypertensive medication at baseline, mean systolic BP, smoking status, BMI, hypercholesterolemia and MRI scanner system.*Bootstrapped 95% CIs. BPV: Blood Pressure Variability, IRR: Incidence Rate Ratio, WMH: White Matter Hyperintensity.

Part IV – General discussion and summary

General discussion

SVD is very common and to some extent present in almost all individuals >65 years[1]. However, the underlying pathophysiology remains poorly understood since most studies are cross-sectional and conducted in older patients with end-stage SVD present. In this thesis, we examined cerebral abnormalities in young patients with hypertension, the most established risk factor for SVD, to catch the first pathological changes predisposing to MRI markers of SVD. We found that young patients with hypertension already had more MRI markers related to SVD compared to controls, mainly WMH. Moreover, we detected more BBB leakage in patients with hypertension, which was associated with the microstructural integrity of the white matter. To further explore within individuals the effect of blood pressure *increase,* we examined the effects of blood pressure increase caused by anti-hypertensive medication withdrawal for diagnostic purposes, followed by its restoration and the attendant blood pressure decrease on BBB integrity using serial MRI. Increase in blood pressure was associated with increased BBB leakage while restoration of the BBB following blood pressure lowering was less evident. Since not only high blood pressure but also large fluctuations in blood pressure can harm the brain, we examined the effect of BPV on the progression of SVD markers over 14 years. We found that in individuals with the highest variability in blood pressure, there was more progression of WMH and more incident lacunes.

In this part I will provide a general discussion of the findings of this thesis, by discussing methodological considerations, limitations of these studies and clinical implications. Finally, I will discuss directions for future research.

Methodological considerations

It is essential to discuss some methodological aspects for the interpretation of our findings. Specifically, I will now discuss several forms of bias and the risk thereof in our studies and the internal and external validity. Moreover, I will discuss examining young adults with hypertension as a model for "early SVD".

Internal validity

Selection bias

Selection bias occurs when study participants are not representative of the target population and the differences in patient characteristics influence the outcome being studied[2]. For example, individuals that are willing to undergo MRI, fill in personal questionnaires and donate blood for study purposes may differ in disease severity, educational level and sex from those who do not wish to participate.

The Hyperintense study had a large study burden and all visits were scheduled during regular working hours. This may have resulted in a selection of participants with more flexible working hours, such as self-employed individuals or those unemployed. However, this is unlikely to have influenced the study outcomes.

Selection bias possibly occurred in the control group. Initially, we contacted all study participants and asked them to refer friends and family as controls, but the response rate was low. We therefore recruited most controls through the personal networks of the researchers, resulting in a group that was more highly educated and included many individuals working in healthcare, who possibly pay greater attention to a healthy lifestyle. The control group may therefore not be fully representative of the general healthy Dutch population. However, this allowed us to include controls quickly and efficiently. Selection bias in controls is challenging to completely avoid since participation is voluntarily and individuals often have a specific motivation to participate in research, such as relatives with the disease of interest or wanting to have a "check" of their brain health.

Attrition bias is a form of selection bias that occurs when participants lost to follow-up differ from those who remain in the study, and the reasons for their drop-out affect the study outcome. In our longitudinal study, only four of the 16 participants completed all four study visits while nine of the 16 completed three measurements and were included in the analyses in **chapter 5**. Some participants were unable to complete all visits before the study ended, whereas others actively withdrew. The reasons for loss to follow-up were diverse and included emigration, surgery for an unrelated medical condition and lack of time. Although attrition bias cannot be fully excluded, the reasons for dropout appear to be random and are unlikely to have influenced our outcomes.

Information bias

Information bias is a type of bias that occurs when measurement errors in key study variables or outcomes are made[2]. Since most of the outcomes in this thesis are based on MRI, we will first discuss accuracy of these methods.

Measurement errors in MRI outcomes

In the Hyperintense study, all MRI scans were performed on a single scanner using the same protocol and head coil, to ensure consistency in MRI measurements. I will now discuss possibilities of measurement errors in outcomes measured with MRI.

Markers of SVD and microstructural integrity

In the Hyperintense study, rating of SVD markers were performed according to established consensus criteria[3] by two trained independent raters to minimize errors. In cases of disagreement, ratings were reviewed in consultation with a neurologist. Although one of the raters was not blinded to participant status, the other rater was and all ratings were jointly reviewed and confirmed. Therefore, the potential impact of lack of blinding is likely minimal. Assessment of WMH on visual rating scales is subject to rater bias and has a ceiling effect, while segmenting WMH to quantify volumes is more accurate and allows detailed quantitative analysis[4]. We therefore employed an automated WMH segmentation method to calculate WMH volumes, after which we manually checked all WMH masks and corrected them where necessary[4]. This minimizes the risk of measurement error.

In the RUNDMC study in **chapter 7**, serial MRI scans were performed on different MRI scanners with different field strengths and acquisition protocols. This is common in long-term follow-up studies due to continuous improvements in MRI hardware and software, but inevitably increases the risk of measurement errors. Several measures were taken to reduce these effects. We did not include microbleed ratings over all time-points since thinner slices inherently lead to better detection of microbleeds and thus a higher incidence. Furthermore, we created difference images to reliably assess incident lacunes during follow-up. Follow-up images were registered to baseline scans and intensity-normalized baseline images were then subtracted from the corresponding follow-up images, after which incident lacunes appear as a hypointense lesion on the background[5]. This approach is much more reliable than human inspection of scans.

In **chapter 7**, the outcome measure was progression of WMH volume over time. This is susceptible to errors because stronger field strength and thinner slices at later time-points improve detection of WMH, leading to an overestimation of WMH progression. All follow-up images were therefore resliced to baseline scans. Furthermore different algorithms to quantify WMH volume at different time-points were used which could introduce measurement error. We think this effect is likely minimal since both algorithms are highly comparable and accurate.

Next to these common SVD markers, we calculated PSMD as a DTI measure of microstructural integrity in **chapter 6** and **chapter 7**. PSMD was shown to have excellent inter-rater, test-retest and inter-scanner reliability[6, 7]. We believe that the risk of measurement error is therefore limited.

DCE-MRI

We used DCE-MRI to measure BBB integrity. In clinical context, DCE-MRI is used to detect pathology characterized by severe BBB loss, such as brain tumours or acute stroke. However, BBB leakage in SVD is much more subtle (about 10 to 100 times less[8]) and thus more challenging to measure. When gadolinium-based contrast is intravenously injected, this has a shortening effect on T1 values which can be measured using a dynamic series of T1-weighted scans over time[9]. This contrast agent (Gadovist) has a molecular weight of 605 g/mol, which can pass a healthy BBB only in a limited amount. When the BBB loses integrity, these molecules can pass the BBB more easily, likely through the degraded tight junctions into the extracellular extravascular spaces[9]. Using pharmacokinetic modelling, we can quantify the BBB leakage rate (*Ki*) and blood plasma volume fraction (*Vp*) by measuring the contrast concentration in a vessel ("vascular input function") and the tissue[10]. Several pharmacokinetic models can be used to analyse DCE-MRI, but the Patlak method is recommended in SVD and was therefore used this thesis[10].

Although DCE-MRI to measure BBB leakage has become increasingly common in SVD research, its technical, clinical and biological validation remains relatively limited. Information on scan–rescan variability is essential when evaluating treatment effects on BBB or, as in **chapter 5**, when performing repeated DCE-MRI measurements in the same participant. Variation between measurements in an individual can be related to methodological factors (hardware drift, differences in noise level) and biological factors unrelated to disease progression (caffeine intake, physical activity, sleep). Only a few studies have assessed reproducibility, in patients with SVD[11] and healthy individuals[12, 13]. Cramer et al. reported an intraclass correlation coefficient (ICC, a measure of the between subject variation) of 0.79-0.82 between BBB measurements and an ICC of 0.77 was reported by Wong et al[11, 13]. An ICC > 0.75 is considered to reflect a good reproducibility. These studies report a coefficient of variation of BBB permeability between 1-14%, which is considered to reflect an excellent to moderate reproducibility. Varatharaj et al. further demonstrated that reproducibility could be improved by extending scan duration and by using venous input functions (in the superior sagittal sinus)[12], both of which were applied in the Hyperintense study. This is especially relevant in studies with repeated measurements, where distinguishing true biological change from methodological or random variability is needed. In cross-sectional studies, such variability is less critical, since random fluctuations are expected to occur in both patients and controls. Due to the relatively large voxel size in DCE-MRI (1x1x2 mm), our ability to measure BBB leakage in very specific brain regions is limited unfortunately. To account for this limitation, we did not analyse leakage

changes at the voxel level, but instead calculated mean values for white and grey matter.

