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Neurociências Clínicas, Neuropsiquiatria e Saúde Mental – ramo clínico

BioRiMiCH: Novos Biomarcadores pré-sintomáticos de Risco para doença Microvascular Cerebral na Hipertensão

BioRiMiCH: novel pre-symptomatic Biomarkers of Risk in Microvascular Cerebral disease in arterial Hypertension

Ana Maria Gonçalves Monteiro

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2024

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DISSERTAÇÃO DE CANDIDATURA AO GRAU DE DOUTOR SUBMETIDA À FACULDADE DE MEDICINA DA UNIVERSIDADE DO PORTO

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DISSERTAÇÃO DE CANDIDATURA AO GRAU DE DOUTOR SUBMETIDA À FACULDADE DE MEDICINA DA UNIVERSIDADE DO PORTO

Esta tese foi elaborada de acordo com os Critérios Curriculares desta Faculdade para Recrutamento na Carreira Académica e Admissão e Classificação a Provas Académicas, em vigor a partir de 21 de julho de 2021, nomeadamente no vertido no ponto III.2. a respeito dos Critérios de Exigência Mínima para Submissão de Pedido de Admissão a Provas de Tese de Doutoramento.

Decorrendo do predito enquadramento, apresenta-se uma tese estruturada pela compilação de trabalhos de investigação, na forma de artigos originais, em que a candidata é 1ª autora e que foram publicados ou aceites para publicação em revistas indexadas com fator de impacto.

Fazem parte desta tese as seguintes publicações:

I – Monteiro A, Castro P, Pereira G, Ferreira C, Sorond F, Milstead A, Higgins JP, Polónia J and Azevedo E (2021) Neurovascular coupling is impaired in hypertensive and diabetic subjects without symptomatic cerebrovascular disease. *Front. Aging Neurosci* 2021; 13:728007. doi: 10.3389/fnagi.2021.728007.

II – Monteiro A, Castro P, Pereira G, Ferreira C, Duque C, Sorond F, Milstead A, Higgins JP, Polónia J and Azevedo E. Could salt intake directly affect the cerebral microvasculature in hypertension? *J Stroke Cerebrovasc Dis* 2022; 31(9):106632. doi: 10.1016/j.jstrokecerebrovasdis.2022.106632.

III – Monteiro A, Castro P, Pereira G, Ferreira C, Polónia J, Lobo M and Azevedo E. Cerebral blood flow regulation and cognitive performance in hypertension. J Cereb Blood Flow Metab. 2024 (aceite para publicação).

Em cumprimento com o disposto no Art.º 8º do Decreto-Lei n.º 388/70, declaro que participei ativamente na conceção dos objetivos de todos os trabalhos que constituem esta tese. Fui responsável pela operacionalização do trabalho e pela recolha e armazenamento dos dados. Fui responsável pela análise e interpretação dos resultados e pela redação da versão inicial dos manuscritos. Participei ativamente na revisão e aprovação das versões finais dos manuscritos e fui responsável pela sua submissão para publicação.

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Ao meu marido, a minha melhor metade, pelo seu apoio incansável, paciência infinita e amor absoluto. A sua inabalável confiança em mim é minha maior motivação, espero ser sempre um motivo de orgulho.

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“Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less.” — Marie Curie

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LIST OF ABBREVIATIONS

Aβ: amyloid-β
ABPM: ambulatory blood pressure monitoring
ACEi: angiotensin-converting enzyme
ACM: artéria cerebral media
ACP: artéria cerebral posterior
ACR: albumin-to-creatinine ratio
AD: Alzheimer´s disease
AGEs: advanced glycation end-products
ALS: arterial spin labeling
Ang II: angiotensin II
ANS: autonomic nervous system
ANV: acoplamento neurovascular
ARB: angiotensin receptor blockers
AVLT: Auditory Verbal Learning Test
BBB: blood-brain barrier
BOLD: blood-oxygenation-level-dependent
BP: blood pressure
CA: cerebral autoregulation
CAA: cerebral amyloid angiopathy
CADASIL: cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CAN: cardiovascular autonomic neuropathy
CBF: cerebral blood flow
CBFV: cerebral blood flow velocity
CBV: cerebral blood volume
CKD: chronic kidney disease
CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration
CMB: cerebral microbleeds
CMI: cortical microinfarcts
CSF: cerebrospinal fluid
CSVD: cerebral small vessel disease
DAN: dorsal attention network
dAC: autorregulação cerebral dinamica
dCA: dynamic cerebral autoregulation
DM: diabetes mellitus
DMN: default mode network
DR: diabetic retinopathy
DTC : Doppler transcraniano
DTI: diffusion tensor imaging
eGFR: estimated glomerular filtration rate
EPVS: enlarged perivascular spaces
$ETCO_2$: end-tidal carbon dioxide
FA: fractional anisotropy
fMRI: functional magnetic resonance imaging
FLAIR: fluid-attenuated inversion recovery image
HbA1c: hemoglobin A1c
HD: hemodialysis
HR: heart rate
HS: high salt
HT: hypertension/hypertensive
IL: interleukin
LF: low frequency
LS: low salt
MAP: mean arterial pressure
MCA: middle cerebral artery
MCI: mild cognitive impairment
MD: mean diffusivity
MMSE: Mini Mental State Examination
MoCA: Montreal Cognitive Assessment
MRI: magnetic resonance imaging
Na^+: sodium
NADPH: nicotinamide adenine dinucleotide phosphate
NAWM: normal-appearing white matter
NBT: n-back test
nDM: non-diabetes mellitus
NO: nitric oxide
NOS: nitric oxide synthase
NGVU: neuro-glial-vascular unit
NVC: neurovascular coupling
NVU: neurovascular unit
$PaCO_2$: partial pressure of carbon dioxide
PASA : posterior-to-anterior shift with aging
PCA: posterior cerebral artery
PET: positron emission tomography
PFC: prefrontal cortex
PVS: perivascular spaces
rCBF: regional cerebral blood flow
RCT: randomized controlled trials
ROS: reactive oxygen species
RSSI: recent small subcortical infarcts
SSBP: salt sensitivity of BP
SVAN: salience-ventral attention network
TCD: transcranial Doppler
TFA: transfer function analysis
VCI: vascular cognitive impairment
VCS: vascular comorbidity score
VD: vascular dementia
VLF: very low frequency
$VRCO_2$: Vasoreactivity to CO_2/ vasorreactividade ao CO_2
VRF: vascular risk factors
WM: white matter
WMH: white matter hyperintensity

ABSTRACT

Background

Cerebral small vessel disease (CSVD) is a major contributor to cognitive decline and stroke, with hypertension (HT) as the most significant risk factor. Despite its clinical importance, reliable biomarkers for early detection and monitoring of CSVD remain elusive, and studies on cerebral microvascular dysfunction in HT are scarce.

Objectives

This work aimed to investigate the utility of studying cerebral blood flow regulation using the transcranial Doppler (TCD), a non-invasive tool, as a biomarker for CSVD in HT patients. The specific objectives were to assess cerebrovascular regulation through dynamic cerebral autoregulation (dCA), cerebral vasoreactivity to CO_2 ($VRCO_2$), and neurovascular coupling (NVC), examining variations in these parameters between HT subjects and healthy controls, as well as the influence of comorbid diabetes mellitus (DM). Furthermore, we aimed to explore the role of renal function and dietary salt consumption as independent predictors of microvascular dysfunction in HT patients. Finally, we wanted to investigate the associations between the TCD parameters and cognitive performance in HT patients and study their relation to imaging markers of CSVD.

Methods and results

We conducted a cross-sectional study in a cohort of 56 HT patients asymptomatic for CSVD, approximately half with comorbid diabetes. This work comprised three papers utilizing the TCD to assess microvascular function. The right middle (MCA) and left posterior (PCA) cerebral arteries were monitored simultaneously to evaluate dCA, $VRCO_2$, and NVC. Neuropsychological evaluation and 1.5 T MRI were performed to explore associations between cerebral blood flow regulation and CSVD markers.

The first study examined TCD metrics in HT patients, with and without DM, in comparison with data from healthy controls and revealed that the NVC is impaired in the PCA of HT patients, particularly the natural frequency parameter, indicating increased rigidity of the cerebral resistance vessels. The impairment was worse with comorbid DM and was independent of MRI markers of CSVD.

The second paper focused on the relationship between renal function, dietary salt consumption, and TCD studies. Notably, dietary salt consumption emerged as an independent predictor of microvascular dysfunction in HT, with the natural frequency parameter in the

PCA territory again showing promise as a surrogate marker for microvascular dysfunction in HT.

The third paper investigated whether the TCD metrics are related to cognitive performance in HT patients and explored them as tools for predicting cognitive decline. We found that enhanced dCA in the MCA predicted poorer memory performance, while, unexpectedly, reduced NVC in the PCA predicted both superior processing speed and memory performance. These findings suggest HT-induced hemodynamic and neural network modifications, which may represent adaptive responses potentially contributing to the preservation of cognitive performance in frontal brain regions or impaired stimulus processing with loss of neurovascular efficiency.

Conclusion

This work underscores the potential of the TCD as a valuable tool for detecting and monitoring CSVD in HT. The NVC parameter natural frequency emerged as particularly sensitive for detecting asymptomatic microvascular dysfunction in the posterior territory. Furthermore, our findings propose an independent predictive role of dietary salt consumption, shedding light on novel avenues for CSVD research and therapeutic interventions. The associations between microvascular hemodynamics and cognitive parameters underline the intricate interplay between cerebrovascular function and cognition and offer an interesting approach for future work focusing on HT-induced cognitive decline.

Overall, this research project led to new findings in CSVD in the context of HT, providing a foundation for future research and clinical applications.

Keywords: Cerebral small vessel disease; Hypertension; Diabetes mellitus; Transcranial Doppler monitoring; Dynamic cerebral autoregulation; Cerebral vasoreactivity to CO_2; Neurovascular coupling; Daily salt intake; Vascular cognitive impairment; Microvascular dysfunction; Natural frequency; Biomarkers.

RESUMO

Introdução

A doença de pequenos vasos cerebrais (DPVC) representa uma das principais causas de declínio cognitivo e acidente vascular cerebral, com a hipertensão (HT) identificada como o fator de risco mais preponderante. Apesar da sua relevância clínica, a deteção precoce e a monitorização da DPVC carecem de biomarcadores confiáveis, e são notavelmente escassos os estudos sobre a disfunção microvascular cerebral na HT.

Objetivos

Este projeto teve como objetivo central investigar a utilidade da avaliação da regulação do fluxo sanguíneo cerebral por Doppler transcraniano (DTC), uma ferramenta não invasiva, enquanto biomarcador para DPVC em indivíduos com HT. Os objetivos específicos compreenderam a avaliação da regulação cerebrovascular através da autorregulação cerebral dinâmica (dAC), da vasorreatividade cerebral ao CO_2 ($VRCO_2$) e do acoplamento neurovascular (ANV). Investigaram-se as disparidades nestes parâmetros entre indivíduos com HT e indivíduos saudáveis, considerando também a influência da diabetes mellitus (DM) comórbida. Além disso, explorou-se o papel da função renal e do consumo de sal na dieta como preditores independentes da disfunção microvascular em pacientes com hipertensão. Investigou-se, ainda, as associações entre parâmetros de hemodinâmica cerebrovascular, desempenho cognitivo e marcadores imagiológicos da DPVC nestes doentes.

Métodos e resultados

O presente estudo, de natureza transversal, foi conduzido numa coorte de 56 doentes hipertensos assintomáticos para DPVC, apresentado cerca de metade DM comórbida. O trabalho compreendeu três artigos utilizando o DTC para avaliar a função microvascular. As artérias cerebral média direita (ACM) e posterior esquerda (ACP) foram monitorizadas simultaneamente para a avaliação da dAC, $VRCO_2$ e ANV. Os doentes realizaram também avaliação neuropsicológica e ressonância magnética de 1,5 T para investigar associações entre a hemodinâmica cerebrovascular e os marcadores de DPVC.

O primeiro estudo examinou métricas de DTC em hipertensos com e sem DM, comparando com dados de controlos saudáveis, e revelou comprometimento significativo do ANV na ACP nos hipertensos, particularmente no parâmetro frequência natural, indicando aumento da rigidez dos vasos de resistência cerebrais. Este comprometimento foi mais acentuado nos hipertensos com DM e foi independente da presença de marcadores imagiológicos de DPVC.

O segundo artigo centrou-se na relação entre a função renal e o consumo dietético de sal e as métricas de DTC. Notavelmente, o consumo de sal emergiu como um preditor independente da disfunção microvascular na HT, e o parâmetro frequência natural no território da ACP demonstrou novamente potencial como marcador para disfunção microvascular nesta doença.

O terceiro artigo analisou a relação entre os parâmetros de DTC e o desempenho cognitivo de doentes com HT, para as explorar como ferramentas preditivas de declínio cognitivo. Observou-se que melhor dAC na ACM previu pior desempenho na memória, enquanto, inesperadamente, menor ANV na ACP previu melhor velocidade de processamento e memória. Esses resultados sugerem possíveis modificações hemodinâmicas e de rede neurais na HT, podendo representar alterações adaptativas, potencialmente contribuindo para a preservação do desempenho cognitivo em regiões frontais, ou pior processamento de estímulos, com perda de eficiência neurovascular.

Conclusão

Este trabalho realça o potencial do DTC como ferramenta útil para a deteção e monitorização do desenvolvimento de DPVC na HT. O parâmetro frequência natural emergiu como particularmente sensível na identificação de disfunção microvascular assintomática no território vascular posterior. Para além disso, os resultados apontam o consumo de sal dietético como um preditor independente de lesão microvascular cerebral, sugerindo novas direções de investigação e intervenções terapêuticas na DPVC. As associações entre hemodinâmica microvascular e parâmetros cognitivos enfatizam a complexa interação entre a função cerebrovascular e a cognição, oferecendo uma hipótese interessante para investigação futura no declínio cognitivo associado à HT.

Em suma, este projeto contribuiu com novos achados no contexto da DPVC na HT, fornecendo uma base para investigação e aplicações clínicas futuras.

Palavras-chave: Doença de pequenos vasos cerebrais; Hipertensão; Diabetes mellitus; Doppler transcraniano funcional; Autorregulação cerebral dinâmica; Vasorreatividade cerebral ao CO_2; Acoplamento neurovascular; Consumo de sal diário; Defeito cognitivo vascular; Disfunção microvascular; Frequência natural; Biomarcadores.

CHAPTER I - BACKGROUND

CEREBRAL SMALL VESSEL DISEASE

Cerebral vascular disease is a major global health concern as the leading cause of death and morbidity.[1] In Portugal, it ranks as the primary cause of years of life lost, with inestimable economic and social consequences.[2] Research suggests cerebral small vessel disease (CSVD) is the most prevalent pathological neurological process.[3] However, CSVD should not be regarded as a benign condition. It is a significant cause of stroke and profoundly affects cognitive function. Additionally, it is the leading cause of functional decline among older adults, causing gait impairment, falls, and neuropsychiatric symptoms, significantly impacting the quality of life and increasing morbidity and mortality.[4-9]

Despite its prevalence, CSVD is still poorly understood due to the inherent challenges in directly accessing small brain vessels, and pathology goes unnoticed until the damage has already occurred. There is an urgent need for improved risk biomarkers to aid in this condition's early diagnosis and management.