Our current knowledge of the BBB is insufficient to fully understand what influences its integrity. In **chapter 5**, we show that blood pressure appears to affect BBB leakage. It has also been suggested that factors such as caffeine intake, physical activity, smoking, and infections may influence BBB integrity, probably alongside many other unknown contributors[14-17]. Given the small sample size of our study, we were unable to adjust for these potential confounders. As a result, it is possible that part of the observed variation reflects biological fluctuations unrelated to blood pressure rather than the effect of hypertension itself.

The question also remains what we are truly measuring when observing an increase (or decrease) in BBB leakage with DCE-MRI. The differences in BBB leakage rates between hypertensive patients and controls in **chapter 4** are relatively modest (~20% higher in WM and ~16% in GM). This may reflect the effect of elevated arterial pressure associated with hypertension, rather than actual structural BBB damage. Furthermore, the clinical relevance of such small increases in BBB permeability remains unclear, although we can hypothesize that this can be harmful if it persists for a longer period. Since our study does not include long-term follow-up, we cannot determine whether these participants will develop SVD, nor whether BBB leakage precedes its onset. Since we also detect BBB leakage in healthy, normotensive young controls, some degree of contrast leakage may be normal rather than a sign of pathology. The BBB is not an impermeable structure and even under normal conditions, small molecules can cross. We have to define “normal” and “abnormal” BBB leakage. Current DCE-MRI methods can detect small differences, but there is no threshold for pathological permeability. What is considered abnormal likely depends on many factors such as age, brain region, comorbidities, and the imaging protocol used. Small amounts of leakage may reflect normal physiology, while larger increases in BBB permeability could be a predisposition to SVD.

Measurement error blood pressure

Since this thesis focuses on the effects of blood pressure on the brain, accurate measurement of blood pressure is essential. In the Hyperintense study, blood pressure was measured three times while participants were in a seated position using a Dinamap automated blood pressure device after five minutes of rest. To minimize measurement error, the same device and protocol were applied across participants. In most cases, blood pressure measurements were performed after the MRI scan. Stress related to the MRI scan may have increased blood pressure, but

this effect was likely similar across participants and is unlikely to have substantially affected our results. However, office-based blood pressure measurements are more susceptible to measurement error and white-coat effects. Ideally, 24-hour ambulatory blood pressure monitoring is used but this would have substantially increased participant burden. While the blood pressure measurements obtained during the study visit may not perfectly reflect true blood pressure, our analyses focus primarily on group differences between patients with hypertension and controls and as such misclassification between patients and controls seems unlikely. These groups were defined on the basis of diagnosis and medical history, rather than on blood pressure values measured on the day of the study visit.

In the RUNDMC study, discussed in **chapter 7**, blood pressure was measured at four visits over 14 years. During these visits, blood pressure was measured three times with the same automated device (Dinamap), which reduces the risk of measurement error. However, given the long duration of the study, different researchers conducted the measurements at each time point. Although all assessments were performed according to the same standardized protocol, slight variations in execution between researchers cannot be completely excluded.

Confounding

Confounding occurs when the real effect of an exposure on the outcome measure of interest is distorted by another factor that is associated with both the exposure and the outcome, but which is not in the causal chain[18]. Although confounding is present in every observational study, we attempted to minimize confounding by applying strict in- and exclusion criteria. For example, we excluded patients with large artery disease and high risk of thromboembolic conditions because this can also cause cerebral damage and particularly mimics of MRI markers of SVD. Unfortunately our control group was younger than the hypertensive group, making it possible that the cerebral abnormalities we observed are already the result of aging instead of hypertension. We have adjusted all statistical models in this thesis for age but residual confounding cannot be fully excluded, since age-related changes in the brain may not be entirely captured by this statistical correction.

Adjustment for BMI in studies examining effects of blood pressure is a methodological challenge. In the Hyperintense study, participants with hypertension had a much higher BMI compared to normotensive controls raising the question of whether the observed effects of blood pressure on the brain are mediated by BMI. However, a variable is only considered a confounder when either related to exposure or outcome, and is not a factor in the causal pathway of the disease[18]. In the case of

BMI and hypertension, it is estimated that at least 75% of the hypertension cases is related to obesity and as such is in the causal chain[19]. Therefore, adjusting for BMI could potentially diminish part of the true effect of hypertension on the brain, and is likely not appropriate in this context.

Another potential confounder is education level. In the Hyperintense study, patients with hypertension were generally lower educated than controls. Individuals with higher IQ and level of education may have more "cognitive reserve", meaning that they are more protected against age-related cognitive decline[12]. It is unknown whether education level affects BBB integrity. As cognition was not an outcome in this study, we cannot directly assess its potential impact, but it is unlikely to have influenced our main measures of BBB leakage.

External validity

External validity is the extent to which the findings of a study are applicable to other populations, settings and situations. We recruited patients with hypertension that were referred to the Radboudumc or Rijnstate hospital with treatment-resistant hypertension or suspicion of secondary hypertension. Most patients with hypertension are primarily treated by their GP where blood pressure is often well controlled with one or two antihypertensive agents. The patients referred to the hospital for further investigation are thus likely to represent a more severe population, with higher blood pressure levels and longer disease duration. The greater SVD burden and increased BBB leakage observed in these patients could therefore be an overestimation. However, blood pressure was on average 147/92 mmHg which is comparable to other studies among patients with hypertension. Most patients with hypertension were taking 1.5 Defined Daily Dosage (DDD) of antihypertensive medication, which is likely similar to other patients with hypertension[20]. However, it should be noted that hypertension care in the Netherlands is generally of high quality, which may limit the applicability of our findings to countries with less accessible healthcare systems[21]. Furthermore, as most participants in our study were white, the results may not be generalizable to populations with different ethnic backgrounds.

Studying young individuals with hypertension as model for early SVD

Studying young adults with hypertension at risk to examine SVD pathology is an innovative approach. Traditionally, research on SVD has focused on end-stage lesions such as WMH, lacunes, and microbleeds. However, it is now evident that these structural abnormalities are likely preceded by a long period, spanning possibly decades, of subtle microvascular dysfunction that is still poorly understood and

challenging to visualize with MRI. To understand what precedes MRI markers of SVD, a possible approach may be to study younger individuals at risk of developing SVD, in whom microvascular injury is likely already present but has not yet progressed to irreversible structural damage. Investigating this earlier stage potentially offers a window for identifying early pathological mechanisms of SVD and for developing interventions at a point where meaningful prevention is still possible. In this thesis, we demonstrated that the first signs of SVD are already measurable decades before the age at individuals are usually studied. However, WMH volume was still very low and approximately 40 times smaller compared to older SVD patients included in **chapter 7**, supporting the idea that indeed this population represents a very early stage of the disease process. Nevertheless, we do not know whether our participants actually develop SVD later in life. Although hypertension is considered the major risk factor to develop SVD, many individuals with hypertension do not develop clinically or radiologically apparent SVD. Moreover, many patients with SVD do not have hypertension[22-24]. Overall, young adults with hypertension are a valuable model to study the earliest stages of SVD, but it is important to recognize that SVD is a multifactorial disease, and hypertension alone does not determine its development. Other vascular, genetic, and lifestyle factors likely contribute to disease onset and progression, and should be taken into account when interpreting these findings.

In a subset of the participants with hypertension, we examined the effects of blood pressure increase and decrease following antihypertensive medication withdrawal. The effect of blood pressure elevation on the brain is challenging to study directly in humans, both for ethical and practical reasons. Early experimental animal studies demonstrated opening of the BBB following acute hypertension, induced either chemically or through saline infusion[25-27]. In the Hyperintense study, we studied patients with hypertension who temporarily had to withdraw antihypertensive medication to examine the cause of their hypertension. This study design is unique, as it allowed us to investigate the effects of blood pressure increase without asking participants to discontinue their medication for research purposes, which would raise ethical concerns. Instead, we took advantage of a clinical 'window' that occurs when patients temporarily stop treatment for diagnostic reasons, providing a rare opportunity to study the brain under elevated blood pressure conditions in humans. To our knowledge, this has never been done before.

However, some practical considerations of this study design should be discussed. We initially aimed to recruit 50 hypertensive patients, as discussed in **chapter 2**, but this was not feasible for a few reasons. First, many eligible patients did not fulfil the in- and exclusion criteria; they were older than 55 years, did not have

to discontinue antihypertensive medication or had an earlier TIA/stroke. Second, the burden of the study protocol was high. Participants were requested to attend four study visits during working hours, limiting their willingness to participate. Moreover, the Radboudumc is a national referral centre for PA, with patients referred from all over the Netherlands. Despite the small size of the Netherlands, travel times exceeding one hour reduced willingness to participate. Third, it was challenging to set up a workflow and protocol. Participants had to be scanned in very specific time windows, before and after discontinuation of medication, on one specific MRI scanner with limited availability. This was often not possible. To overcome these problems, we added a second study, including young patients with hypertension aged 18-40 years and normotensive controls who only had one study visit. Moreover, we started recruiting patients referred to a second hospital to increase the number of eligible patients.