Anatomy of the cerebral small vessel network

Cerebral small vessels stem from medium-sized arteries from the superficial subarachnoid circulation. Additionally, at the base of the brain, arterial perforators arise directly from the circle of Willis and its proximal branches (Figure 1).[10,11] While pial arteries form a highly anastomotic network, the deep penetrating vessels have very few collaterals, and the occlusion of a single vessel is sufficient to cause an ischemic lesion. The cortical and the basal systems merge in the deepest areas of the subcortical white matter (WM) in what is known as watershed territories. These regions are particularly vulnerable to hemodynamic insufficiency due to low perfusion pressure.[12]

Pial arterioles are densely innervated by autonomic and sensory fibers originating from peripheral autonomic and sensory ganglia, which run at the adventitia-media border.[13,14] Their role is not entirely understood, as inactivation of autonomic innervation does not affect the magnitude of somatosensory evoked vasodilatation, but they may protect the blood vessels from excessive vasodilation during extreme hemodynamic conditions, such as in acute hypertension.[15,16] Perivascular innervation has been implicated in generating resting-state functional magnetic resonance imaging (fMRI) connectivity signals in surgically disconnected brain regions, suggesting a role in synchronizing hemodynamic signals across the brain.[16] Furthermore, they may also contribute to the integrity of the vascular wall.[17] The autonomic nervous system (ANS) may also have a role in the relative distribution of the cerebral blood flow (CBF) to areas with different degrees of neuronal activity (functional hyperemia).[18]

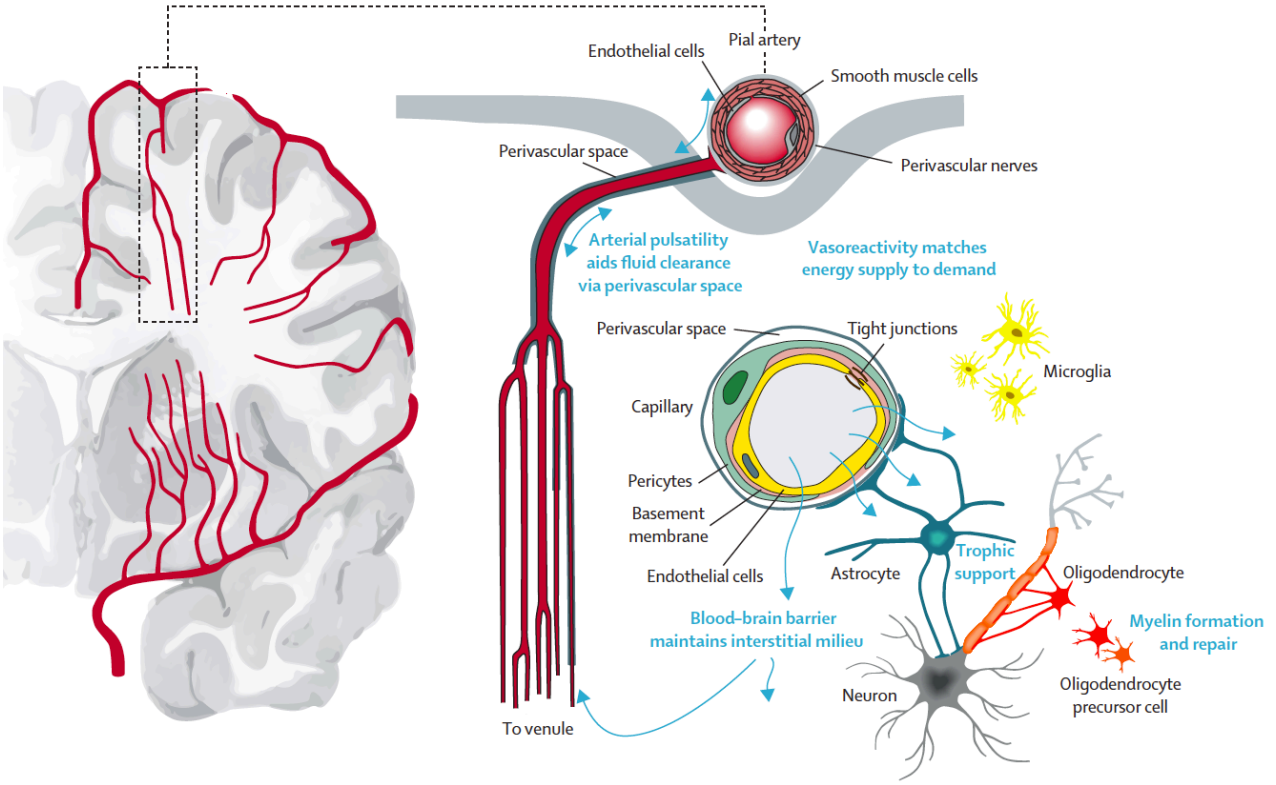

Figure 1. Cerebral small vessel anatomy and structure.

Arterioles penetrate the brain and branch into capillaries. They are each surrounded by a perivascular space connected to the cerebrospinal fluid. Capillary endothelial cells, including their connecting tight junctions, a specialized basement membrane, pericytes, and astrocyte endfeet collectively form the blood-brain barrier (BBB). The BBB is essential for maintaining the interstitial milieu. Astrocytes maintain the interstitial fluid balance, provide energy to neurons, and relay signals between neurons and other cells to the vasculature to adapt blood flow to energy demand. Oligodendrocytes form and repair myelin around axons and provide metabolic and trophic support. Reproduced with permission. Source: Small vessel disease: mechanisms and clinical implications. By Wardlaw JM, el al. Lancet Neurol. 2019; 18(7): 684-96. Copyright © 2019 Elsevier Ltd.

Penetrating vessels are surrounded by perivascular spaces (PVS), which are part of the glymphatic system. In rodents, cerebrospinal fluid (CSF) from the basal cisterns and cerebral convexities enters the perivascular spaces and flushes the interstitium.[19] This perivascular compartment has gained much interest as a major pathway for removing toxic byproducts of brain activity and as a housing space for innate immune cells involved in the brain's immune homeostasis.[16]

As the arterioles penetrate the parenchyma, they gradually lose the multiple layers of smooth muscle cells and adventitia, and the perivascular nerves become sparser. As they run deeper into the brain, the glial and the vascular basement membranes become fused, obliterating the perivascular space, and astrocytic endfeet encase the arterioles.[14,16,20,21]

At the capillary level, the vascular endothelium is enveloped by a specialised basal membrane, and one-third of its surface is covered by pericytes, which replace the smooth muscle cells.[16] These two cell types are also surrounded by astrocytic endfeet, and together

they form the blood-brain barrier (BBB). At this level, endothelial cells exhibit a low rate of vesicular transport, increased expression of tight junction molecules that exclude free transport of substances over 400 kDa, and a vast repertoire of bidirectional molecular transporters regulating the molecular exchange between the blood and the brain.[12,22] In the past decades, the focus of the metabolic regulatory capacity was the BBB, but more recently, a broader functional structure has been recognized: the neuro-glial-vascular unit.[23]

The neuro-glial-vascular unit (NGVU)

The interaction between the brain and the vasculature is remarkably complex. The brain is a highly metabolically demanding organ requiring high energy levels, and the coordination between the blood supply and the demand is paramount to avoid tissue damage.[24] To meet the high metabolic demand of the brain, cerebral autoregulation (CA) ensures a constant CBF despite changes in arterial pressure and increased neuronal activity leads to functional hyperemia in a process called neurovascular coupling (NVC).[21]

The concept of a neurovascular unit (NVU) emerged to emphasize the complex and unique anatomical and functional relationship between the brain tissues and the vasculature.[25] Rather than distinct entities, endothelial cells, neurons, pericytes, astrocytes, oligodendrocytes, and other glial cells are organized and interconnected into millions of functional units – neuro-glial-vascular units – that manage blood supply, regulate fluid and nutrient entry into the interstitium, maintain and repair myelin and remove waste products (Figure 2).[23] It is widely believed that the NGVU plays a key role in the resilience to acute insults and the chronic pathological processes leading to neurodegeneration and dementia. Understanding these relationships is essential for better recognizing the earliest stages of microvascular dysfunction and the mechanisms leading to the different manifestations. The NGVU has been recently recognized as an important biomarker and possible therapeutic target for cerebrovascular disease.[26]

Neurons are the driving agents initiating increases in CBF during functional hyperemia. It has been long known that sustained increases in neural activity leads to increases in local vascular density, and later studies demonstrated that neural activity evokes CBF increases highly restricted to the activated network.[16] In addition to the metabolically driven vascular response induced by excitatory neurons, recent studies suggest a role of interneurons in neurovascular coupling, a neurogenically driven vascular response.[27-29]

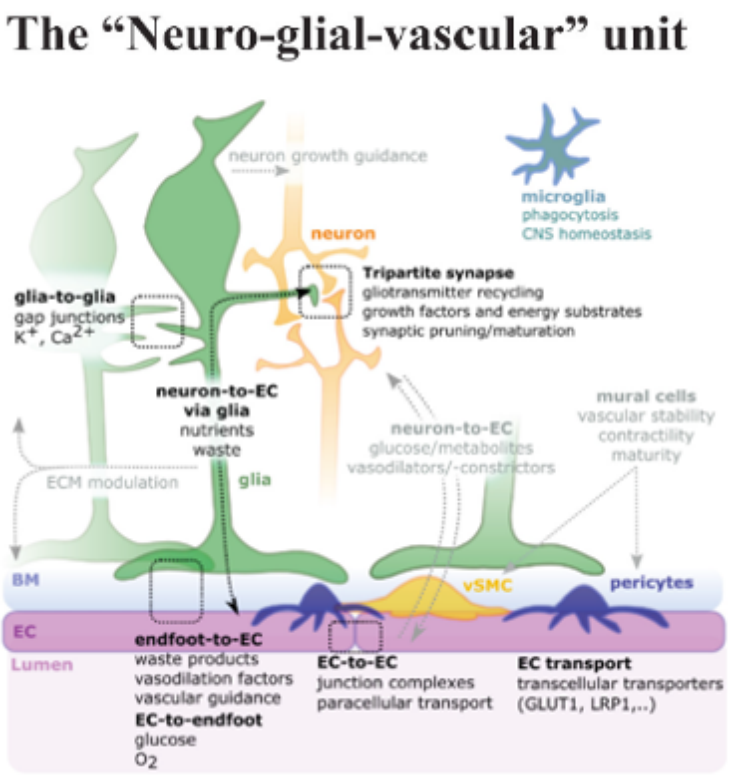

Figure 2. Schematic of the neuro-glial-vascular-unit (NGVU) in health and disease.

The NGVU is a hetero-cellular complex formed by neurons, glia, vascular smooth muscle cells, pericytes, microglia, and endothelial cells (EC). For NGVU functionality, various direct (i.e., glia-to-glia, tripartite synapse, endfoot-to-EC, EC-to-endfoot) and indirect (i.e., neuron-to-EC via glia, neuron-to-EC via mural cells, microglia) pathways need to be considered. Adapted from Kugler EC, et al.[22] Reproduced with permission. Source: The "Neuro-Glial-Vascular" Unit: The Role of Glia in Neurovascular Unit Formation and Dysfunction. By Kugler EC, et al. Front. Cell Dev. Biol. 2021; 9:732820. Copyright © 2021 Kugler.

Endothelial cells are known to regulate CBF in response to chemical and mechanical signals.[30,31] During cortical activation, signals generated by neuronal activity are retrogradely conveyed to upstream arterioles remote from the activation area to coordinate blood flow adjustments efficiently.[32,33] Besides their role in the BBB, recent evidence suggests a crucial role of the endothelium in relaying activity-induced neurovascular signals to upstream vessels.[34,35]

Astrocytes physically connect the endothelium and the neurons. With this close interaction, nutrients are delivered, and waste products are passed to the microglia or bloodstream. Since neurons have no glucose storage and rely on oxidative metabolism, the glucose stored in astrocytes renders them central players in maintaining the high neuronal metabolic needs.[22] Additionally, the astrocytic endfeet membranes are enriched in aquaporin-4 water channel proteins, which regulate fluid flow through the interstitial space.[23] Aquaporin-4 molecules might relocate to the outer side of astrocytic endfeet in areas of damage, as seen in white matter hyperintensities.[19] Astrocytes also contribute to the

functionality of the BBB, modulate neurotransmission, and impact neurogenesis.[22] More recently, astrocytes have been shown to participate in neurovascular coupling and in regulating basal CBF and resting neuronal activity (vascular-neuronal coupling).[36,37]

Pericytes provide vascular structural support, contribute to the maintenance of the BBB, and influence vessel diameter by responding to vasoactive signals.[16] They seem vital in sensing neuronal activity to regulate cerebral perfusion, contributing to functional hyperemia.[38,39]

Oligodendrocytes form myelin and support axons by supplying energy. When injured, they can be replaced by the maturation of precursor cells. Studies have shown that dysfunctional endothelial cells block oligodendrocyte precursor cell maturation, impairing myelination and repair.[40,41]

Pathophysiology of cerebral small vessel disease

Cerebral small vessel disease is a chronic, progressive condition and refers to any damage to the small vessels, 50-400 μm in diameter, supplying the WM and deep grey matter structures. It includes the small perforating arteries and arterioles, the capillaries, and probably venules and small veins, but this term is most often used to refer to arterial vessel pathology.[11,42]

The pathophysiologic mechanisms of CSVD are yet unclear. The European small brain vascular disease expert group classifies CSVD into subtypes based on the pathological characteristics: 1) arteriolosclerosis, 2) cerebral amyloid angiopathy (sporadic and hereditary), 3) inherited or genetic small vessel disease (other than cerebral amyloid angiopathy), 4) inflammatory/immunologically mediated small vessel disease, 5) venous collagenosis, 6) other small vessel diseases (e.g., post-radiation angiopathy and non-amyloid microvessel degeneration in Alzheimer's disease).[11]

Type 1, arteriolosclerosis, also known as age-related and vascular risk-factor-related small vessel disease, is the most prevalent form of CSVD and thus will be the focus of this work. The pathological hallmarks of type 1 CSVD are the loss of smooth muscle cells from the tunica media, deposits of fibro-hyaline material, narrowing of the lumen, and thickening of the vessel wall (lipohyalinosis), which may progress to fibrinoid necrosis and microaneurysm formation, resulting in vessel rupture and haemorrhage (intracerebral haemorrhage and microbleeds) (Figure 3).[43]