Despite these practical considerations, this is an interesting model to study high blood pressure. In the fourteen participants that joined the study, blood pressure increased in eleven participants, on average with 11/4 mmHg. Although this is a substantial increase, it is not necessarily a dramatic one. The clinical significance of such a change remains uncertain, since it is unclear how much blood pressure increase is meaningful in this context. Nevertheless, it is important to be cautious when increasing blood pressure, as even modest increases in blood pressure could be risky in vulnerable populations.

General discussion of study findings

After discussing the methodological considerations, I will now interpret the findings of this thesis within a broader context.

SVD and BBB leakage in young patients with hypertension

Hypertension is considered the major vascular risk factor for sporadic SVD. Although the overall prevalence of SVD markers was low, we detected more WMH, lacunes and cerebral microbleeds in young patients with hypertension compared to normotensive controls (**chapter 3).** I will now discuss the sequence of pathological events from hypertension to BBB leakage that may lead to MRI markers of SVD.

There are likely various ways by which hypertension contributes to SVD. Hypertension causes structural alterations in the vascular wall, contributing to arterial stiffening (arteriolosclerosis), especially in subcortical areas[28]. The resulting increase in pulse

pressure and mechanical stress leads to adaptive changes aimed at protecting the downstream microcirculation, a process known as vascular remodeling[29]. Moreover, hypertension causes microvascular rarefaction, a reduction in microvascular density, suggested to result from increased pressure and hypoperfusion[29, 30]. Next to these structural alterations, hypertension can induce endothelial damage. Endothelial cells constitute a part of the BBB, which surrounds the small vessels of the brain (arterioles, capillaries and venules) and tightly controls the environment of the brain[31]. A healthy BBB ensures controlled "traffic" of molecules and ions across the vessel wall; passive through diffusion and active through transcytosis, carrier-mediated transport and receptor-mediated transport[32]. Moreover, a healthy BBB protects the central nervous system from potentially harmful substances in the blood. In **chapter 4** we show that despite the low SVD burden, young patients with hypertension had lower BBB integrity. This is also observed in older hypertensive patients and women with pre-eclampsia (hypertension during pregnancy)[33, 34]. This supports the idea that high blood pressure is associated with BBB disruption.

The clinical consequences of subtle BBB disruption, like in patients with hypertension and SVD, are poorly understood since most studies lack long-term follow-up. However, we know from other diseases such as Multiple Sclerosis (MS), stroke and infections that (acute) BBB disruption can severely damage the brain[35, 36]. When the BBB becomes leakier, reactive, harmful plasma components may enter the brain and induce oedema and neuroinflammation[32]. Increased extracellular fluid is observed in WMH, which could be the result of BBB disruption[37, 38]. In the young patients with hypertension in this thesis, we observe more WMH, higher PSMD and more BBB leakage. Although this supports the idea that BBB disruption causes increased extracellular fluid and damages the WM, we cannot exclude reverse causality, meaning that BBB disruption can either be the result or the cause of structural brain damage. It is likely that these processes reinforce each other in a vicious cycle: BBB leakage leads to extracellular fluid accumulation and inflammation, which in turn further damages vascular integrity and BBB function. In **chapter 6**, BBB integrity was associated with PSMD independent of visible damage (i.e. WMH volume), which could indicate that BBB disruption is an early pathological process rather than a consequence of existing WMH. Longitudinal studies are essential to determine the role of BBB disruption in (progression of) SVD but are rarely done. One longitudinal study found baseline BBB permeability in the WMH penumbra to be associated with increased microstructural damage two years later[39]. Unfortunately BBB leakage was only measured once in this study, while it would be particularly interesting to assess changes in BBB disruption over time and the association with tissue damage. This finding needs to be replicated in other longitudinal studies especially given the small sample size (n=43)[39].

BBB leakage in response to blood pressure changes

In this thesis, we found increased BBB leakage when blood pressure increased. The mechanisms leading to increased BBB leakage following blood pressure increase are likely different from the structural vessel wall alterations caused by chronic hypertension. I will now discuss several mechanisms could explain the increase in leakage of contrast agent with increase in blood pressure.

First, an increase in blood pressure may directly damage the structural integrity of the BBB, as discussed in the previous section. However, it remains uncertain whether a relatively short period of elevated blood pressure, such as 4–6 weeks in the Hyperintense study, is sufficient to cause detectable structural damage. Second, increase in blood pressure could, when exceeding the autoregulation threshold, increase arterial pressure, which could increase cerebral blood flow and intracranial pressure. This would facilitate the movement of small molecules such as contrast agent from the blood plasma across the BBB, independent of true BBB disruption. However, the general hypothesis is that autoregulation protects the brain from fluctuations in blood pressure and keeps cerebral blood flow relatively stable[40]. Although it has been suggested that autoregulation is impaired in patients with chronic hypertension, a recent review concluded there is no convincing evidence that autoregulation is impaired in these patients[40]. Supporting this, several studies have shown that starting antihypertensive treatment and blood pressure lowering had no effect on CBF[40]. It could be suggested that the blood pressure elevations in this study exceeded the upper limit of autoregulation[41]. Nevertheless, the commonly accepted systolic threshold of >180 mmHg (after which autoregulation becomes impaired) was exceeded in only two participants. Interestingly, we do observe an increase in *Vp* with increase in blood pressure. It is important to note that *Vp* reflects the fraction of tissue occupied by intravascular plasma, rather than cerebral blood flow itself. An elevation in *Vp* may therefore indicate increased intravascular volume or capillary filling in response to higher arterial pressure, rather than an increase in perfusion. Taking this together, it is unlikely that increased contrast extravasation is caused by increased intracranial pressure.

Finally, elevated blood pressure may mechanically expand cerebral vessels, increasing the total endothelial surface area. A larger surface area provides more opportunity for small molecules to cross the BBB, independent of structural barrier damage. In this scenario, the observed increase in leakage could reflect hemodynamic and physical changes in the small vessels rather than damage to the BBB. It is important to note that, regardless of whether the increased leakage of contrast is caused by structural BBB damage, elevated arterial pressure,

increased endothelial surface area, or a combination of these factors, the resulting extravasation of molecules can be harmful for the brain and should be prevented.

In **chapter 5**, we did not observe an immediate reduction in BBB leakage following blood pressure lowering in all participants. If the increased BBB permeability was solely due to elevated arterial pressure, one would expect a strong reduction once blood pressure is normalized. Since this is not the case for all individuals, it suggests that hypertension may induce structural changes in the BBB that persist over time, even after blood pressure has been lowered. In women with pre-eclampsia, which is high blood pressure during pregnancy, more BBB leakage was observed years after the pregnancy[33]. This supports the idea that temporary elevation in blood pressure can alter the structure of the BBB for a longer period, although we cannot be certain that high blood pressure during pregnancy is the cause of observed BBB differences. Another explanation is the longer interval between MRI scans, which increases the likelihood that external factors, such as infections, could also influence BBB integrity in the meantime. Moreover, this observation is based on a small subset of participants (n=7) who experienced a reduction in blood pressure after medication restart, and therefore these findings need to be confirmed in a larger cohort.

Blood pressure variability and SVD

Not only high blood pressure, but large BPV is also shown to contribute to mortality, SVD and dementia risk[42]. In **chapter 7**, we found that patients with SVD with the largest BPV had the most progression of WMH and more incident lacunes compared to individuals with the smallest BPV. In healthy individuals, variation in blood pressure is needed to maintain adequate perfusion in response to metabolic demands and external factors[43]. An example of this is "nocturnal dipping", a decrease in blood pressure during the night, related to the (in) activity of the sympathetic nervous system[43]. Abnormally large fluctuations in blood pressure could be a marker of impaired cardiovascular regulation but could also represent a cardiovascular risk factor itself. As described above, hypertension causes atherosclerosis and arterial stiffening. This stiffening can lead to reduced elasticity of the vessels, impairing the capacity to regulate blood pressure and thus increase BPV[44]. On the other hand, increased BPV is also suggested to cause mechanical stress on vessel walls and contribute to increased arterial stiffness. So this is a vicious cycle where BPV contributes to and results from cerebrovascular damage. Furthermore, it is suggested that elevated BPV is associated with cerebral hypoperfusion, again contributing to WM damage[45]. Interestingly, we and others found that the effect of BPV was independent of mean blood pressure, suggesting

that fluctuations in blood pressure may be more harmful for the brain than constant high blood pressure[42]. This is supported by results from the SPRINT-MIND trial, where higher BPV was associated with dementia risk despite strictly controlled mean blood pressure levels[46].