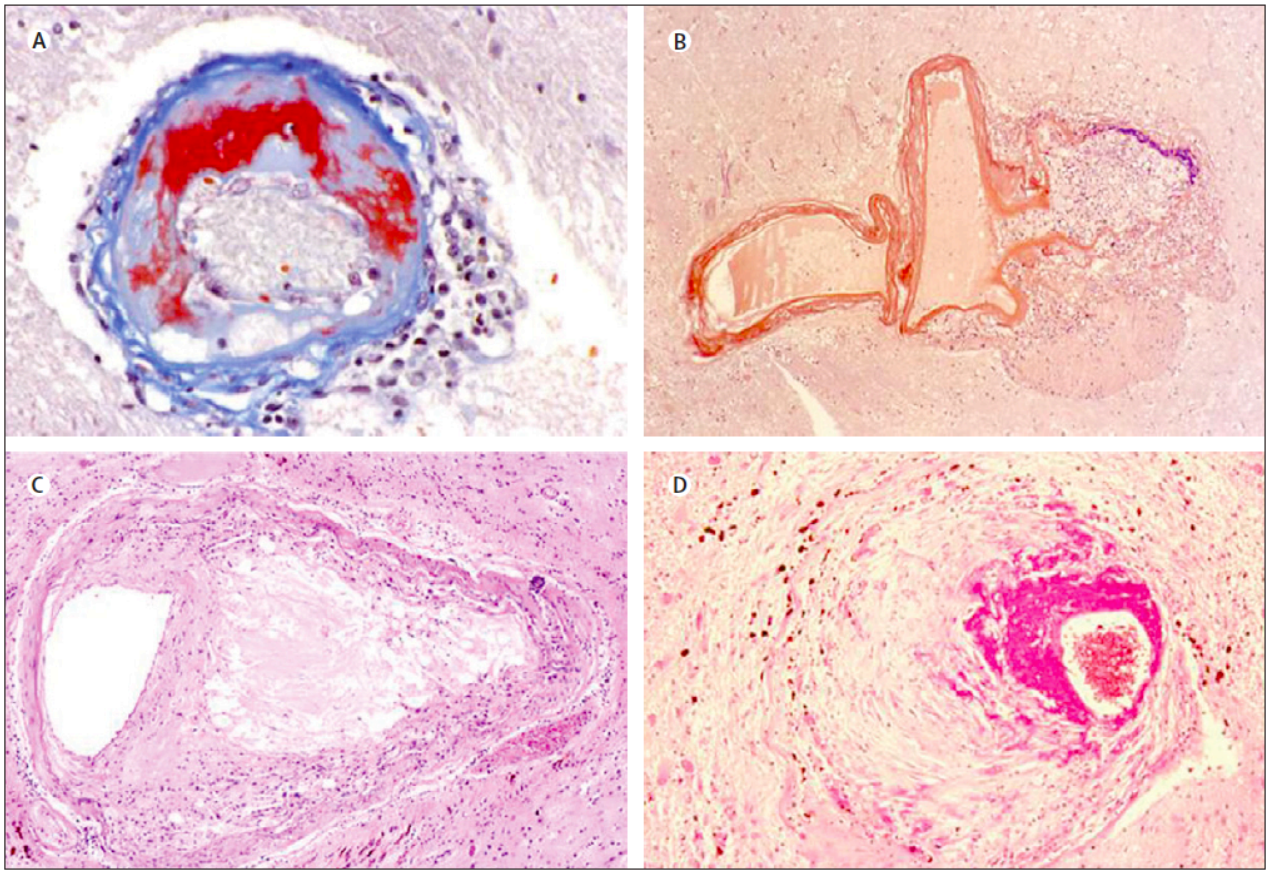

Figure 3. Pathological features of type 1 cerebral small vessel disease.

(A) Lipoyalinosis (basal ganglia ×100). (B) Microaneurysm in the right thalamus. Fibrinoid necrosis of the aneurysmal wall is almost ready to rupture. Phosphotungstic acid haematoxylin stain. Original magnification ×25. (C) Microatheroma (basal ganglia ×20). (D) Fibrinoid necrosis (pons ×20). Reproduced with permission. Source: Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. By Pantoni, L. Lancet Neurol 2010; 9:689–701 Copyright © 2010 Elsevier Ltd.

Although arteriolosclerosis is a marker of vessel injury and probably accelerates tissue damage, there is some debate on what initiates the microvascular abnormalities and how dysfunction of small vessels wall causes brain damage.[23] The mechanisms underlying hemorrhagic forms of CSVD are more evident if the vessel wall damage reaches the point of rupture, but the etiology and pathogenesis of some ischemic lesions are more hypothetical.

The sympathetic perivascular innervation exerts a trophic effect on the vascular wall, which is required for cerebrovascular hypertrophy. Elevated intravascular pressure leads to the release of growth factors and oxidative stress, and leads to reduced availability of nitric oxide (NO), which has antiproliferative properties (Figure 4).[44] In endothelial NO synthase (NOS) null-mice, hypertrophy persists even if ligation of the carotid artery prevents increased pressure, thus indicating that hypertrophy can occur without the mechanical stimulus on the vascular wall.[45]

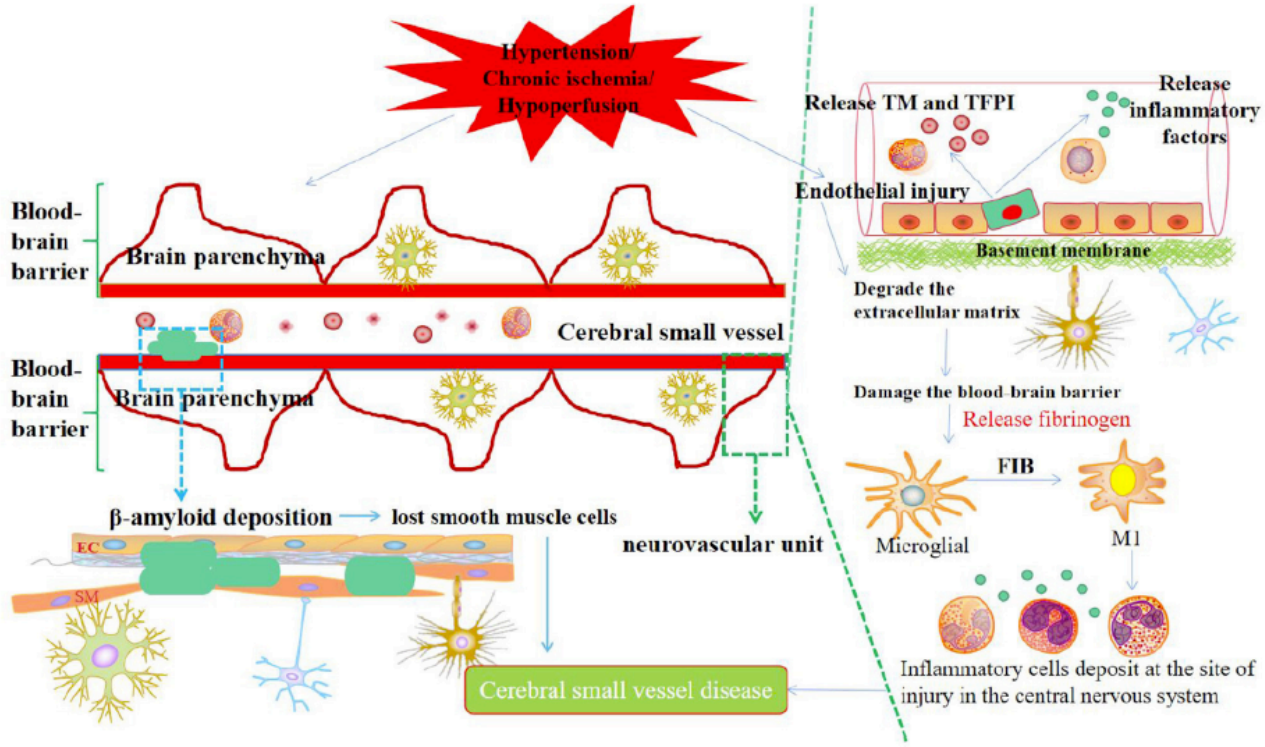

Figure 4. Pathological characteristics of cerebral small vessel disease.

Blood-brain barrier (BBB) dysfunction is a potential mechanism of early brain injury. The compromised BBB allows entry of potentially detrimental plasma components and immune cells into the brain parenchyma, further promoting BBB breakdown and white matter damage via neuroinflammation and direct neurotoxic effects. Fibrinogen leakage causes microglia activation, amyloid-ß plaque formation, and pericyte loss. Reproduced with permission. Source: Cerebral Small Vessel Disease: Neuroimaging Features, Biochemical Markers, Influencing Factors, Pathological Mechanism and Treatment. By Ren, B. et al. Front. Neurol. 2022; 13:843953. Copyright © The Authors 2022.

Blood-brain barrier/neuro-glial-vascular unit dysfunction

BBB dysfunction appears to be a critical step in CSVD. It is present in the hippocampus of patients with early cognitive dysfunction,[46] and in patients with stroke or CSVD features on neuroimaging, including in normal-appearing WM (NAWM).[23,47,48] Increased BBB permeability is associated with higher white matter hyperintensity (WMH) burden on MRI (a neuroimaging correlate of CSVD, see below) and cognitive decline,[49] and there was a significant correlation between functional outcome and increased BBB permeability in a longitudinal study with CSVD patients.[47] BBB damage leads to leakage of fluid, protein, and other plasma constituents, causing interstitial edema, contributing to the thickening of arteriole walls, and promoting inflammation.[23,49] Fibrinogen leakage causes microglia activation, peripheral macrophage recruitment, and blocking of oligodendrocyte precursor cell maturation. Furthermore, it binds to amyloid-ß, promoting plaque formation and pericyte loss, providing a possible mechanistic link between CSVD and Alzheimer´s disease (AD) development.[50] Pericyte degeneration is observed in several neurodegenerative disorders,

including AD and mild cognitive impairment (MCI).[39] Loss of pericytes has been demonstrated in aged hypertensive mice, with marked disruption of autoregulation.[51] Pericyte-deficient mice develop reduced capillary CBF responses to neuronal stimulus, resulting in neurovascular uncoupling in early stages, despite normal neuronal, endothelial, smooth muscle cell, and astrocyte-dependent mechanisms of flow regulation, suggesting that pericyte loss contributes to vascular dysregulation.[52]

Cerebral blood flow disruption

Compromised CBF is another expression of endothelial dysfunction, but the relationship between low CBF and CSVD development is complex. Vessel lumen restriction is thought to lead to a state of chronic hypoperfusion of the WM, eventually resulting in the degeneration of the myelinated fibers as a consequence of oligodendrocyte death.[11] Alternatively, acute occlusion of a perforating arteriole leads to focal and acute ischemia, with pannecrosis, the postulated mechanism for lacunar infarcts.[53,54] This theory remains unproven despite having been proposed many years ago.[11] The arteriolar endothelium continues into the capillaries, and the parenchymal injury seen in CSVD is not confined to the periarteriolar space.[23] Furthermore, longitudinal studies showed conflicting results regarding whether low CBF predicts WMH burden.[23,55] CBF might reflect the loss of viable tissue rather than causing tissue damage.[23]

Inflammation and oxidative stress

Interestingly, after transient global ischemia, perineural spaces, WM, and oligodendrocytes are the first to suffer, and neuronal death occurs several days after the initial damage. Moreover, microglial activation occurs early in the process, protracting until 30 days after the initial ischemia. This activation is believed to be involved in the progression of the ischemic damage.[56] Periventricular macrophages are a major source of vascular oxidative stress and key contributors to neurovascular and cognitive dysfunction in models of chronic hypertension.[16] Oxidative stress has a prominent role in the pathogenesis of CSVD. It leads to endothelial dysfunction, exacerbates inflammation, and dysregulates the neurovascular unit, worsening BBB disruption, causing neurovascular uncoupling and, ultimately, neuronal damage.[44,56] Reduced cerebral vasoreactivity to CO_2 ($VRCO_2$) has been attributed to increased reactive oxygen species (ROS) and reduced NO in hypertensive and diabetic patients with endothelial dysfunction.[57]

Perivascular spaces (PVS)

PVS are thought to result from the obstruction of the glymphatic system by catabolites, proteins, and cell debris, contributing to fluid and plasma cell leakage, which may potentiate perivascular inflammation.[56] Aging and microinfarcts impair perivascular clearance, suggesting that failure of the glymphatic system is involved in brain dysfunction in these conditions.[58,59] PVS in MRI studies are associated with several CSVD-related features, including hypertension, inflammatory cytokines, and cognitive decline.[60] In addition, PVS become more prevalent with increasing WMH, microbleeds, and cerebral amyloid angiopathy and associate with lacunar stroke (vs other stroke types).[60] Furthermore, PVS associate with BBB dysfunction and increased intracranial and extracranial vascular stiffness. Therefore, PVS may be important markers of several pathological processes that lead to CSVD.[23]

Etiology/Vascular risk factors

The exact causes of CSVD are not entirely understood, but it is believed that a combination of risk factors contributes to its development. Hypertension (HT) is the strongest risk factor for CSVD.[61,62] Several other vascular risk factors (VRF) have been proposed, the most well-established being diabetes mellitus (DM), smoking and hyperlipidemia.[63] The prevalence of CSVD also increases with age, with no known significant gender or racial differences.[64]

Hypertension

HT is a critical health issue in Portugal, affecting 42% of adults.[65] It is the leading cause of stroke and a major risk factor for cognitive decline.[61,62] Globally, over 1 million people suffer from HT, making it a widespread concern.[66] HT patients are at increased risk for developing WMH, lacunes, perivascular spaces, and microbleeds, which are recognized imaging hallmarks of CSVD.[63,67] Chronic high blood pressure (BP) leads to structural (mal)adaptations in the cerebral circulation, as mentioned above (see "Pathophysiology of cerebral small vessel disease"), leading to arterial stiffening, vascular occlusions, and changes in the mechanical and hemodynamic properties of blood vessels.[44,62] Functional hyperemia is attenuated in animal models of hypertension and has been demonstrated in HT patients.[44] Oxidative stress and endothelial dysfunction are important mediators in the pathogenic effects of HT, contributing to the impairment of neurovascular responses.[44,62,68] Recent research revealed that HT may trigger low-grade vascular inflammation and microglia activation, both associated with CSVD.[69]

Diabetes mellitus

DM is the 8th leading cause of disability worldwide, and its impact has increased significantly in the last 30 years.[1] DM-induced chronic vasculopathy includes macrovascular disorders, such as cardiovascular and cerebrovascular large vessel disease, and microvascular disorders, including nephropathy, retinopathy, and neuropathy, that can ultimately affect brain health.[70] Patients with type 2 DM have a particularly high incidence of lacunar stroke,[71,72] and an increased risk of developing WMH.[63] Furthermore, studies have implicated DM as a risk factor for cognitive impairment and dementia, possibly related to CSVD.[72,73] However, the mechanism by which cognitive decline occurs and whether it can be explained by dysfunction of the NGVU remains to be elucidated.[73]