The mechanisms underlying BPV likely also depend on the interval between the blood pressure measurements. In **chapter 7**, we measured visit-to-visit BPV and the interval between the blood pressure measurements was long (3-5 years). This variability is thought to be primarily due to behavioural and environmental factors and compliance to antihypertensive therapy[47]. While the long intervals between measurements resemble the situation in clinical practice where blood pressure is assessed only periodically, many factors can influence blood pressure during these intervals. The emergence of devices that can measure blood pressure cuffless and continuously therefore has great potential for assessing BPV since blood pressure is highly dynamic. While office-based blood pressure measurements only provide snapshots, cuffless devices can capture fluctuations in blood pressure throughout daily activities and sleep[48]. Cuffless pulse wave-based devices to assess BPV are currently not recommended to assess BPV since the methodology is not sufficiently validated[43]. With broader adoption and validation, cuffless blood pressure devices could eventually become the preferred approach for evaluating both average blood pressure and BPV.

Clinical relevance

The studies conducted in this thesis are mostly exploratory and conducted in relatively small samples of patients. The clinical relevance of investigating blood pressure–related brain changes in young patients lies primarily in improving our understanding of early, subclinical mechanisms of SVD. While the immediate clinical implications are limited, these insights emphasize the importance of proper blood pressure control.

The Dutch Health Council recently advised to focus primarily on targeting modifiable risk factors to reduce dementia incidence, especially hypertension. In a recent large trial, it was demonstrated that blood pressure reduction led to 15% lower incidence of all-cause dementia after four years, emphasizing the major health burden that hypertension poses[49]. Although hypertension is more prevalent in the elderly, an estimated 10–25% of individuals aged 30–49 years in the Netherlands are hypertensive with most of them being unaware of this[50]. This

prevalence is rising, likely due to unhealthy lifestyle habits and increasing obesity rates[51]. Several studies have shown that midlife hypertension is a much stronger risk factor for dementia than late-life hypertension[52, 53]. However, awareness and treatment remain much lower in young adults compared to older patients, as hypertension is often asymptomatic and blood pressure is not routinely measured in healthy adults[51]. Population-wide screening could increase the number of hypertension diagnoses, but would also place substantial demands on our health care system, since more patients would require monitoring and treatment[21]. Such programs should therefore only be considered when health care systems have sufficient resources to cope with the extra patients. The WHO recommends that blood pressure is measured 'opportunistically' when patients first present to health care facilities and periodically thereafter[21]. Raising awareness among young adults about the consequences of hypertension is crucial. Importantly, it has been estimated that achieving 50% blood pressure control worldwide would generate financial benefits 18 times greater than the associated costs[21].

Different classes of antihypertensive medications are widely used, with different mechanisms of action. It is suggested that these distinct classes have varying effects on cerebral microvascular health and SVD. In a post-hoc analysis of the SPRINT-MIND trial, ACE inhibitors were associated with less WMH progression[54]. However, the more recent TREAT-SVDs trial found no differences in cerebrovascular reactivity after treatment with amlodipine, losartan or atenolol, although the treatment period was short[55]. It is suggested that the differential effects of drug classes are related to differences in effect on BPV[55]. To my knowledge, no studies have examined differential effects of antihypertensive medication on BBB function. The sample size of the Hyperintense study was unfortunately too small to examine this. Since patients with hypertension already require medication, selecting the drug class that is most beneficial for both blood pressure control and endothelial function would be a feasible strategy without adding treatment burden.

Future perspectives

Improving reliability and reproducibility of DCE-MRI

While DCE-MRI is currently the most validated method to assess subtle BBB leakage in SVD, there is no standardized protocol available. Greater harmonization of both imaging protocol and data analysis pipeline would increase reproducibility and allow comparison across study sites. It has been recommended to establish an international working group that works on an open-source consensus protocol, but

to our knowledge, this is not available yet[10]. Moreover, scanning times are relatively long (>15 minutes recommended) to allow sufficient contrast to leak to detect a signal[10]. To address this, recent studies have applied non-standard protocols in which one pre-contrast and two post-contrast T1-weighted images are acquired after 20–30 minutes, while other sequences are performed between acquisitions[56]. This is time-efficient and would make DCE-MRI more clinically feasible, and this should be replicated in other studies.

Non-invasive measurement of BBB integrity

Even with increased efforts to improve the robustness of DCE-MRI, this technique has substantial limitations. Contrast injection is invasive, can cause allergic reactions and is not safe in patients with renal insufficiency[57]. Especially older patients can therefore not always safely undergo DCE-MRI. Arterial Spin Labeling (ASL) could offer a new MRI technique to measure BBB leakage non-invasively. In ASL, water within arterial blood is "labeled" after which it crosses the BBB and can be measured in the brain tissue[58]. DCE-MRI and ASL measure different properties of the BBB, since DCE-MRI primarily reflects passive contrast leakage across the endothelial layer, whereas ASL reflects active water transport from perivascular spaces to the interstitial space[57]. To my knowledge, no studies have examined BBB leakage with ASL and DCE-MRI simultaneously, so it remains unknown to what extent the two methods provide overlapping information. ASL could represent a less invasive alternative for repeated measurements to monitor effects of treatment on BBB leakage, but more research is needed to establish its accuracy and reliability in detecting BBB alterations. An even less invasive approach would be to assess SVD-related changes without conducting MRI, since MRI is expensive, time-consuming and not widely available. There has been growing interest in using the retina as a window to the brain. Retinal imaging allows the evaluation of microvascular alterations, which may reflect similar pathological processes occurring in the brain[59]. Retinopathy is associated with lacunar stroke, WMH and vascular dementia[59]. Recently, retinal vessel reactivity in response to a CO_2 challenge was found to be strongly related to SVD[60]. It is suggested that the BBB and the blood-retinal barrier have similar structures[59] but to my knowledge, no studies have assessed blood-retinal barrier as a surrogate marker for BBB integrity.

Pharmacological modification of BBB integrity

Next to trials focused on preventing SVD through conventional risk factors, there are not many therapeutic trials in SVD. Since endothelial dysfunction is a major contributor to BBB damage, targeting this is a promising approach to diminish SVD. The recent MINERVA trial found no effect minocycline on BBB permeability after

three months[61]. Interestingly, they found a very poor correlation between baseline BBB leakage and after three months, emphasizing that more information on the reproducibility of DCE-MRI is needed [61].

One therapeutic agent that is specifically interesting in this context is metformin. This drug is generally used in patients with type 2 diabetes, but has many other potentially beneficial effects next to glucose lowering[62]. In observational studies, use of metformin in patients with diabetes reduced development of cognitive dysfunction, lowered the risk of incident dementia and reduced SVD burden[63-65]. Metformin is suggested to improve endothelial function and BBB integrity, although this is mainly demonstrated in animal studies[66-68].This is an example of drug repurposing: exploring new uses for existing therapeutics, expanding their applications to benefit a larger patient population. This approach is faster, less expensive and risky and has higher success rates than traditional drug development approaches[69].

Conclusion

By using advanced MRI techniques in young patients with hypertension, we demonstrated that not only structural SVD markers were more prevalent in these individuals, but increased BBB leakage was also observed and associated with reduced microstructural white matter integrity. Moreover, a short-term increase in blood pressure following temporary discontinuation of antihypertensive medication resulted in a further increase in BBB leakage. Future studies should investigate the long-term consequences of BBB disruption on brain structure and function, and explore whether restoring BBB integrity could serve as a therapeutic target to prevent or slow SVD progression.

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Summary

PART I – INTRODUCTION

Cerebral small vessel disease (SVD) refers to pathologies affecting the smallest vessels of the brain, mostly linked to ageing and cardiovascular risk factors such as hypertension and obesity. Individuals with SVD have an increased risk of stroke, cognitive and motor impairment and dementia. Despite the major health burden of SVD, there is currently no treatment, likely because the pathophysiology is poorly understood. Since most studies are conducted in older individuals with end-stage SVD lesions visible on MRI, little is known about early pathological alterations in the small vessels that ultimately lead to cerebral damage. To learn more about early pathological mechanisms in SVD, studying young and middle-aged adults at risk of developing SVD is crucial. Hypertension is the major risk factor for SVD, making young individuals with hypertension a particularly valuable population to investigate early, potentially reversible brain changes associated with SVD.