Aging

The incidence of CSVD increases with aging, and it is present to some extent in virtually every individual aged 60 years or older.[64,74] VRF have a greater impact on older individuals, indicating an acceleration of CSVD progression with increased age.[63] There is increasing evidence that advanced age and chronic HT may reduce the ability to self-regulate CBF in response to various systemic blood pressure levels.[75] This highlights the importance of early prevention, diagnosis, and treatment of modifiable VRF for managing CSVD. Besides the accumulation of VRF over time, other mechanisms explain CSVD development with aging. With advanced age, changes in BBB transport systems, decreased CSF turnover, and altered basement membrane composition occur.[76] Furthermore, aging also affects the cellular components of the NGVU, with senescence of endothelial cells, loss of pericytes, and modifications of astrocytes towards more inflammatory phenotypes.[76] Age-related inflammation – inflammaging – is an emerging concept that refers to chronic, low-grade inflammation that occurs with aging, resulting from this cellular and immune senescence, mitochondrial dysfunction, and defective autophagy.[77] Inflammaging is increasingly considered a risk factor for CSVD.[49]

Tobacco

Smoking is a well-established risk factor for cognitive decline and doubles the risk of both AD and non-AD dementia.[78,79] Individuals who smoke are more likely to develop lacunar infarcts and WMH, which are hallmarks of CSVD.[63] Studies have also shown that smoking leads to lower microstructural integrity of the cerebral WM, both in WMH and in NAWM.[80] In addition, smoking exacerbates the effects of HT in the cerebral microvasculature, which can

also contribute to the development of CSVD.[81] Impaired neurovascular coupling has been observed in young chronic smokers.[82] Although epidemiological and clinical studies have shown the impact of smoking in the cerebral microvasculature, comprehensive toxicological and mechanistic studies are still scarce. Nevertheless, smoking likely disrupts the BBB by promoting oxidative stress and inflammation and by altering tight junctional protein expression through nicotinic receptors.[83,84]

Dyslipidemia

Dyslipidemia has been established as a risk factor for large vessel disease and stroke However, its role in the development of CSVD is still controversial.[85] Animal studies have shown that hyperlipidemia can lead to impaired cerebral autoregulation and an increased risk of CSVD.[86,87] Additionally, a recent meta-analysis has found an association between hyperlipidemia and the development of CSVD neuroimaging markers, suggesting it is a relevant VRF for CSVD.[63]

Chronic kidney disease

Chronic kidney disease (CKD) has been suggested as a risk factor for CSVD. Patients with CKD have increased risk of stroke and cognitive decline,[88-90] as well as higher stroke severity and a higher probability of a poor outcome after stroke.[91,92] Recent studies have shown increased prevalence and severity of MRI markers of CSVD with deteriorating estimated glomerular filtration rate (eGFR) in CKD patients.[93]

Other risk factors for CSVD have been suggested, most requiring further confirmation, including increased salt intake, obesity, obstructive sleep apnea, chronic heart failure, coronary artery disease, atrial fibrillation, and peripheral artery disease.[42,94-99]

Clinical picture

Historically, CSVD was considered a benign condition, but it is now recognized as a significant cause of morbidity and mortality worldwide. It is defined by a combination of clinical and neuroimaging markers and can range from asymptomatic to debilitating. The presenting symptoms and clinical course of CSVD are highly variable and depend on several factors, including the type, extent, and localization of the vascular lesions, the degree of secondary neurodegeneration, resilience factors, and comorbid conditions.[23] Severe WM

changes independently and strongly predict rapid global functional decline in older adults even before any disabeling complaints occur.[100]

Stroke

CSVD is a prevalent cause of ischemic stroke, accounting for 25% of cases.[4] It is the major cause of lacunar strokes, which are small subcortical ischemic lesions occurring mainly in the basal ganglia, thalamus, pons, and subcortical white matter, resulting from CSVD pathology concerning lenticulostriate branches extending from the middle and anterior cerebral arteries, thalamoperforating branches extending from the posterior cerebral artery, and paramedian branches extending from the basilar artery.[42] Despite their small size, lacunar strokes are not benign and can lead to permanent neurological deficits and significant mortality rates.[101,102] Additionally, CSVD is the primary cause of spontaneous intracerebral haemorrhage, typically located in deep brain structures, with the highest mortality rate among strokes at approximately 40%. Survivors of these types of strokes are often left with severe disabilities.[103] Silent CSVD is even more common, occurring 6-10 times more often than stroke. It is estimated that there are about 10 silent brain changes for every symptomatic stroke.[42] Furthermore, up to 20% of asymptomatic elderly people have evidence of incidental lacunes on MRI, which more than doubles the risk of subsequent stroke and dementia.[64]

Cognitive impairment

CSVD is the main cause of vascular cognitive impairment (VCI) and dementia (VD).[104] There is remarkable heterogeneity in cognitive symptoms among patients with the same burden of CSVD markers on brain imaging: it may go unnoticed or result in only mild functional deficits, usually attributed to age, or it can be a progressive condition evolving into overt dementia.[11,105] CSVD can cause VCI and VD through several mechanisms. The accumulated burden of WM disease causes progressive disruption of neuronal networks.[12] Moreover, several studies have shown that strategic lacunar infarcts, especially in the frontal area and thalamus, can cause cognitive decline.[67,106-108] Recurrent or multiple infarcts, leading to considerable stroke cumulative volume (>100 mL), is also a recognized cause for VCI/VD, coined as multi-infarct dementia.[109] Brain imaging markers of CSVD (including WMH, lacunes, microbleeds, recent small subcortical infarcts, cortical microinfarcts and brain atrophy, discussed below) have all been shown to increase the risk of VCI/VD.[104] Cognitive decline associated with CSVD typically manifests as executive dysfunction and altered processing

speed, attention, and memory.[104,110] It is also increasingly recognized that CSVD contributes to the emergence and progression of AD.[4,9,12,111]

Gait impairment and falls

Gait disturbance is the second most prevalent consequence of CSVD, after cognitive decline. Gait and balance impairment are prevalent among the elderly and increase the risk of falls, institutionalization, and mortality.[112] The term "frontal gait disorder" is commonly used to describe the apraxic gait that develops with aging, and CSVD is a leading cause of this disturbance.[113] Studies have shown decreased functional connectivity in regions mainly located in the sensorimotor network and frontoparietal network.[114] The burden of CSVD has a significant association with the development of vascular parkinsonism in the elderly, which is characterized by bradykinesia, muscle rigidity, and shuffling gait.[115,116] Studies show loss of WM integrity in the basal ganglia-thalamocortical circuit, leading to the disconnection of the basal ganglia and motor cortices, which may ultimatlely lead to parkinsonism.[116] Moreover, impaired cerebrovascular regulation has been found to be associated with slow gait speed in older adults.[117,118]

Neuropsiquiatric symptoms

There is increased incidence of neuropsychiatric symptoms in patients with CSVD. Apart from cognitive decline, discussed above, a meta-analysis showed increased incidence of apathy, fatigue, and delirium with worsening WMH.[8] Studies also suggest associations with anxiety, emotional lability, and psychosis, but these associations require further investigation.[8] Reduced WM integrity in the limbic–cortical–thalamic–striatal network is probably associated with the development of emotional and cognitive symptoms.[119]

Neuroimaging correlates

Since most CSVD is asymptomatic, neuroimaging is central to its diagnosis and characterization. However, since the small vessels cannot be visualized directly, diagnosis relies on surrogate markers. The neuroimaging markers of CSVD include WMH of presumed vascular origin, lacunes of presumed vascular origin, enlarged perivascular spaces, recent small subcortical infarcts, cerebral microbleeds and brain atrophy.[120] In addition, small vessel pathology is not confined to visible lesions, and more sensitive MRI methods can show changes occurring in the NAWM and grey matter (Figure 5).[23]

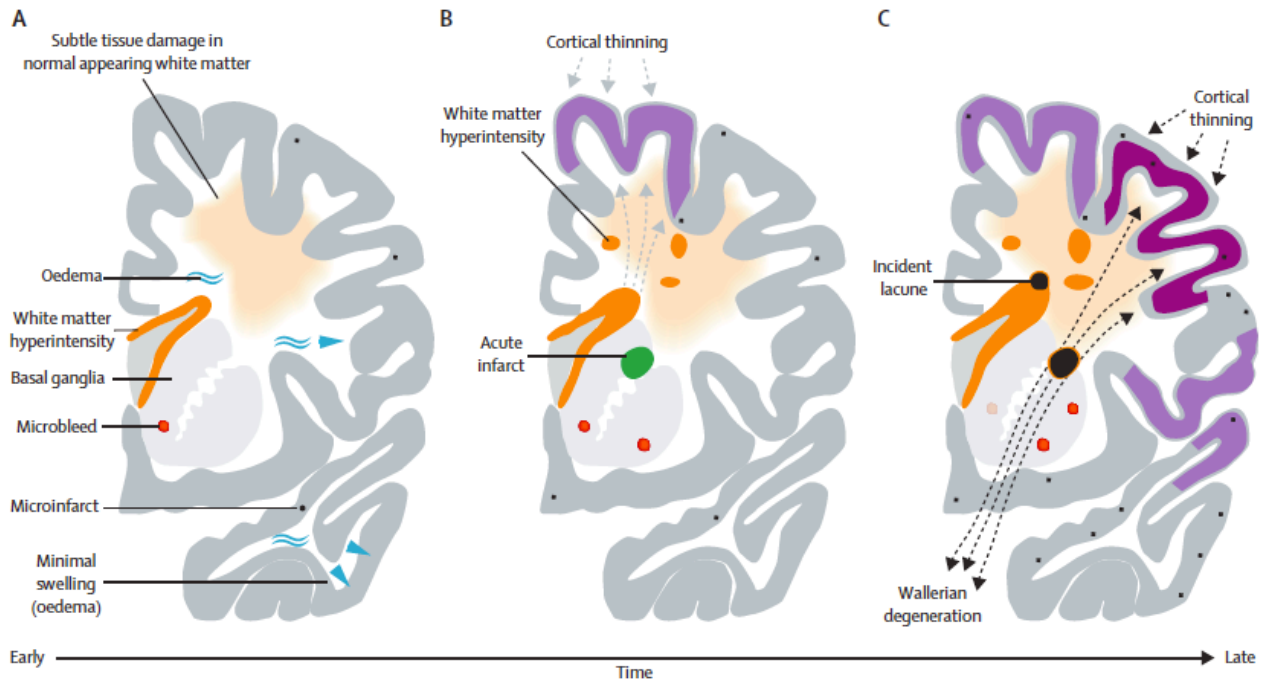

Figure 5. Schematics of CSVD effects on the whole brain.

Left to right is the progression of small vessel disease-related lesions detectable on MRI. Incident lesions, such as infarcts and microbleeds, might occur at variable time points during disease progression or be absent. Acute infarcts and white matter hyperintensities (visible on fluid-attenuated inversion recovery and T2-weighted MRI sequences) cause secondary loss of grey and white matter in connected brain regions and regions with less direct connections, resulting in cortical thinning, brain atrophy, and neurodegeneration. (A) Interstitial fluid increases produce subtle changes in normal white matter, leading to white matter hyperintensity formation. (B) White matter hyperintensities progress, leading to secondary cortical thinning. Acute small subcortical infarcts might appear. (C) White matter hyperintensities, lacunes, microbleeds, secondary cortical thinning, and long tract degeneration worsen. Reproduced with permission. Source: Small vessel disease: mechanisms and clinical implications. By Wardlaw JM, et al. Lancet Neurol. 2019; 18(7): 684-96. Copyright © 2019 Elsevier Ltd.

White matter hyperintensities

WMH of presumed vascular origin are confluent, bilateral, and symmetrical areas in the hemispheric WM that appear hyperintense on T2-weighted and fluid-attenuated inversion recovery images (FLAIR) (Figure 6).[11] They are commonly categorized into periventricular hyperintensities and deep white matter hyperintensities.[121] Hyperintensities can also occur in the basal ganglia and the brainstem.[120] Although their pathogenesis may be multifactorial, WMH of presumed vascular origin are strongly associated with aging, VRF and cerebrovascular disease.[119] Studies have shown increased fluid content with increasing WMH burden and it is present both in WMH and in NAWM, with a decreasing gradient from WMH to adjacent NAWM to more distant brain tissue, consistent with BBB dysfunction.[23,49] The presence and burden of WMH increases the risk for stroke, gait disturbance, cognitive decline and other neuropsyquiatric symptoms.[8,67,75,105,119,122]

Lacunes

Lacunes are defined as CSF-filled cavities of 3-15 mm in diameter, with CSF-signal and a surrounding rim of FLAIR hyperintensity on MRI (Figure 6). As they are usually associated with the occlusion of perforating arteries, their maximal diameter may exceed 15 mm depending on the orientation of the artery and the imaging plane.[119] Less frequently, lacunes may result from cavitation from previous deep intracerebral hemorrhage.[120] Most lacunes are incidental findings on neuroimaging rather than clinically established stroke and are associated with cognitive decline, vascular parkinsonism, increased risk of stroke, as well as increased risk of post-stroke depression and cognitive decline in patients who subsequently experience lacunar stroke.[11,105,119,122]

Perivascular spaces (PVS)

PVS are defined as round or tubular fluid-filled spaces with CSF signal intensity on all MRI sequences, commonly found on the centrum semiovale and basal ganglia (Figure 6). They generally have small diameters, less than 2 mm, but may become enlarged (EPVS) and more likely to be confused with lacunes, although they lack the hyperintense rim on FLAIR images.[119,123] As discussed above, the incidence of PVS is increased in the presence of VRF, which are especially associated with EPVS.[23,60,119] The clinical implications of PVS are still controversial, but cumulative evidence shows that EPVS burden is associated with vascular dementia and AD, post-stroke depression and cognitive impairment, and with the progression of other CSVD markers.[119]

Recent small subcortical infarcts

The connection between typical acute lacunar stroke syndromes and recent small subcortical infarcts (RSSI) is firmly established.[124] Unlike their asymptomatic counterparts, symptomatic RSSI have a predilection for the internal capsule, where the corticospinal and spinothalamic tracts are tightly packed.[119] RSSI can be readily identified on MRI scans as hyperintensities with a diameter of less than 20 mm on axial FLAIR images, with the hyperintense signal seen on diffusion weighted imaging serving as a distinction from old infarcts.[119] As already discussed, these small lesions are believed to stem from sudden blockage of a penetrating artery, and may cause sudden cognitive deficits when occurring in strategic locations such as the thalamus.[23,119] The secondary loss of cortex and WM in connected brain regions that follows results in a cascade of consequences, including cortical thinning, brain atrophy, and neurodegeneration.[23,125]

Cerebral microbleeds

Cerebral microbleeds (CMB) are small, round hypointense lesions with a 'blooming effect' often identified incidentally on T2*-weighted gradient-recalled echo and susceptibility-weighted imaging (Figure 6).[126] The exact mechanisms behind the formation of CMB remain debatable. Most CMB result from true microhemorrhages caused by vessel wall disruption, while there is evidence that some may represent hemorrhagic transformations of previous infarcts. Furthermore, microbleed mimics reflecting vasculopathy rather than parenchymal hemorrhage, including dissection, microaneurysms, or vessel wall thickening, may be confused with CMB.[105] CMB location can also provide valuable insights into their underlying causes. Deeply located microbleeds in the putamen and thalamus are typically associated with HT, while cortical microbleeds and superficial siderosis are usually attributed to cerebral amyloid angiopathy.[43] CMB have been linked to an increased risk of stroke, both intracerebral hemorrhage and ischemic stroke, and increased mortality.[126] In addition, studies have suggested a connection between CMB and dementia, apathy, and fatigue, although these associations are not consistently observed across all studies.[8,126]

Cortical microinfarcts

Cortical microinfarcts (CMI) are small ischemic lesions observed in cortical watershed regions. CMI are common in brain autopsies, especially in patients with dementia and CSVD.[127] Affected individuals are estimated to have hundreds to thousands of CMI, which disrupt structural brain connections.[128] Hence, they could be a mechanistic link between cerebrovascular disease and cognitive decline.[127] CMI can be detected by diffusion-weighted imaging and high-resolution structural MRI, but these modalities cannot capture the entire CMI burden.[128] The pathophysiology of CMI is not fully clear, but they appear to result from CSVD, microemboli and hypoperfusion.[128] CMI are very common in patients with lacunar infarcts, and are associated with higher WMH volume and brain atrophy.[129-131]

Brain atrophy

Brain atrophy occurs in normal aging and many pathological processes, but several studies have reported an association between the presence and severity of CSVD and brain atrophy.[23,120] Several locations have been described, including global atrophy, central atrophy, hippocampal atrophy and focal cortical thinning in regions connected to subcortical infarcts.[132,133] Brain atrophy may mediate, at least in part, the cognitive consequences of CSVD.[134,135] A recent review strongly supports that brain atrophy is an important measure for

studying CSVD.[135] However, how vascular lesions mediate secondary brain atrophy is still debatable.[120] Some evidence suggests that HT is a cause of cortical atrophy, but there is a need for studies that directly test these associations in well-characterized HT-associated CSVD cohorts.[136]

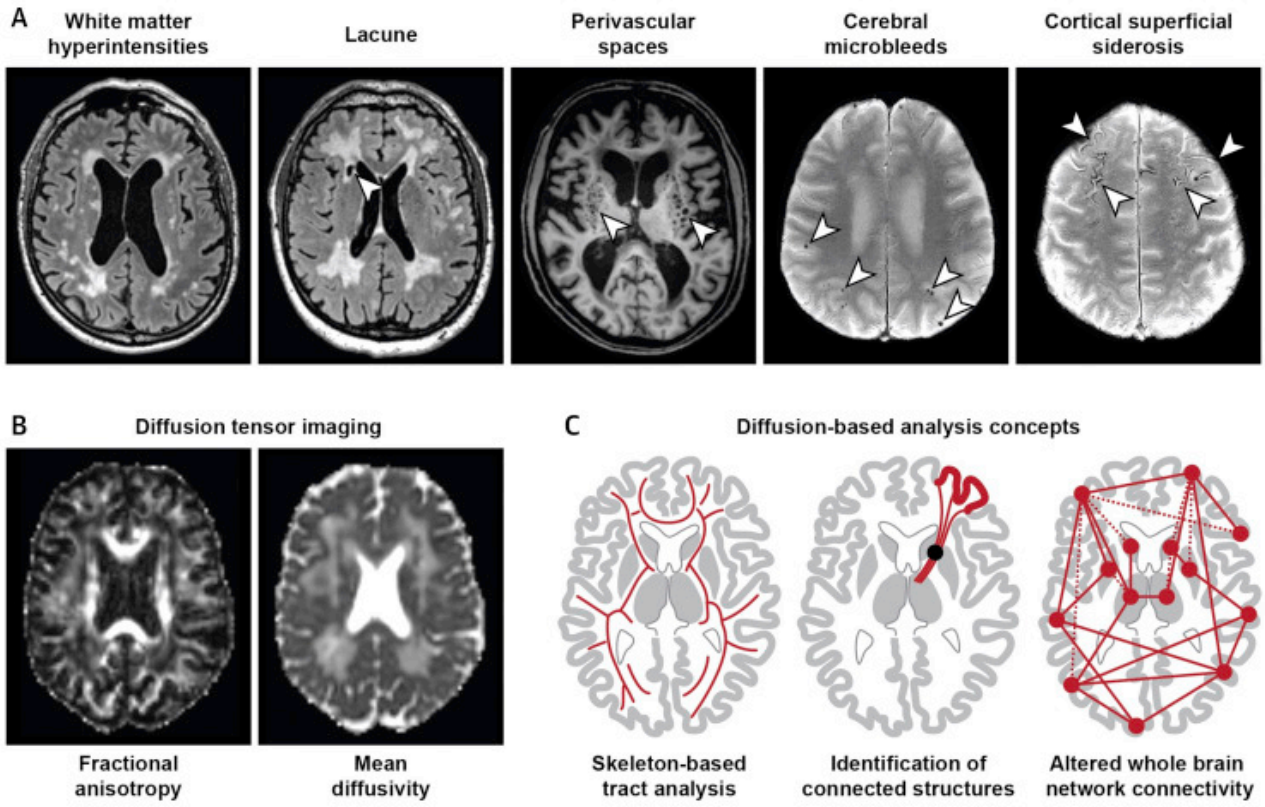

Figure 6. MRI correlates of cerebral small vessel disease.

(A) Signal alterations on conventional MRI (visible lesions) in CSVD. The arrowheads indicate the pathology mentioned at the top of each brain scan. (B) Diffusion tensor imaging shows increased water diffusivity in the brain of a CSVD patient. (C) Diffusion imaging can be analyzed using different strategies, including regional and global approaches. Reproduced with permission. Source: Vascular cognitive impairment and dementia: JACC Scientific Expert Panel. By Iadecola C, et al. Am Coll Cardiol. 2019; 73(25): 3326–3344. Copyright © 2019 Elsevier Ltd.

Therapeutic considerations

As discussed above, CSVD is an insidious disease with imaging changes often preceding noticeable symptoms, which precludes early diagnosis and treatment. In addition, no effective treatments specifically target the underlying pathogenesis of CSVD, leading to a focus on managing associated VRF to slow disease progression. However, the effectiveness of VRF-targeted interventions in preventing CSVD remains inconclusive due to varying evidence from randomized controlled trials (RCT). Differences in trial design, target populations, and endpoint selection contribute to the difficulty in providing definitive evidence.[137] One major limitation lies in the prolonged subclinical course of CSVD, making it challenging to determine

the optimal timing for initiating interventions to prevent disease development and progression. Additionally, the diverse manifestations of CSVD, ranging from acute ischemic and hemorrhagic events to insidious cognitive decline and neuroimaging changes, pose challenges in adopting a uniform approach for symptomatic treatment and secondary prevention. Ideally, interventions should aim to prevent CSVD, emphasizing the need for viable early biomarkers to target in RCT.

Regarding antihypertensive treatments, trials have shown mixed results in reducing cognitive decline. While some studies, such as SYST-EUR, PROGRESS, and HOPE, demonstrated benefits, others, including PRESERVE, SPS3, MRC, SHEP, SCOPE, and HYVET-COG, failed to provide clear evidence of efficacy.[12,137] Although antihypertensive therapy has been shown to reduce WMH progression, this effect has not been consistent across studies, and there seems to be no impact on brain atrophy.[137-139] The use of antihypertensives significantly reduces the incidence of stroke, but the effects on incident lacunes and microbleeds remain unknown.[138-141] Also, multiple studies have failed to produce a robust benefit on the incidence and progression of cognitive decline and dementia.[12,137] The recent SPRINT-MIND trial indicated that intensive BP lowering reduced the risk of MCI and dementia and slowed WMH progression in older adults without diabetes or stroke. However, no significant difference in brain volume or dementia risk was observed compared to standard BP management over four years.[142]

Evidence supporting the benefits of treating diabetes or hyperglycemia in reducing cognitive decline and other CSVD manifestations is limited.[12] A recent cross-sectional study suggested that long-term use of metformin may be protective against total CSVD burden and cognitive impairment.[143]

Similarly, the effects of statins on dementia incidence, cognitive decline, and WMH progression have shown mixed results.[12,49,137] The VITATOPS trial reported less WMH progression in patients with previous stroke and severe WMH on pre-stroke statin therapy.[144] Additionally, atorvastatin reduced stroke recurrence in the subgroup of patients with lacunar ischemic stroke.[145] However, a recent Post Hoc analysis of the SPRINT-MIND trial indicated that patients treated with statins were more likely to experience increased WMH burden over four years.[146]

Behavioral and lifestyle interventions have shown potential benefits in observational studies but have not been fully corroborated in RCT.[12] Observational data on smoking cessation suggest a reduced risk of cognitive decline, but interventional studies have not validated this benefit.[147] Several ongoing RCT aim to build upon existing data in this area.[137]

Other potential CSVD treatments are under investigation, with a significant focus on targeting inflammation and oxidative stress.[137,148,149] However, despite notable advancements in research in CSVD, a noticeable deficit in investment in the search for treatment still persists for this disease.

Considering the available evidence, an effort should be made to diagnose and treat VRF promptly and to educate patients on healthy lifestyles, but further, longer RCT are needed, designed specially to assess the long-term effects of these interventions on the development and progression of CSVD.

TOOLS FOR STUDYING MICROVASCULAR INTEGRITY

There is an unmet need for the evaluation and follow-up of patients at risk for microvascular disease. CSVD markers such as WMH take years to develop, and this window of ongoing damage would be the perfect opportunity to halt the pathological cascade if detected early. The American Heart Association/American Stroke Association recommends further research to identify individuals at higher risk for silent CSVD, to allow cost-effective use of targeted neuroimaging screening and to study therapeutic measures to reduce the incidence of adverse outcomes such as stroke and cognitive decline.[150]

Advanced neuroimaging techniques

Although the MRI is currently the gold standard for the diagnosis of CSVD, conventional MRI findings have been found to overestimate disease severity and not always correlate with functional status.[151] Advanced MRI techniques, such as diffusion tensor imaging (DTI), have shown promise in providing more sensitive and specific measures of CSVD. DTI measures the diffusion of water molecules within WM tracts, revealing microarchitectural changes in lesional and NAWM. The most common derived measures are fractional anisotropy (FA) and mean diffusivity (MD). FA decreases and MD increases as WM integrity is lost and diffusion is no longer restricted to a single direction along the axonal paths.[152] There is growing evidence that DTI, particularly the MD component, is a more sensitive biomarker of WM injury than T2 or FLAIR sequences.[67] Studies have shown a relationship between DTI measures in NAWM and CA measured by transcranial doppler (TCD).[153] These metrics are more recently being converted into maps of connectivity or 'connectome', that can be used to study the connections between brain networks and their efficiency.[152,154] Despite its promise, DTI has several disadvantages. It is a complex, expensive, operator-dependent and time-consuming imaging

technique, requiring specialised software and expertise to analyse and interpret data that may not be available in clinical settings.[155] It is sensitive to noise and artifacts, and the main measures are influenced by age, sex and underlying pathology, which may render data interpretation challenging in the context of CSVD.[152,155]

Neurovascular dysfunction has been shown to precede clinical and imaging manifestations of the disease.[156-158] Hence, the key to preventing the irreversible consequences of CSVD may lay in the study of the functional integrity of the microvasculature. Cerebral hemodynamics has been studied with a variety of techniques, including positron emission tomography (PET), fMRI using blood-oxygenation-level-dependent (BOLD) signal and arterial spin labeling (ASL) perfusion.

Perfusion PET imaging can be used to measure CBF responses to neuronal activation. It is an invasive procedure which uses radiolabled tracers and requires an invasive arterial blood sampling procedure, which is technically challenging and inherently noisy. Also, it has low special resolution and is not readily accessible in most clinical settings.[159,160] BOLD-fMRI measures changes in blood oxygenation in response to neural activity, which indirectly measures changes in CBF and cerebral blood volume (CBV). It is used to study microvascular hemodynamic status because it provides high spatial resolution images and can study changes in CBF in specific brain regions.[136,154,161] ALS allows non-invasive measurement of tissue perfusion by magnetic labelling of arterial blood water. Unlike BOLD-fMRI, ASL provides a direct measure of CBF and is not influenced by changes in blood oxygenation or other confounding factors.[162] Recently, velocity-selective ASL perfusion fMRI has been tested as a potential strategy to study cerebral microvasculature in disease states (such as critical arterial stenosis and acute stroke) without the limitation of arterial transit delay that occurs with standard ASL. This technique may be particularly suited for studying cerebral vasoreactivity but requires further investigation. ALS is also promising for fMRI, since perfusion and blood volume fMRI are more specific to the parenchyma than BOLD.[163] Additionally, by combining ASL with BOLD imaging, a technique referred to as calibrated BOLD, the change in cerebral metabolic rate of oxygenation from neuroactivation can be calculated.[164,165] Despite their merits, BOLD and ALS have several disadvantages: they are expensive, with complex set-up, acquisition and processing, and are not readily available in clinical settings. Hence, they are not adequate modalities for screening asymptomatic CSVD.

The transcranial Doppler in the study of cerebral hemodynamics

The TCD is a relatively inexpensive and non-invasive technique that can be used to measure cerebral blood flow velocity (CBFV).[166] Unlike MRI and PET-based studies, it provides

high temporal resolution, which allows the assessment of fast changes in flow parameters resulting from changes in neural activity. It has the advantage of being portable and is not contraindicated in patients with implanted medical devices or the critically ill. It is available in most hospital settings, although it requires specialized personnel. TCD has some important limitations. It has limited spatial resolution, which is restricted to proximal branches of the arteries of the circle of Willis at the base of the brain, although it is sensitive to detect distal hemodynamic changes. In addition, about 10% of the healthy population exhibits an insufficient temporal ultrasound window and cannot be studied by this method.[167] Nevertheless, TCD could be used as a cost-effective tool for detecting microvascular disfunction in at-risk individuals.