The overall aim of this thesis was to examine these early pathological alterations in the brain in young patients with hypertension, at risk of developing SVD, to learn more about the pathogenesis underlying SVD. Moreover, we aimed to explore the effect of fluctuations in blood pressure on progression of SVD.

The studies in **chapters 3-6** are based on the Hyperintense study, of which we presented the protocol in **chapter 2.** This prospective, observational cohort study was designed to examine cerebrovascular and structural abnormalities, possibly preceding SVD, in young adults with hypertension. The study aimed to include 50 hypertensive adults (18-55 years) who temporarily discontinued antihypertensive medication for diagnostic purposes. Patients were recruited in the outpatient clinics of the Department of Internal Medicine of the Radboud University Medical Centre (Radboudumc) and Rijnstate hospital. Main exclusion criteria were a history of ischemic/haemorrhagic stroke or transient ischemic attack (TIA), large-artery disease and cardioembolism, major other (neurological) diseases and contraindications for 3T MRI and contrast injection. MRI and clinical data was collected at four timepoints: before medication withdrawal (baseline), once antihypertensives are largely or completely withdrawn (T=1), when patients have restarted medication (T=3) and reached target blood pressure and 1 year later (T=4). The 3T MRI protocol included conventional structural sequences and advanced techniques to assess various aspects of microvascular integrity, including blood-brain barrier function using DCE-MRI, white matter integrity and microperfusion.

PART II – MRI ABNORMALITIES IN YOUNG PATIENTS WITH HYPERTENSION

In **chapter 3** we examined the prevalence of common SVD MRI markers, i.e. WMH, lacunes and cerebral microbleeds, in patients with hypertension and normotensive controls included in the Hyperintense study. Although the overall SVD burden was low in both groups, we observed a significantly higher WMH volume in the patients with hypertension compared to the controls. While none of the normotensive controls showed evident periventricular WMH (Fazekas >0), this was observed in approximately 18% of the patients with hypertension. Deep WMH (Fazekas >0) were more commonly observed, in approximately 58% of the patients with hypertension and 14% of the controls. Lacunes and microbleeds were both rare and only observed in the patients with hypertension. One lacune was observed in one patient with hypertension, and microbleeds were present in three patients with hypertension. Interestingly, we did not observe an association between duration of hypertension and WMH volume. This study demonstrated that the first SVD MRI markers could already be detected in young patients with hypertension.

Disruption of the blood-brain barrier (BBB), a protective layer surrounding the smallest vessels, is suggested to be involved in SVD pathogenesis. When this layer loses its integrity, harmful plasma proteins may infiltrate the brain and cause injury, over time manifesting as structural damage observed on MRI. Loss of BBB integrity is observed in patients with end-stage SVD and dementia, making it difficult to determine whether BBB dysfunction precedes SVD MRI markers. If so, improving BBB integrity may be a promising target for early intervention in SVD. In **chapter 4**, we examined whether BBB integrity is impaired in patients with hypertension compared to normotensive controls. We measured BBB leakage in 59 patients with hypertension (mean age of 35.8 years) and 21 healthy controls (mean age of 29.9 years). Patients with hypertension had 17% significantly more BBB leakage in the white matter and 15% in the grey matter compared to controls. This association remained significant after adjusting for SVD score. This study demonstrated BBB disruption in young patients with hypertension independent of pre-existing SVD MRI markers, suggesting a possible role of BBB dysfunction in SVD pathogenesis.

To examine whether blood pressure directly affects the integrity of the BBB, we investigated BBB leakage in young patients with hypertension following blood pressure increase and decrease in the Hyperintense study in **chapter 5**. Fourteen middle-aged hypertensive adults who temporarily discontinued antihypertensive medication for diagnostic purposes were included. DCE-MRI was done at three timepoints: before medication withdrawal (visit 1), during withdrawal (visit 2)

and after medication restart (visit 3). After medication withdrawal, blood pressure increased in 11/14 participants (79%). Increase in blood pressure between visit 1-visit 2 was significantly associated with increase in BBB leakage in the white matter. Following medication restart, blood pressure decreased in seven participants, with BBB leakage decreasing in four of them. There was no association between decrease in blood pressure and BBB leakage between visit 2-visit 3. In conclusion, blood pressure increase was associated with increased BBB leakage. Reduction in BBB leakage following medication restart was observed in most, but not all participants. This suggests that BBB leakage is dynamic and influenced by blood pressure.

After demonstrating that patients with hypertension have more BBB leakage than normotensive controls, we aimed to examine whether BBB leakage is linked to early, microstructural white matter changes. In **chapter 6**, we therefore examined the association between BBB integrity and Peak Width of Skeletonized Mean Diffusivity (PSMD), a marker of white matter damage, in young patients with hypertension and normotensive controls. We included the same 59 patients with and 21 healthy controls included in the Hyperintense study as described earlier. DCE-MRI was used to measure BBB leakage and multi-shell diffusion tensor imaging was used to examine PSMD. Patients with hypertension had significantly higher PSMD, i.e. lower white matter microstructural integrity, compared to controls. Moreover, BBB leakage in the white matter was associated with higher PSMD. This supports the hypothesis that BBB dysfunction is an early feature of SVD and highlights the importance of early prevention and intervention in hypertension.

PART III – BLOOD PRESSURE VARIABILITY

Blood pressure is not stable and fluctuates continuously throughout the day. This is referred to as blood pressure variability (BPV). Not only high blood pressure, but also increased BPV, thus larger fluctuations in blood pressure, is a strong risk factor for SVD-related brain damage. It is still unclear whether increased BPV is a direct cause of SVD or simply a result of changes in the blood vessel walls associated with SVD. Studying the effect of BPV on the progression of the disease, instead of solely on cross-sectional data, can help clarify its role in the progression of SVD. In **chapter 7**, we examined whether blood pressure variability (BPV) is associated with more progression of SVD. Specifically, we examined whether visit-to-visit BPV is associated with WMH progression over 14 years and MRI markers after 14 years. We included 199 participants with SVD from the Radboud University Nijmegen Diffusion tensor Magnetic resonance imaging Cohort (RUNDMC) who underwent baseline assessment in 2006 and follow-up in 2011, 2015 and 2020. BPV

was calculated as coefficient of variation of BP at all visits. We found that systolic BPV was associated with faster progression of WMH and higher risk of incident lacunes. There was no association between systolic BPV and GM and WM volumes, Peak Skeleton of Mean Diffusivity (PSMD) or microbleed count after 14 years. In conclusion, visit-to-visit systolic BPV was associated with increased progression of WMH volumes and higher risk of incident lacunes over 14 years in participants with SVD. Future studies are needed to examine causality of this association.

Dutch summary

DEEL I – INLEIDING

Cerebral small vessel disease (SVD) verwijst naar pathologieën die de kleinste bloedvaten van de hersenen aantasten, meestal gerelateerd aan veroudering en cardiovasculaire risicofactoren zoals hypertensie en obesitas. Individuen met SVD hebben een verhoogd risico op het krijgen van een beroerte, cognitieve en motorische stoornissen en dementie. Ondanks de grote maatschappelijke en individuele last van SVD is er momenteel geen behandeling beschikbaar, waarschijnlijk omdat de pathofysiologie nog onvoldoende wordt begrepen. Aangezien de meeste studies worden uitgevoerd bij oudere personen in het eindstadium van SVD, waarbij SVD-laesies al zichtbaar zijn op MRI, is er weinig bekend over vroege pathologische veranderingen in de kleine bloedvaten die uiteindelijk leiden tot hersenbeschadiging. Om meer te weten te komen over vroege pathologische mechanismen bij SVD, is het van cruciaal belang om (jong) volwassenen te bestuderen die risico lopen op het ontwikkelen van SVD. Hypertensie is de belangrijkste risicofactor voor SVD, waardoor jonge personen met hypertensie een bijzonder waardevolle populatie vormen om vroege, mogelijk omkeerbare hersenveranderingen in verband met SVD te onderzoeken.

Het overkoepelende doel van dit proefschrift was om deze vroege pathologische veranderingen in de hersenen te onderzoeken bij jonge patiënten met hypertensie die risico lopen op het ontwikkelen van SVD, om zo meer te weten te komen over de pathogenese die ten grondslag ligt aan SVD. Bovendien wilden we het effect van schommelingen in de bloeddruk op de progressie van SVD onderzoeken.

De studies in de **hoofdstukken 3-6** zijn gebaseerd op de Hyperintense studie, waarvan het protocol in **hoofdstuk 2** wordt gepresenteerd**.** Deze prospectieve, observationele cohortstudie was opgezet om cerebrovasculaire en structurele afwijkingen, die mogelijk voorafgaan aan SVD, te onderzoeken bij jonge volwassenen met hypertensie. De studie had als doel 50 volwassenen met hypertensie (18-55 jaar) te includeren die tijdelijk hun antihypertensiva staakten voor diagnostische doeleinden. Patiënten werden geïncludeerd in de poliklinieken van de afdeling Interne Geneeskunde van het Radboudumc en het Rijnstate ziekenhuis. De belangrijkste exclusiecriteria waren een voorgeschiedenis van ischemische/hemorragische beroerte of voorbijgaande ischemische aanval (TIA), aandoeningen aan de grote slagaders en cardio-embolie, andere ernstige (neurologische) aandoeningen en contra-indicaties voor 3T MRI en contrastinjectie. MRI en klinische gegevens werden verzameld op vier tijdstippen: vóór het staken

van de medicatie (baseline), zodra de antihypertensiva grotendeels of volledig waren gestaakt (T=1), toen de patiënten de medicatie weer waren begonnen (T=3) en de streefbloeddruk hadden bereikt en 1 jaar later (T=4). Het 3T MRI-protocol bestond uit conventionele structurele sequenties en geavanceerde technieken om verschillende aspecten van de microvasculaire integriteit te beoordelen, waaronder de bloed-hersenbarrièrefunctie met behulp van DCE-MRI, de integriteit van de witte stof en microperfusie.

DEEL II – MRI-AFWIJKINGEN BIJ JONGE PATIËNTEN MET HYPERTENSIE

In **hoofdstuk 3** hebben we de prevalentie onderzocht van veelvoorkomende SVD-MRI-markers, d.w.z. witte stof afwijkingen (WMH), lacunes en cerebrale microbloedingen, bij patiënten met hypertensie en normotensieve controles in de Hyperintense studie. Hoewel de totale hoeveelheid SVD op MRI in beide groepen laag was, zagen we een significant hoger WMH volume bij de patiënten met hypertensie in vergelijking met de controles. Terwijl geen van de normotensieve controlegroep duidelijke periventriculaire WMH (Fazekas >0) vertoonden, werd dit wel waargenomen bij ongeveer 18% van de patiënten met hypertensie. Diepe WMH (Fazekas >0) werden waargenomen bij ongeveer 58% van de patiënten met hypertensie en 14% van de controlegroep. Lacunes en microbloedingen waren beide zeldzaam en werden alleen gezien bij de patiënten met hypertensie. Bij één patiënt met hypertensie werd één lacune waargenomen en bij drie patiënten met hypertensie waren microbloedingen aanwezig. Interessant genoeg zagen we geen verband tussen de duur van hypertensie en het WMH volume. Deze studie toonde aan dat de eerste tekenen van SVD al bij jonge patiënten met hypertensie konden worden gedetecteerd.

Verstoring van de bloed-hersenbarrière (BBB), een beschermende laag rondom de kleinste bloedvaten, zou mogelijk een rol spelen bij het ontstaan van SVD. Wanneer deze laag zijn integriteit verliest, kunnen schadelijke plasma-eiwitten de hersenen binnendringen en schade veroorzaken, wat na verloop van tijd zichtbaar wordt als structurele schade op MRI scans. Verlies van de integriteit van de BBB wordt waargenomen bij patiënten met SVD en dementie, waardoor het moeilijk is om te bepalen of BBB dysfunctie voorafgaat aan SVD MRI-markers. Als dat het geval is, kan het verbeteren van de integriteit van de BBB een veelbelovend doelwit zijn voor vroegtijdige interventie bij SVD. In **hoofdstuk 4** hebben we onderzocht of de integriteit van de BBB bij patiënten met hypertensie is aangetast in vergelijking met normotensieve controles. We hebben de lekkage van de BBB gemeten bij 59 patiënten met hypertensie (gemiddelde leeftijd 35,8 jaar) en 21 gezonde controles (gemiddelde leeftijd 29,9 jaar). Patiënten met hypertensie hadden 17% meer

BBB-lekkage in de witte stof en 15% in de grijze stof in vergelijking met de controlegroep. Dit verband bleef significant na correctie voor de SVD score, een maat voor hoe veel SVD zichtbaar is in het brein. Deze studie toonde BBB lekkage aan bij jonge patiënten met hypertensie, onafhankelijk van reeds bestaande SVD MRI-markers, wat wijst op een mogelijke rol van BBB dysfunctie in de pathogenese van SVD.

Om te onderzoeken of de bloeddruk de integriteit van de BBB rechtstreeks beïnvloedt, hebben we in het Hyperintense onderzoek in **hoofdstuk 5** de hoeveelheid BBB lekkage onderzocht bij jonge patiënten met hypertensie na een stijging en daling van de bloeddruk. Veertien volwassenen van middelbare leeftijd met hypertensie die tijdelijk hun antihypertensiva hadden gestaakt voor diagnostische doeleinden, werden in het onderzoek opgenomen. DCE-MRI werd uitgevoerd op drie tijdstippen: vóór het staken van de medicatie (bezoek 1), tijdens het staken (bezoek 2) en na het hervatten van de medicatie (bezoek 3). Na het staken van de medicatie steeg de bloeddruk bij 11 van de 14 deelnemers (79%). De stijging van de bloeddruk tussen bezoek 1 en bezoek 2 was significant geassocieerd met een toename van BBB lekkage in de witte stof. Na hervatting van de medicatie daalde de bloeddruk bij zeven deelnemers, waarbij bij vier van hen de BBB lekkage ook afnam. Er was geen verband tussen de daling van de bloeddruk en de BBB lekkage tussen bezoek 2 en bezoek 3. We concludeerden dat de stijging van de bloeddruk gepaard ging met een toename van de BBB-lekkage. Bij de meeste, maar niet alle deelnemers werd een afname van de BBB-lekkage waargenomen na hervatting van de medicatie. Dit suggereert dat BBB integriteit dynamisch is en wordt beïnvloed door de bloeddruk.

Nadat we hadden aangetoond dat patiënten met hypertensie meer BBB lekkage hebben dan normotensieve controles, wilden we onderzoeken of BBB integriteit verband houdt met vroege, microstructurele veranderingen in de witte stof. In **hoofdstuk 6** hebben we daarom het verband onderzocht tussen de integriteit van de BBB en de piekbreedte van de geskeletiseerde gemiddelde diffusiviteit (PSMD), een marker voor schade aan de witte stof, bij jonge patiënten met hypertensie en normotensieve controles. We hebben dit onderzocht in dezelfde 59 patiënten met hypertensie en 21 gezonde controles die eerder in het Hyperintense onderzoek mee hadden gedaan. DCE-MRI werd gebruikt om BBB lekkage te meten en multi-shell diffusietensorbeeldvorming werd gebruikt om PSMD te onderzoeken. Patiënten met hypertensie hadden een significant hogere PSMD, d.w.z. een lagere microstructurele integriteit van de witte stof, in vergelijking met de controlegroep. Bovendien was BBB lekkage in de witte stof geassocieerd met een hogere PSMD.

Dit ondersteunt de hypothese dat BBB dysfunctie een vroeg kenmerk is van SVD en benadrukt het belang van vroege preventie en interventie bij hypertensie.

DEEL III – BLOEDDRUKVARIABILITEIT

De bloeddruk is niet stabiel maar fluctueert continu gedurende de dag. Dit wordt bloeddrukvariabiliteit (BPV) genoemd. Niet alleen hoge bloeddruk, maar ook verhoogde BPV, dus grotere schommelingen in de bloeddruk, is een sterke risicofactor voor SVD-gerelateerde hersenschade. Het is nog onduidelijk of verhoogde BPV een directe oorzaak is van SVD of slechts een gevolg van veranderingen in de bloedvatwanden die gepaard gaan met SVD. De rol van BPV in de progressie van SVD is eveneens onduidelijk. In **hoofdstuk 7** hebben we onderzocht of de variabiliteit van de bloeddruk geassocieerd is met een grotere progressie van SVD. We hebben met name onderzocht of BPV van bezoek tot bezoek geassocieerd is met progressie van WMH over een periode van 14 jaar en MRI-markers na 14 jaar. We hebben 199 deelnemers met SVD opgenomen uit de Radboud University Nijmegen Diffusion tensor Magnetic resonance imaging Cohort (RUNDMC), die in 2006 de eerste meting ondergingen en in 2011, 2015 en 2020 een follow-up. BPV werd berekend als de variatiecoëfficiënt van de bloeddruk bij alle bezoeken. We ontdekten dat een hogere systolische BPV geassocieerd was met een snellere progressie van WMH en een hoger risico op lacunes. Er was geen verband tussen systolische BPV en witte en grijze stof volumes, PSMD of het aantal microbloedingen na 14 jaar. We concludeerden dat systolische BPV geassocieerd was met een verhoogde progressie van WMH-volumes en een hoger risico op incidentele lacunes gedurende 14 jaar bij deelnemers met SVD. Er is meer onderzoek nodig om de causaliteit van dit verband te onderzoeken.