The TCD measures blood flow velocities in the basal cerebral arteries as a perfusion correlate. Aaslid and coworkers first described this technique.[168] The CBFV can be continuously monitored in the cerebral basal distribution arteries, such as the M1 segment of the middle cerebral artery (MCA), A1 segment of the anterior cerebral artery, or the P1 or P2 segments of the posterior cerebral artery (PCA), using bilateral pulse-wave 2-MHz ultrasonic probes secured with a headframe.[167] Usually, for vasoregulation studies, bilateral MCA or PCA are monitored, but data from the two arterial territories can also be recorded simultaneously, although the sides used must be consistent throughout the study, as perfusion properties can vary between the left and right sides.[169,170]

Three fundamental mechanisms drive the adaptation of the CBF: cerebral autoregulation, cerebral vasoreactivity, and neurovascular coupling. An integrative evaluation of cerebrovascular function should involve assessing all of these mechanisms. The TCD can be used to study these mechanisms, as the caliber of the insonated arteries remains relatively constant under normal physiological conditions.[171,172] Therefore, in the absence of significant arterial disease, the CBFV measured at the base of the brain reflects the distal microvessels´ resistance. In response to stimuli such as variations in BP, cognitive challenges, or changes in CO_2 levels, changes in the caliber of the distal small vessels ensue. These changes, in turn, induce variations in CBV and, consequently, in CBFV, given the stable caliber of the insonated vessels (Figure 7).

CEREBRAL AUTOREGULATION

CA is an intrinsic ability that enables the brain to maintain an adequate blood flow in response to fluctuations in systemic BP, at least in non-pathological circumstances. This mechanism protects the brain from hypo or hyperperfusion, ensuring the CBF matches the

metabolic demand.[173] Reduced effectiveness of CA can jeopardize the continuous supply of oxygenated blood to the brain.

Static CA refers to the steady-state, long-term relationship between the mean arterial pressure (MAP) and CBF. It was classically considered that the CBF is maintained relatively flat within a broad range of MAP (60-150 mmHg).[174] However, subsequent data have challenged this concept and demonstrated that CA efficacy depends on the severity, speed, and direction of the changes in perfusion pressure and that the CA plateau may be much shorter (approximately 10 mHg).[15] Hence, CA should not be analyzed only as a static phenomenon.

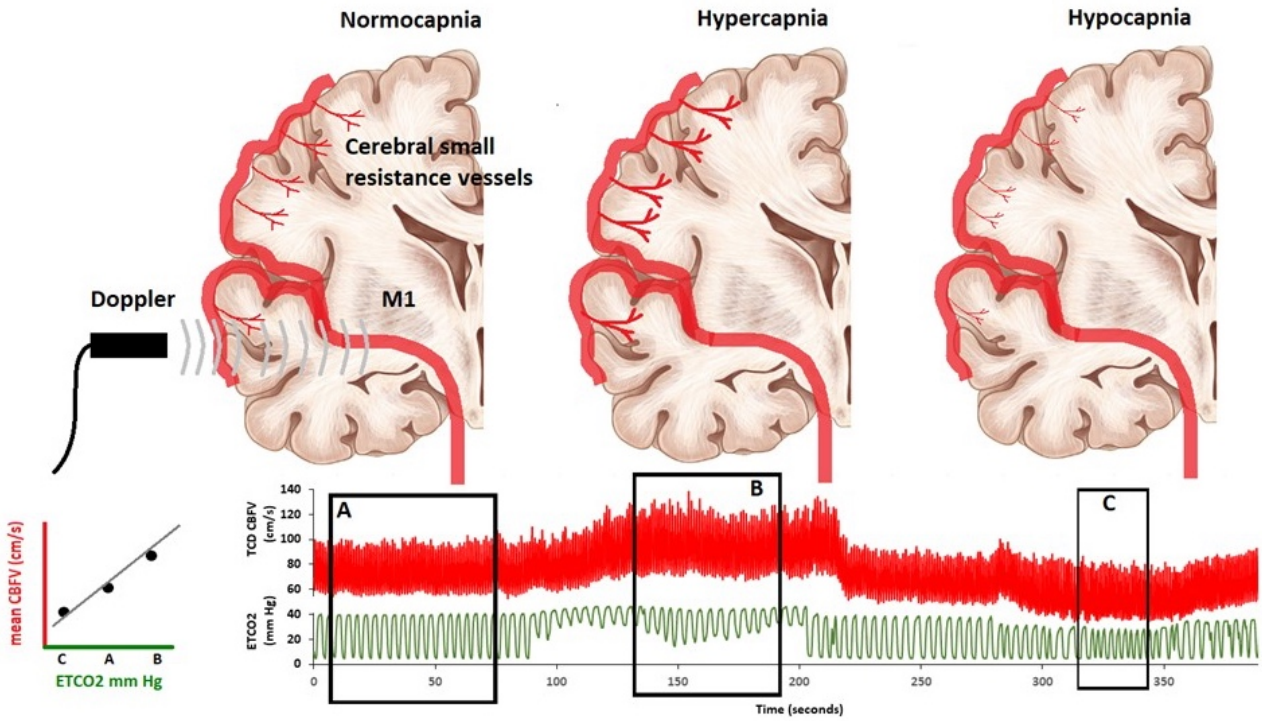

Figure 7. CBFV changes during vasoreactivity to CO_2 testing.

Compared to normocapnia (A), in hypercapnia the small resistance vessels dilate and blood flow velocity measured proximally at the MCA increases (B). The opposite occurs with hypocapnia (C). CBFV: cerebral blood flow velocity; $ETCO_2$: end-tidal carbon dioxide; MCA: middle cerebral artery. Reproduced with permission. Source: Acute Neurologic Injury in ICU: Vasomotor Reactivity Testing by Transcranial Doppler (TCD/TCCS) in Neurosonology in Critical Care. By Castro P. and Azevedo E. Copyright © 2022 Springer Nature.

Dynamic cerebral autoregulation (dCA) refers to the active response of the cerebral blood vessels to spontaneous or induced acute fluctuations in BP.[175,176] The cerebral arterioles are not fast enough to counteract higher frequency oscillations in BP and function as a high-pass filter, allowing oscillations in the BP >0.20 Hz to pass through to the CBF while dampening slower frequency oscillations.[177]

Traditional techniques to assess dCA challenged the cerebrovascular system by inducing acute changes in the BP, either pharmacologically or with maneuvers such as the Valsalva, the sit-to-stand, or using pressurized thigh cuffs. However, these interventions were not always practical or safe in clinical practice, especially when evaluating the elderly or the critically ill.[178] Furthermore, other physiological processes potentially confounded the results (e.g. sympathetic activation, cortical activation, increased partial pressure of carbon dioxide ($PaCO_2$)). Hence, subsequent research focused on developing methods for assessing dCA based on spontaneous fluctuation of BP and CBF at rest.[179]

Although no universally accepted method exists, transfer function analysis (TFA) has become popular.[180] TFA models dCA as a linear system, with the MAP as input and CBF as output. While this is a simplification, since changes in MAP lead to changes in cerebrovascular resistance and variables other than MAP affect CBF, it provides a useful framework for quantifying dCA.[178] The TFA uses the Fast Fourier Transform to calculate the auto-spectral density and cross-spectral density of the MAP and CBF signals using Welch´s method, and the transfer function, H(f), is then calculated by the following equation:[178]

$$H(f) = Sxy(f)/Sxx(f),$$

where Sxx(f) is the auto-spectral density of the MAP and Sxy(f) is the cross-spectral density between the MAP and CBF signals.

TFA quantifies dCA as the gain, phase, and coherence components of the spontaneous changes in MAP, analyzing the degree to which these changes are reflected in the CBF (Figure 8). The values are presented in very low (VLF, 0.02–0.07 Hz), low (LF, 0.07-0.20 Hz), and high (0.20–0.50 Hz) frequencies. As mentioned above, the dCA operates in the VLF and LF.[178] **Gain** measures how much the CBF oscillations are amplified or attenuated relative to the MAP oscillations at each frequency. **Phase** measures of the time lag between the oscillations of the MAP and CBF at each frequency. A higher phase indicates that there is asynchrony between the two oscillations. Lower gain and higher phase mean that the cerebral arteries effectively keep a steady blood flow irrespective of the MAP. **Coherence** measures how strongly the MAP and CBF signals are linearly related. A coherence value close to 1 indicates that they are strongly linearly related, while a coherence value close to 0 indicates that they are weakly related or unrelated. This value is needed to assess the statistical reliability of the gain and phase estimates and, hence, to help identify conditions where they may not be reliable (e.g., in the presence of unrelated noise in real-life measurements).[178,181] The degrees of freedom are calculated to obtain the minimum value of coherence that is significantly different from zero.[178]

Monitoring protocol

To evaluate spontaneous dCA, it is crucial to monitor various physiological parameters simultaneously. These include continuous BP, $PaCO_2$, and heart rate (HR), which are important for accurately interpreting the results. Noninvasive measurement of continuous BP on a beat-to-beat basis is possible with devices such as the Finometer® (FMS, Amsterdam, The Netherlands). This device, widely used in research, measures brachial pressure using a finger-cuff and corrects for finger pressure and hydrostatic height relative to the heart level. These measurements are clinically validated and are similar to intra-arterial recordings.[182] To account for the effects of $PaCO_2$ on dCA and BP, it is necessary to simultaneously measure end-tidal carbon dioxide ($ETCO_2$) with infrared capnography. This can be used as a surrogate for $PaCO_2$.[178] The HR can be obtained using a three-lead electrocardiogram. Figure 9 illustrates the patient setting for dCA monitoring. The analog signals of the CBFV and BP waveforms, $ETCO_2$, and HR are synchronized and stored in digital format using a data acquisition device such as the Powerlab® (AD Instruments, Oxford, UK), used for this project (Figure 10).

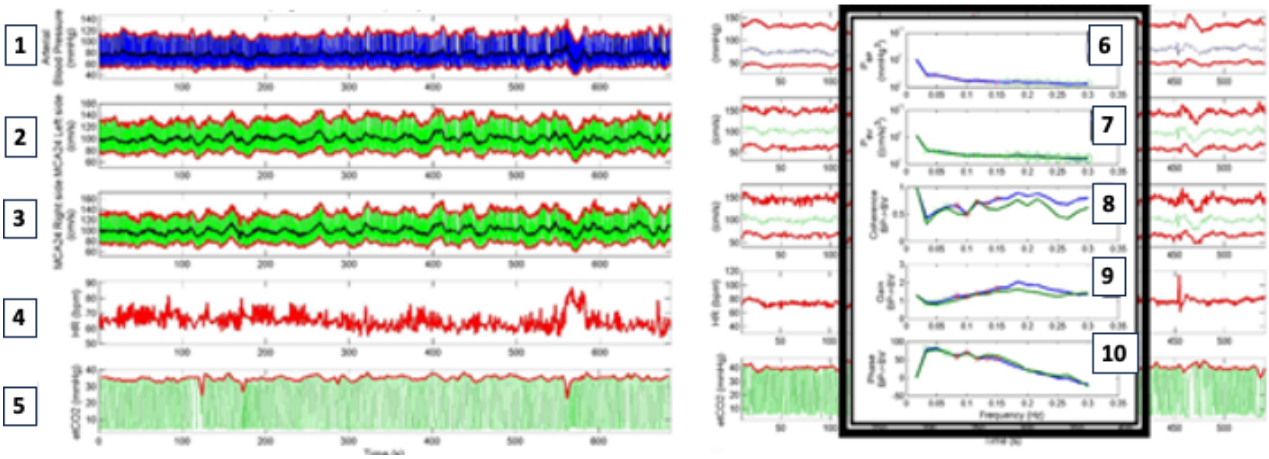

Figure 8. Transfer function analysis

The MAP and MFV are derived from the time series of continuous monitoring of BP (1) and CBFV (2 and 3), respectively. The spontaneous oscillation of these physiological variables is noteworthy. The transfer function analysis (TFA) quantifies the influence of the MAP (input) on the MFV (output) in the frequency domain from its spectra (6 and 7, respectively). The TFA produces 3 parameters: coherence (8), gain (9), and phase shift (10). Reproduced with permission. Source: Castro P., unpublished personal data, calculated with private software based on Matlab®.

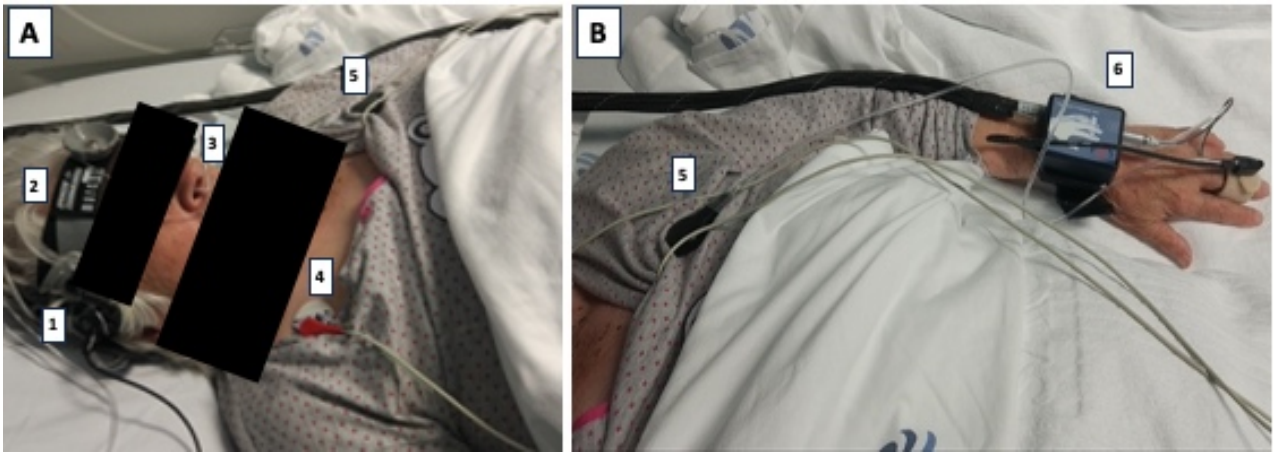

Figure 9. Patient setting for dCA monitoring.

(A) With the patient lying supine, TCD probes (1) are secured bilaterally with a headframe (2). A nasal cannula (3) is also placed and secured in the headframe for continuous $ETCO_2$ measurement. Three electrocardiogram leads (4) are placed to obtain a lead II electrocardiographic line. (B) Continuous blood pressure measurement with finger plethysmography (6) using the Finometer® device allows beat-to-beat monitorization of the arterial blood pressure (ABP) curve. A height correction unit (5) is placed at the heart level to correct possible drifts in ABP caused by unwanted hand movements.