Appendices

A1. List of abbreviations

Abbreviations	
ACE	Angiotensin-converting enzyme
AD	Alzheimer's disease
AIC	Akaike information criterion
ARB	Angiotensin receptor blocker
ARR	Aldosterone/renin ratio
BBB	Blood-brain barrier
BET	Brain extraction tool
BMI	Body mass index
BP	Blood pressure
BPV	Blood pressure variability
CAA	Cerebral amyloid angiopathy
CADASIL	Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CCB	Calcium channel blocker
CSF	Cerebrospinal fluid
CI	Confidence interval
CV	Coefficient of variation
DCE	Dynamic Contrast-Enhanced
DDD	Defined daily dose
DTI	Diffusion tensor imaging
DWI	Diffusion-weighted imaging
eGFR	Estimated glomerular filtration rate
ESH	European society of hypertension
FA	Fractional anisotropy
FDR	False discovery rate
FLAIR	Fluid-attenuated inversion recovery
FSL	Functional magnetic resonance imaging of the brain software library
GP	General practitioner
GRE	Gradient echo sequence
GM	Grey matter
ICC	Intraclass correlation coefficient
ICV	Intracranial volume
IVIM	Intravoxel incoherent motion
IRR	Incidence rate ratio
IQR	Interquartile range

Abbreviations	
Ki	Leakage rate
LME	Linear mixed effects
OBPM	Office-based blood pressure measurement
OR	Odds ratio
MARS-WMH	MIAC automated region segmentation for white matter hyperintensities
MD	Mean diffusivity
METC	Medisch-ethische toetsingscommissie
MNI	Montreal neurological institute
MP2RAGE	Magnetization-Prepared 2 Rapid Acquisition Gradient Echoes
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
NA or N/A	Not applicable
NAWM	Normal appearing white matter
PA	Primary aldosteronism
PSMD	Peak width of skeletonized mean diffusivity
Q	Quartile
Radboudumc	Radboud university medical centre
RAS	Renin-angiotensin system
ROI	Region of interest
Rs-fMRI	Resting-state functional MRI
RUNDMC	Radboud university Nijmegen diffusion tensor magnetic resonance imaging cohort
SAMSEG	Sequence adaptive multimodal segmentation
SD	Standard deviation
STRIVE	STandards for ReportIng Vascular changes on nEuroimaging
SVD	Small vessel disease
SWI	Susceptibility-weighted imaging
TE	Echo time
TIA	Transient ischemic attack
TOAST	Trial of ORG 10172 in acute stroke treatment
TR	Repetition time
Vp	Blood plasma volume fraction
WHO	World health organization
WM	White matter
WMH	White matter hyperintensities

A2. Research data management

Ethics and privacy

Medical and ethical approval

This thesis is based on the results of research involving human participants, which were conducted in accordance with relevant national and international legislation and regulations, guidelines, codes of conduct and Radboudumc policy. The recognized Medical Ethics Review Committee 'METC Oost-Nederland' has given approval to conduct these studies (Hyperintense study: NL75003.091.20, RUNDMC study: NL69678.091.19). The institutional ethical review committee CMO Radboudumc, Nijmegen, the Netherlands has given approval to conduct these studies (CMO Radboudumc dossier number: 2020-6936, Biobank: 2020-6937).

Privacy measures

According to Dutch legislation, data collection from electronic patient files was performed by personnel with a treatment relationship with the patient or by the researcher(s) upon consent by the study participant. The privacy of the participants in these studies was warranted by the use of pseudonymization. The pseudonymization key was stored on a secured network drive that was only accessible to members of the project who needed access to it because of their role within the project. The pseudonymization key was stored separately from the research data. The privacy of the participants in these studies was warranted by the use of fully anonymous data.

Informed consent

Informed consent was obtained from participants to collect and process their data for this research project. Consent was also obtained for sharing and reuse of the (pseudonymized) data for future research.

Data collection and storage

Data collection

Data for all chapters in this thesis were collected in the Radboudumc hospital and not re-used from other sources. Data and questionnaires for studies in this theses were collected through electronic Case Report Forms (eCRF) of a prospective data collection in Castor EDC. Data were converged from (electronic) health records or Castor EDC to SPSS R studio version 4.3.1. Clinical data for all studies were extracted and added in the eCRF in Castor EDC from electronic health records (EPIC).

Data storage

Data from chapter 2-7 were stored and analyzed on the Neurology department server and are only accessible by project members working at the Radboudumc. These secure storage options safeguard the availability, integrity and confidentiality of the data. Paper (hardcopy) data is stored in secure cabinets at the Neurology department.

Data sharing according to the FAIR principles

All research data that are presented in this thesis were well archived according to the FAIR (Findable, Accessible, Interoperable and Reusable) principles. The studies in chapter 2, 3 and 7 are published open access, while the study in chapter 4 is published with restricted access. Chapters 5 and 6 have been submitted for publication and are currently under review.

All datasets are archived with restricted access in a Data Sharing Collection (DSC) in the Radboud Data Repository. Details of this repository are shown in the Table below:

Chapter	Title	DSC
2-7	Midlife hypertension and the brain	DOI: https://doi.org/10.34973/0dn9-b909

These datasets were published with restricted access. Requests for access will be checked by the professor of the Neurology Department against the conditions for sharing the data as described in the signed Informed Consent. Data will be archived for 15 years in the Radboud Data Repository after termination of the study.

A3. About the author

Esther Janssen was born on the 2nd of October, 1995, in Woerden in the Netherlands. She grew up in Montfoort, a small village near Utrecht. After graduating high school in 2013, she started the Bachelor Psychobiology at the University of Amsterdam and moved to Amsterdam.

After completing this, she started with the Master Psychopharmacology and Pathophysiology, a track of the Biomedical Sciences – Neurobiology program also at the University of Amsterdam. During this two year Master program she conducted a research internship at the Department of Anatomy of the Radboudumc in Nijmegen. Supervised by Amanda Kiliaan and Maximilan Wiesmann, she studied abnormalities in post-mortem tissue of patients with hypertension. To learn more about this topic, she conducted her second Master's research project in Edinburgh, supervised by Joanna Wardlaw.

After obtaining her Master's degree in 2019, she started working as a research assistant at the Department of Psychiatry of the Vrije Universiteit Amsterdam. She returned to Nijmegen and started her PhD supervised by Frank-Erik de Leeuw at the Radboudumc in 2021. She currently lives in Nijmegen.

A4. List of publications

Publications in this thesis

1. **Janssen E.,** ter Telgte A., Verburgt E., de Jong J.J.A., Marques J.P., Kessels R.P.C., Backes W.H., Maas M.C., Meijer F.J.A., Deinum J., Riksen N.P., Tuladhar A.M., de Leeuw F.E. The Hyperintense study: Assessing the effects of induced blood pressure increase and decrease on MRI markers of cerebral small vessel disease: Study rationale and protocol. *Eur Stroke J.* 2022 Sep 1;7(3):331–338.
2. Snijders M.H.*, **Janssen E.*,** Verburgt E., ter Telgte A., van den Berg T.N.A., Maas M.C., Meijer F.J.A., Tuladhar A.M., Riksen N.P., Deinum J., de Leeuw F.E.
3. On the origin of cerebral small vessel disease: MRI markers of cSVD in young adults with hypertension. *Cereb Circ Cogn Behav.* 2025;9:100397.
4. **Janssen E.,** de Jong J.J.A., Verburgt E., ter Telgte A., van den Berg T.N.A., Marques J.P., Maas M.C., Meijer F.J.A., Tuladhar A.M., Riksen N.P., Deinum J., Backes W.H., de Leeuw F.E. Higher blood-brain barrier permeability in middle-aged adults with hypertension. *Hypertension.* 2025 Dec;82(12):2172–2182.
5. **Janssen E.,** van Dalen J.W., Cai M., Jacob M.A., Marques J., Duering M., Richard E., Tuladhar A.M., de Leeuw F.E., Hilkens N.A. Visit-to-visit blood pressure variability and progression of white matter hyperintensities over 14 years. *Blood Press.* 2024 Dec;33(1):2314498.