To obtain spontaneous dCA by TFA, it is essential to follow specific recommendations. The evaluations should be conducted in a temperature-controlled environment, ideally between 22-24°C. Subjects should abstain from caffeinated substances and heavy meals for at least 4 hours before the examination, avoid exercise and alcohol ingestion for a minimum of 12 hours, and remain in a quiet environment without excessive acoustic and visual stimulation during the evaluations. Baseline measurements should be obtained supine or seated with uncrossed legs after a 15-minutes rest period. Robust estimates of TFA parameters require recordings of spontaneous fluctuations of BP and CBFV lasting at least 5 minutes.[178]

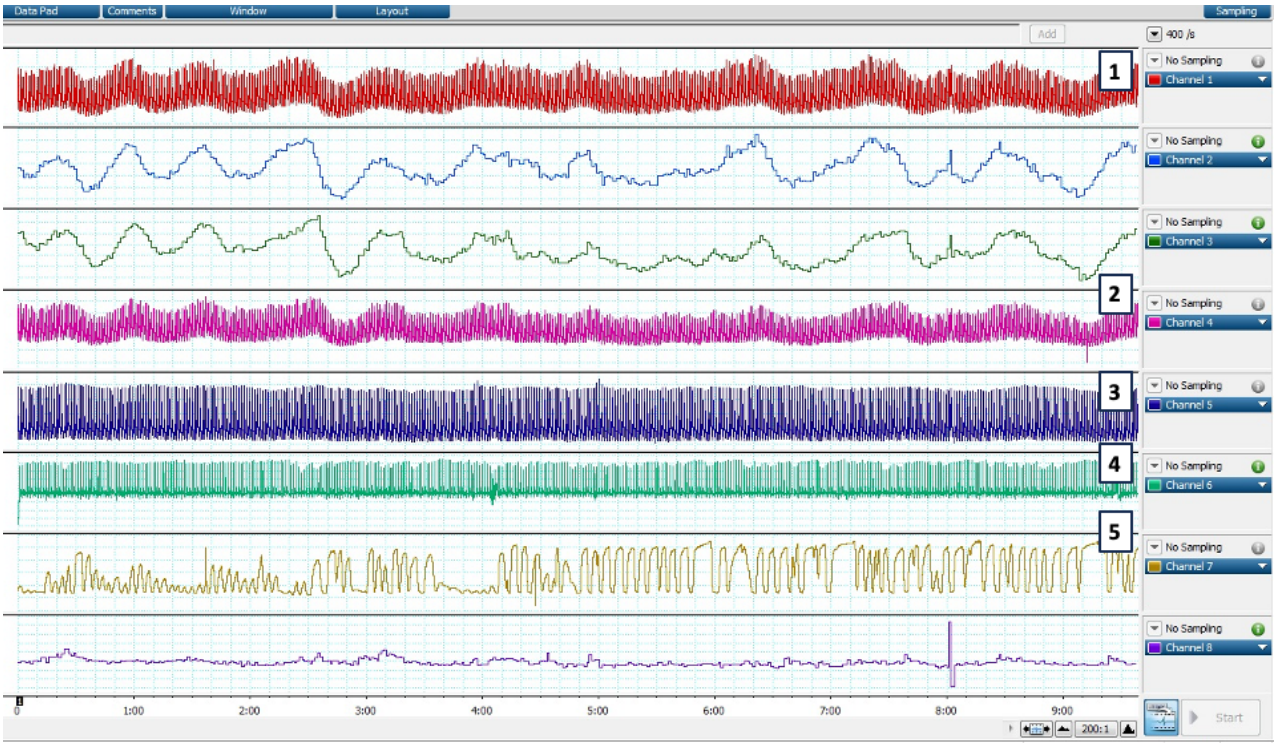

Figure 10. Multimodal integration

Analog signals from Powerlab® during supine rest. (1) CBFV in the right MCA (cm/s), (2) CBFV in the left PCA (cm/s), (3) blood pressure (mmHg), (4) electrocardiogram (mV), and (5) capnography (mmHg). Example from one of the subjects from this project. Personal unpublished data.

CEREBRAL VASOREACTIVITY

Cerebral vasoreactivity reflects the adaptation of the resistance vessels´ diameter in response to variations in the $PaCO_2$ and pH. As the $PaCO_2$ levels rise, indicating increased tissue oxygen demand, the cerebral arterioles dilate, increasing CBF. This dilation is considered a measure of the cerebrovascular endothelial function and is seen as the "cerebrovascular reserve".[183,184] The underlying idea behind assessing cerebral vasoreactivity is based on the premise that in pathological conditions, as in critical arterial stenosis and occlusion, the resistance vessels may already be at maximum dilation, resulting in a diminished response to the vasodilatory stimulus.[184,185]

Monitoring protocol

Vasoreactivity to CO_2 ($VRCO_2$) is assessed by measuring changes in CBF and $ETCO_2$ levels in response to variations in $PaCO_2$ This can be achieved through a hypocapnic stimulus, such as volitional hyperventilation and/or a hypercapnic stimulus, such as inhalation of an air/CO_2 mixture (carbogen, 5% CO_2) or breath holding.[181,183] $VRCO_2$ is calculated as the ratio of the percentage change in CBFV to the change in $ETCO_2$ (Figure 11).[181]

The setup for assessing $VRCO_2$ is similar to the one described for dCA. Baseline recordings are obtained after 15 minutes of rest in a low-stimulus environment. Individuals are then monitored through successive 1-2 minutes steps of rest, followed by a hypercapnic stimulus (until reaching a plateau of $ETCO_2$, 7-10 mmHg above baseline), another rest period (breathing room air until normocapnia is reached), and finally a hypocapnic stimulus (until reaching a plateau of $ETCO_2$, 7-10 mmHg below baseline).[186]

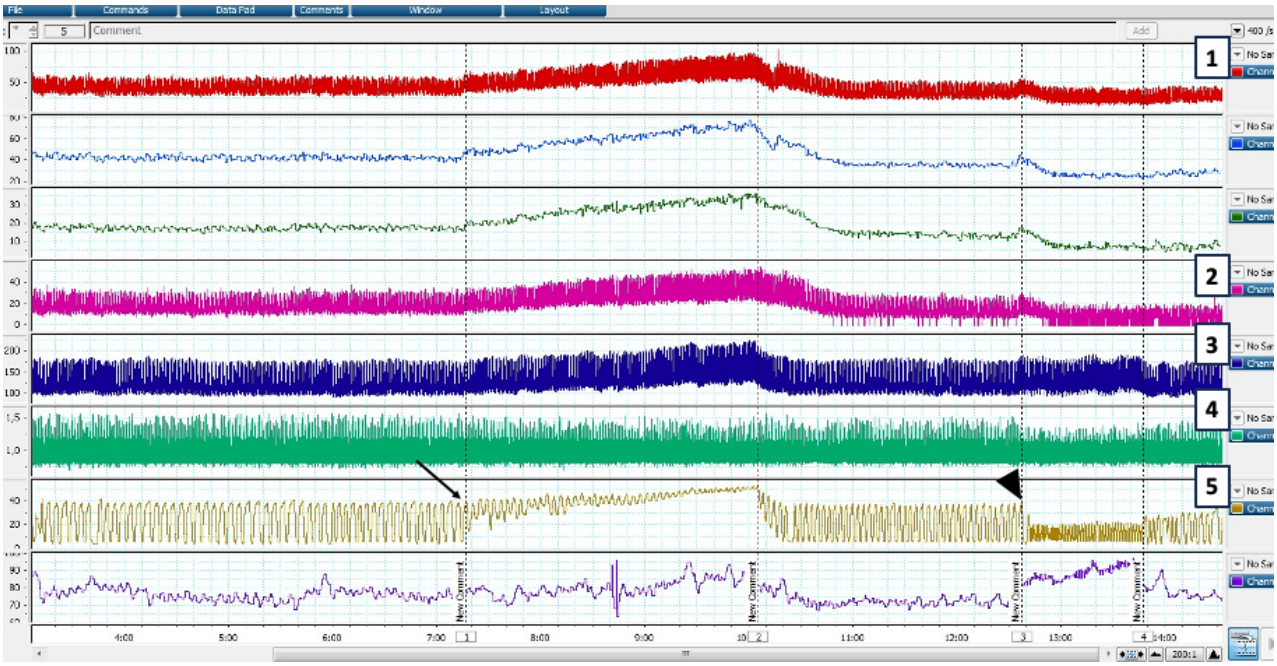

Figure 11. Vasoreactivity protocol.

Analog signals form Powerlab® during the vasoreactivity protocol. The arrow marks the beginning of the hypercapnic stimulus (carbogen inhalation), and the arrowhead marks the beginning of the hypocapnic stimulus (hyperventilation). (1) CBFV in the right MCA (cm/s), (2) CBFV in the left PCA (cm/s), (3) blood pressure (mmHg), (4) electrocardiogram (mV), and (5) capnography (mmHg). Example from one of the subjects from this project. Personal unpublished data.

NEUROVASCULAR COUPLING

NVC is the process by which transient changes in neural activity lead to prompt adjustments in local CBF. An alteration of the cerebrovascular resistance regulates the CBF increase as the arterioles in the precapillary bed dilate in response to several vasoactive substances.[20] The mechanisms governing NVC have been discussed in the "The neuro-glial-vascular unit" section.

These local increases in CBF, termed functional hyperemia, are typically considered to have the purpose of supplying the needs of the highly metabolically active neurons. However, this may not be the case. There is evidence that the baseline levels of CBF are more than

adequate to supply oxygen and nutrients to maximally activated neurons, and the elicited functional hyperemia oversupplies the requirements of the activated neurons. Also, NVC operates during sleep at even higher levels than in awakeness.[187] Animal models have demonstrated that NVC is inverted or absent in many brain areas, not reflecting metabolic activity.[188,189] A recent review suggests that NVC may play several non-exclusive roles in neurosignaling and maintaining homeostasis without a direct role in supplying oxygen and glucose to active brain regions (which would be limited by the brief nature of the NVC response).[187] It may serve in 1) replenishing the active brain areas with neuromodulators with oxygen-limited synthesis (such as acetylcholine and norepinephrine); 2) temperature homeostasis; 3) stabilizing and optimizing the cerebrovascular structure; 4) CSF production and circulation; 5) indirect signaling of neurons by relaying chemical signals from systemic sources and 6) direct neuro-modulatory functions (the hemo-neuro hypothesis), via mechanical and chemical signaling (by varying NO and oxygen concentrations, which modulate neural excitability).[187,190]

Although our understanding of the specific functions of NVC is currently constricted, it is clear from pathology that NVC has an essential physiological role. NVC disruption adversely impacts brain function and is present in several neuropathological processes.[20,191] Consequently, studying NVC in the healthy and diseased brain can offer valuable insights into disease processes and potentially serve as a target for therapeutic interventions.

The brain's functional anatomy allows for the easy and reliable examination of NVC by measuring the effects of sensorimotor or cognitive stimulation on CBFV. CBFV is recorded in the basal cerebral arteries as a perfusion correlate of changes in local vascularisation. This technique was first developed by Aaslid in 1987 and has since been widely used.[192] Studies have demonstrated a close relationship between regional changes in CBF and corresponding alterations in CBFV.[193]

Monitoring protocol

NVC is recorded mainly in the MCA or the PCA, contingent on the stimulus applied. Several stimuli have been used to evaluate the MCA, depending on the aim of the study, including motor, sensory, cognitive, and emotional tasks.[184] Visual paradigms are used for the PCA, including flickering lights, a flickering checkerboard, text reading, and a rotating colored optokinetic drum.[184,194] A minimum of 5 cycles of rest-stimulation should be recorded, and the average value calculated to eliminate spontaneous variations of the CBFV.[167,195,196] In healthy individuals, these tasks will lead to an average of 10-20% increase in CBFV in the PCA and a 5-8% increase in the MCA.[166] The relative changes in CBFV are then analyzed (normalized

CBVF) since the absolute value depends on the insonation angle, which cannot be estimated or corrected.[167] The hemodynamic data from each hyperemic recording is extracted beat-by-beat using an acquisition system, including the RR interval, systolic, diastolic and mean BP, and mean, peak and minimum CBFV. $ETCO_2$ is also extracted breath-by-breath. After extraction, data from each subject and condition are placed in a separate spreadsheet that includes all the repeated NVC cycles collected. The software automatically coalescences all trials from each subject into one contour for each outcome metric of interest for analysis.[166]

Visual paradigm

The selective activation of the occipital lobe can be achieved using a simple visual paradigm, such as the flickering checkerboard paradigm (Figure 12). Briefly, a black and white checkerboard pattern is presented on a computer screen. The pattern alternates rapidly, producing a flickering effect that stimulates the primary visual cortex. Conventionally, after a 10-15 minute period of quiet rest, 5-10 cycles are repeated, where each cycle consists of a 20-30 second period of eyes closed followed by 30-40 seconds of eyes opened.[166] The evoked response can be calculated as $\frac{maximumCBFV-baselineCBFV}{baselineCBFV} \times 100\%$.[183]

Rosengarten and colleagues proposed a method for analyzing and modeling the vascular dynamics of NVC using a control system approach. In this approach, the increased cortical activity evoked by the visual paradigm serves as the input, and the time course of CBFV in the PCA serves as the output. Control system analysis is a valuable tool for studying biological systems regulated by feedback control mechanisms, as it identifies critical parameters that influence system behavior. The visually evoked flow response curve is described with four parameters according to the second-order linear function (Figure 13):[197]

$$G(s) = \frac{K(1+T_v s)}{\frac{s^2}{\omega^2} + 2\frac{\xi}{\omega}s + 1},$$

where "K" represents the gain, "Tv" the rate time, "ω" the natural frequency, and "ξ" the attenuation.[197] **Rate time** is the initial steepness of the CBFV increase, **natural frequency** represents the oscillatory properties of the system, **attenuation** describes dampening and tonus features, such as the elastic properties of the wall vessel, and **gain** corresponds to the relative CBFV difference between the resting stage and the steady-state level during visual stimulation.[198]

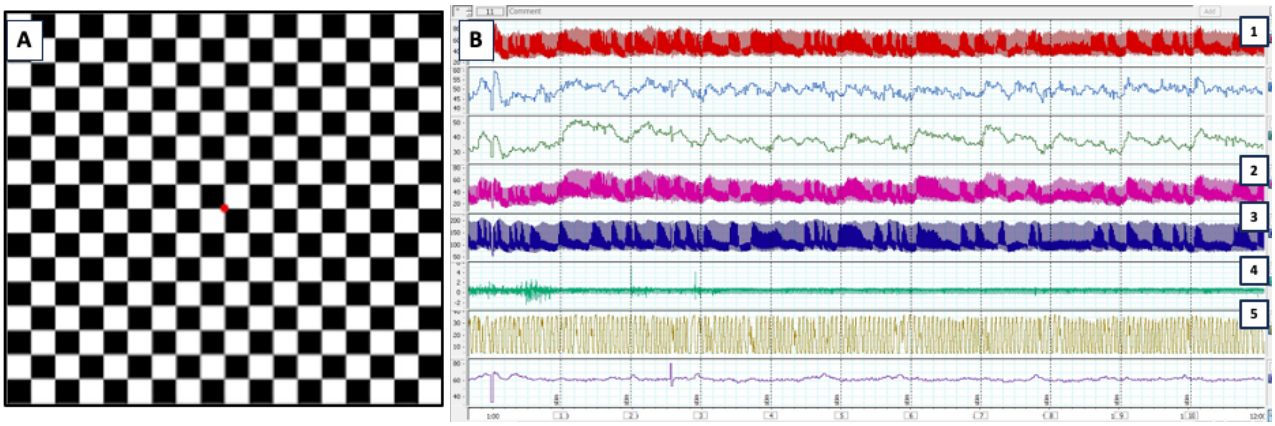

Figure 12. Flickering checkerboard paradigm

(A) Checkerboard pattern with a red fixation point. (B) Analog signals obtained with Powerlab® during the flickering checkerboard. The dotted lines mark the stimulus application (eyes opened). (1) CBFV in the right MCA (cm/s), (2) CBFV in the left PCA (cm/s), (3) blood pressure (mmHg), (4) electrocardiogram (mV), and (5) capnography (mmHg). Example from one of the subjects from this project. Personal unpublished data.