Articles submitted in this thesis

1. **Janssen, E.,** de Jong, J. J.A., Verburgt, E., ter Telgte, A., van den Berg, D., Maas, M.C., Meijer, F.J.A., Tuladhar, A.M., Deinum, J., Riksen, N.P., Backes, W.H., de Leeuw, F. E. Blood-Brain Barrier disruption in response to blood pressure changes in adults with hypertension: a serial MRI study.
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Publications not in this thesis

1. Hamilton O., Backhouse E., **Janssen E.,** Jochems A., Maher C., Ritakari T., Stevenson A., Xia L., Deary I., Wardlaw J. Cognitive impairment in sporadic cerebral small vessel disease: A systematic review and meta-analysis. *Alzheimers Dement.* 2020 Nov 13;17:665–685.
2. Blair G.W., **Janssen E.,** Stringer M.S., Thrippleton M.J., Chappell F., Shi Y., Hamilton I., Flaherty K., Appleton J.P., Doubal F.N., Wardlaw J.M. Effects of cilostazol and

isosorbide mononitrate on cerebral hemodynamics in the LACI-1 randomized controlled trial. *Stroke.* 2022;53:29–33.

3. Verburgt E.*, **Janssen E.*,** Jacob M.A., Cai M., ter Telgte A., Wiegertjes K., Kessels R.P.C., Norris D.G., Marques J.P., Duering M., Tuladhar A.M., de Leeuw F.E. Role of small acute hyperintense lesions in long-term progression of cerebral small vessel disease and clinical outcome: A 14-year follow-up study. *J Neurol Neurosurg Psychiatry.* 2023;94(2):144–150.
4. Rudilosso S., Stringer M.S., Thrippleton M., Chappell F., Blair G.W., Jaime Garcia D., Doubal F., Hamilton I., **Janssen E.,** Kopczak A., Ingrisch M., Kerkhofs D., Backes W.H., Staals J., Duering M., Dichgans M., Wardlaw J.M. Blood-brain barrier leakage hotspots collocating with brain lesions due to sporadic and monogenic small vessel disease. *J Cereb Blood Flow Metab.* 2023;43(9):1490–1502.
5. **Janssen E.,** de Leeuw F.E. Getting to the Heart of the Heart-Brain Connection: Association or Causation? *Neurology.* 2023 Jul 11;101(2):57–58.
6. Solé-Guardia G., Luijten M., **Janssen E.,** Visch R., Geenen B., Küsters B., Claassen J.A.H.R., Litjens G., de Leeuw F.-E., Wiesmann M., Kiliaan A.J. Deep learning-based segmentation in MRI-(immuno)histological examination of myelin and axonal damage in normal-appearing white matter and white matter hyperintensities. *Brain Pathol.* 2025 Mar;35(2):e13301
7. Stringer M.S., Blair G.W., Kopczak A., Kerkhofs D., Thrippleton M.J., Chappell F.M., Maniega S.M., Brown R., Shuler K., Hamilton I., Garcia D.J., Doubal F.N., Clancy U., Sakka E., Poliakova T., **Janssen E.,** Duering M., Ingrisch M., Staals J., Backes W.H., van Oostenbrugge R.J., Biessels G.J., Dichgans M., Wardlaw J.M. Cerebrovascular function in sporadic and genetic cerebral small vessel disease. *Ann Neurol.* 2025 Mar;97(3):483–498.
8. Voorter P.H.M., Stringer M.S., van Dinther M., Kerkhofs D., Dewenter A., Blair G.W., Thrippleton M.J., Jaime Garcia D., Chappell F.M., **Janssen E.,** Kopczak A., Staals J., Ingrisch M., Duering M., Doubal F.N., Dichgans M., van Oostenbrugge R.J., Jansen J.F.A., Wardlaw J.M., Backes W.H. Heterogeneity and penumbra of white matter hyperintensities in small vessel diseases determined by quantitative MRI. *Stroke.* 2025 Jan;56(1):128–137.
9. **Janssen E.,** de Leeuw F.E. Progression in the understanding of cerebral small vessel disease regression. *Neurology.* 2025 Feb 25;104(4):e213391.
10. van Lith T.J.*, **Janssen E.*,** van Dalen J-W., Li H., Koeneman M., Sluis W.M., Wijers N.T., Wermer M.J.H., Huisman M.V., van der Worp H.B., Meijer F.J.A., Tuladhar A.M., Bredie S.J.H., de Leeuw F.-E.Higher blood pressure variability during hospitalisation is associated with lower cerebral white matter integrity in COVID-19 patients. *Blood Press.* 2025 Dec;34(1):2493828.

*Shared first authorship

A5. PhD portfolio

Activity	Organizer	Year	ECTS*
Basiscursus Regelgeving en Organisatie voor Klinische onderzoekers (BROK) certificate	Radboudumc	2021	1.5
Writing Scientific Articles	Radboud University	2021	3.4
Toolkit Advanced (f)MRI	Donders Institute	2022	2
Design and Illustration	Radboud University	2022	0.9
Projectmanagement for PhD candidates	Radboud University	2022	1.9
Conference Lyon, France	ESO**	2022	2.5
Statistics for clinical researchers	Radboudumc	2023	2
Analysing longitudinal and multilevel data using R	Radboudumc	2023	3.4
Conference Munich, Germany	ESO**	2023	2.5
Career development for PhD candidates & postdocs – the next step in my career	Radboudumc	2023	0.7
Scientific integrity	Radboudumc	2024	0.7
Conference Basel, Switzerland	ESO**	2024	2.5
Edinburgh Stroke Research Workshop	ESO**	2024	2.5
Conference Helsinki, Finland	ESO**	2025	2.5

*1 ECTS (European Credit Transfer System) equals a workload of 28 hours

** European Stroke organization

A6. Donders Graduate School for Cognitive Neuroscience

For a successful research Institute, it is vital to train the next generation of scientists. To achieve this goal, the Donders Institute for Brain, Cognition and Behaviour established the Donders Graduate School in 2009. The mission of the Donders Graduate School is to guide our graduates to become skilled academics who are equipped for a wide range of professions. To achieve this, we do our utmost to ensure that our PhD candidates receive support and supervision of the highest quality.

Since 2009, the Donders Graduate School has grown into a vibrant community of highly talented national and international PhD candidates, with over 500 PhD candidates enrolled. Their backgrounds cover a wide range of disciplines, from physics to psychology, medicine to psycholinguistics, and biology to artificial intelligence. Similarly, their interdisciplinary research covers genetic, molecular, and cellular processes at one end and computational, system-level neuroscience with cognitive and behavioural analysis at the other end. We ask all PhD candidates within the Donders Graduate School to publish their PhD thesis in de Donders Thesis Series. This series currently includes over 750 PhD theses from our PhD graduates and thereby provides a comprehensive overview of the diverse types of research performed at the Donders Institute. A complete overview of the Donders Thesis Series can be found on our website: https://www.ru.nl/donders/donders-series

The Donders Graduate School tracks the careers of our PhD graduates carefully. In general, the PhD graduates end up at high-quality positions in different sectors, for a complete overview see https://www.ru.nl/donders/destination-our-former-phd. A large proportion of our PhD alumni continue in academia (>50%). Most of them first work as a postdoc before growing into more senior research positions. They work at top institutes worldwide, such as University of Oxford, University of Cambridge, Stanford University, Princeton University, UCL London, MPI Leipzig, Karolinska Institute, UC Berkeley, EPFL Lausanne, and many others. In addition, a large group of PhD graduates continue in clinical positions, sometimes combining it with academic research. Clinical positions can be divided into medical doctors, for instance, in genetics, geriatrics, psychiatry, or neurology, and in psychologists, for instance as healthcare psychologist, clinical neuropsychologist, or clinical psychologist. Furthermore, there are PhD graduates who continue to work as researchers outside academia, for instance at non-profit or government organizations, or in pharmaceutical companies. There are also PhD graduates

who work in education, such as teachers in high school, or as lecturers in higher education. Others continue in a wide range of positions, such as policy advisors, project managers, consultants, data scientists, web- or software developers, business owners, regulatory affairs specialists, engineers, managers, or IT architects. As such, the career paths of Donders PhD graduates span a broad range of sectors and professions, but the common factor is that they almost all have become successful professionals.

For more information on the Donders Graduate School, as well as past and upcoming defences please visit:

http://www.ru.nl/donders/graduate-school/phd/

A7. Dissertations of the Cerebrovascular Research Program

- Liselore Snaphaan. Epidemiology of post stroke behavioral consequences. Radboud University Nijmegen, 12 March 2010
- Karlijn F. de Laat. Motor performance in individuals with cerebral small vessel disease: an MRI study. Radboud University Nijmegen, 29 November 2011
- Anouk G.W. van Norden. Cognitive function in elderly individuals with cerebral small vessel disease. An MRI study. Radboud University Nijmegen, 30 November 2011
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