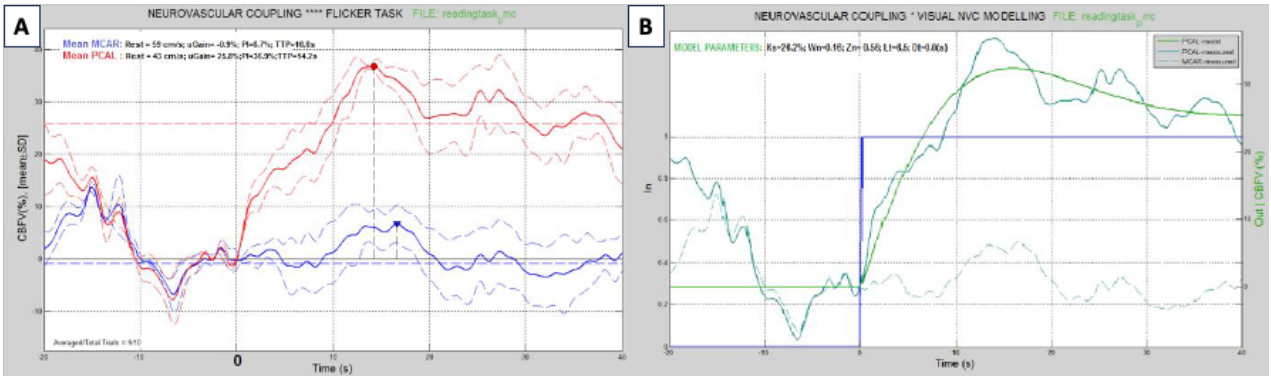

Figure 13. Neurovascular coupling – visual paradigm

(A) Left PCA mean CBFV (red) and right MCA mean CBFV (blue). The CBFV increases rapidly in the PCA after the visual stimulus, with a visually evoked flow response of 37%, while the visually evoked flow response in the MCA is 7%. (B) Modeled curve as proposed by Rosengarten et al.[197] Example from one of the subjects from this project. Personal unpublished data.

Executive function paradigm

The N-back test (NBT) is a cognitive task commonly used to study NVC in the MCA territory by eliciting the activation of the prefrontal cortex (Figure 14). After a 10-15 minutes period of quiet rest, the participant is presented with a sequence of letters, displayed every 2 seconds. The participant is then asked to indicate whether the current letter matches the one presented 1 item ago (1-back task) or 2 items ago (2-back task). The results are normalized with a control task involving identifying the letter "X," performed before each N-back task.[118] The NVC is calculated using the equation:

$$\frac{(CBFVnbt - CBFVx)}{CBFVx} \times 100\%,$$

where CBFVnbt stands for the relative CBFV increase during the N-back task, and CBFVx stands for the CBFV during the control task. The 2-back task results are the most reliable since they induce a higher CBFV rise and potentially represent the NVC more accurately.

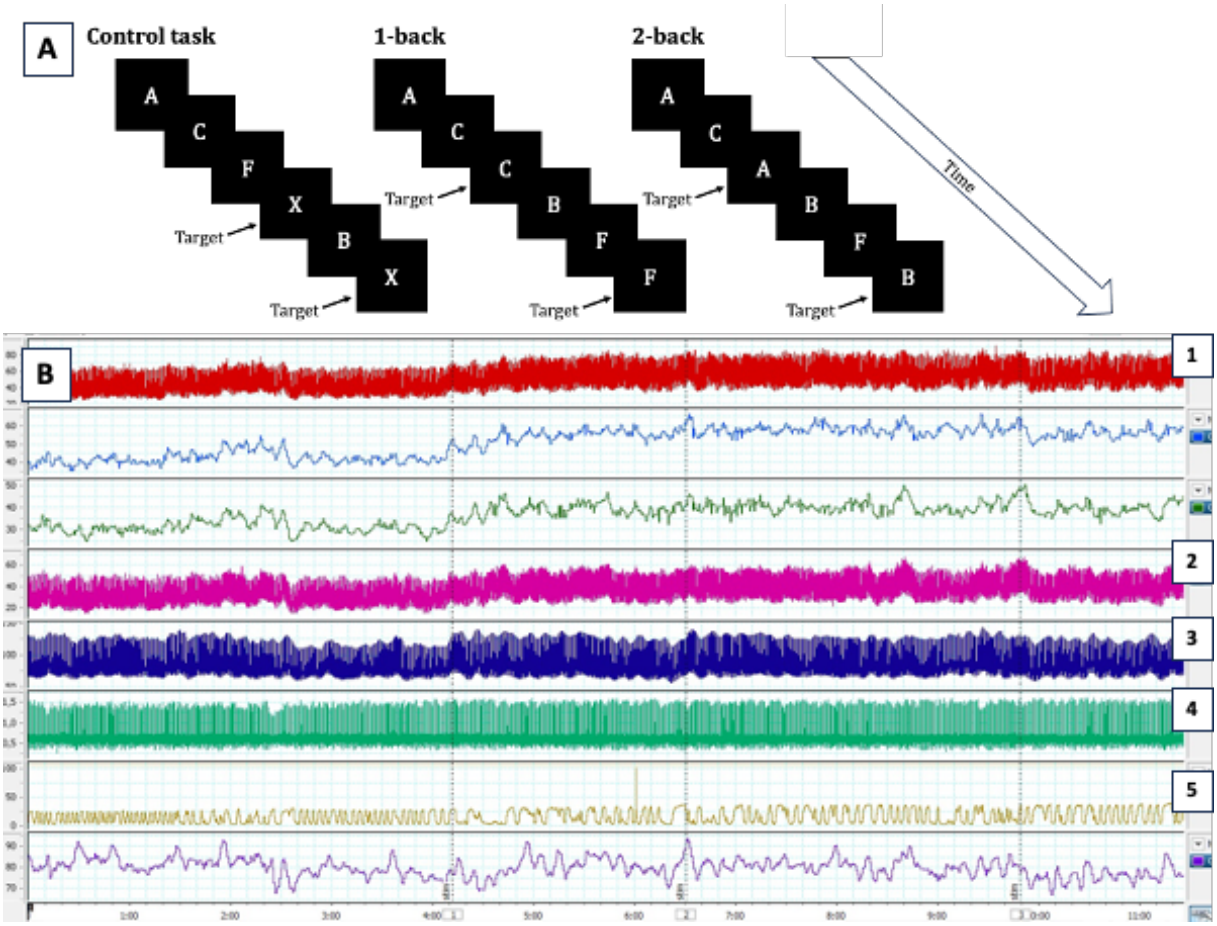

Figure 14. Neurovascular coupling – N-back test

(A) Illustration of the n-back paradigm. The letters are presented in 2-second intervals and last for 2 seconds on the screen. The control task (finding X) is presented before the 1-back task and again before the 2-back test. (B) Analog signals obtained with Powerlab® during the N-back test. (1) CBFV in the right MCA (cm/s), (2) CBFV in the left PCA (cm/s), (3) blood pressure (mmHg), (4) electrocardiogram (mV), and (5) capnography (mmHg). Example from one of the subjects from this project. Personal unpublished data.

CURRENT CHALLENGES IN CEREBRAL SMALL VESSEL DISEASE

Despite its high prevalence and debilitating consequences, there is a lack of effective interventions for CSVD. As discussed previously, current management strategies primarily focus on addressing underlying risk factors, but there is a pressing need for more research to identify and develop new therapeutic options. Given the chronic and progressive nature of CSVD, effective treatments will likely require early intervention and long-term use. Developing such interventions is complicated by the lack of understanding of the underlying mechanisms of CSVD and the need for reliable, safe, and affordable biomarkers for the disease. Furthermore, there is considerable variability in the selection and definition of endpoints for CSVD trials, primarily relying on traditional imaging markers and clinically relevant changes.

HT is by far the most important modifiable VRF for CSVD.[43] However, the relationship between HT and the brain is complex, and evidence suggests a role for the brain in developing HT.[199] Diagnosis and pharmacological treatment for HT currently rely on measuring systolic and diastolic BP, and high-normal BP is not recommended for pharmacological intervention. Additionally, brain imaging for HT patients without neurological symptoms is not supported due to cost and availability issues.[200] Nevertheless, evidence suggests early cerebrovascular and cognitive dysfunction in HT before reaching diagnostic BP levels.[199] Worse cognitive performance has been demonstrated even in prehypertensive young adults during sleep deprivation compared to normotensives.[26] There is evidence of impaired cognition in the high-normotensive range in midlife, an effect lost in late life.[201] Long-term BP variability from healthy young adulthood is associated with worse verbal memory and psychomotor speed in midlife.[202] Recent genetic analyses from large consortia also indicate that some patients with more severe CSVD may be susceptible to even small BP elevations.[203] It would be useful to identify these individuals for whom more intensive BP control may be recommended. Consequently, finding early and intermediate markers of CSVD in HT is crucial for refining targeted prevention and management strategies, selecting patients for clinical trials, and evaluating prognosis.

The brain is an extraordinarily metabolically active organ, requiring precise and dynamic mechanisms for regulating CBF and maintaining the health and longevity of neurons. Changes in cerebral hemodynamics could be the key to an early diagnosis of cerebral small vessel damage due to HT. In chronic HT, the cerebral autoregulatory curve is right-shifted to prevent the transmission of increased systemic pressure to the cerebral circulation.[204] Interestingly, studies on dCA have reported no significant differences or even better dCA in HT patients compared to normotensive individuals.[205-210] These studies indicate that, despite numerous structural and functional vascular changes, HT patients can keep the CBF adequate to basal

neuronal metabolic demand and protect the brain tissue from damage related to pressure variability. Some studies have shown an association between increased CSVD burden and impaired dCA.[211-213] Guo *et al.* found that dCA impairment is diffuse and sustained in patients with lacunar infarction.[214] In contrast, other studies have failed to find a correlation between dCA and traditional imaging markers of CSVD but found less effective dCA with greater damage in NAWM.[153,215] A study found better dCA in the posterior circulation with increased WMH burden.[216]

$VRCO_2$ has been less studied, but impaired $VRCO_2$ has been demonstrated in HT patients compared to normotensive individuals.[57,205,217-219] However, intact $VRCO_2$ has also been shown in recently diagnosed, untreated HT.[220] In addition, the effects of antihypertensive medication on $VRCO_2$ have also shown mixed results.[221,222] $VRCO_2$ was found to be impaired in patients with imaging markers of CSVD when compared to controls.[223,224] Nonetheless, the $VRCO_2$ decline was dissociated from the radiological progression of CSVD markers over 24 months, and the progressive endothelial dysfunction occurred despite well-controlled HT.[224] In studies using MRI techniques, impaired $VRCO_2$ has also been associated with increased CSVD burden and even progression of WMH.[225,226] However, Hajjar and colleagues demonstrated reduced $VRCO_2$ in HT patients irrespective of previous stroke or WMH burden, suggesting that this impairment can precede the clinical and imaging manifestations.[218] A study in presymptomatic patients with a genetic form of CSVD demonstrated a significant $VRCO_2$ decrease in the occipital lobe, also suggesting that it may be a useful biomarker for the early detection of microvascular pathology.[227]

Studies have suggested a causative role of sympathetic activation in initiating sustained BP increases, leading to HT. Repeated activation related to stress would cause sensitization and amplification of the sympathetic responses, leading to cardiovascular and cerebrovascular adaptations that exceed the brain's metabolic demands.[228,229] These adaptations, in addition to causing sustained cardiovascular stress responses, appear to redistribute the CBF to areas involved in the stress response, reducing the CBF available to cognitive processing areas.[199] This could explain the reductions in cognitive performance in young individuals with elevated BP preceding diagnostic levels of HT. It could also explain the normal or improved dCA observed in HT patients and the early endothelial damage, resulting in the blunted CBF in response to varying CO_2 levels observed in HT patients, despite normal dCA.

Studying functional hyperemia in response to cognitive tasks could be a more sensitive marker for early HT-induced cerebrovascular damage. Few studies have investigated NVC in HT patients, mainly using expensive imaging modalities.[62] A study in prehypertensive midlife

individuals using ALS has shown less CBF response to executive cognitive challenge in frontostriatal and frontoparietal regions.[230] In older HT patients, the same technique demonstrated reduced CBF within WMH but not in the surrounding NAWM, suggesting that markers of CSVD may relate better with local microvascular changes than with more generalised perfusion deficits.[231] In a cohort of community-dwelling seniors, NVC was assessed using TCD, and slower walkers demonstrated impaired NVC, irrespective of WMH burden and HT diagnosis.[118] The same group showed an association between better NVC in the MCA and better cognitive function and preserved WM integrity in elderly individuals.[232]

More studies reflecting the microstructural integrity and function of cerebral vasculature are needed to further add to the current understanding of the pathophysiology of microvascular damage in HT, particularly in earlier stages. Functional hyperemia has been less explored in the current literature, especially in the posterior circulation, and could provide a tool for monitoring disease progression and response to therapeutic measures.

As discussed in the "Etiology/Vascular risk factors" section, there is growing evidence of the increased prevalence of cerebrovascular disease in patients with chronic kidney disease (CKD). However, it is unclear whether there is a causative relation or if it is due to the shared traditional VRF.[88,90,233]. In addition, studies suggest that renal dysfunction increases the risk of cognitive impairment.[89,90,233]

This cerebrorenal correlation may derive from small vessel disease. The juxtamedullary afferent arterioles in the kidney and the perforating arteries in the brain share similarities in their hemodynamic proprieties. They are low-resistance vessels exposed to high-pressure fluctuations with a high vascular tone.[234] Both need highly effective myogenic, metabolic and neural responses to counter transmural pressure changes and regulate local blood flow.[235] Hence, markers of renal dysfunction could provide information about cerebral small vessel dysfunction in at-risk patients. Recent studies have shown increased prevalence and severity of MRI markers of CSVD with deteriorating eGFR in CKD patients, suggesting that eGFR may be a valuable predictor of CSVD.[93,233] Likewise, microalbuminuria has been associated with cortical thinning, WMH and cognitive decline.[236,237,203,238] Albuminuria results from endothelial damage to the kidney and is increasingly considered a marker of pressure-induced injury and generalized endothelial dysfunction.[239]

In spontaneously hypertensive rats, both renal and cerebral autoregulation are impaired before the development of stroke and death.[240,241] These studies suggest that the same hemodynamic dysfunction occurs in both vascular beds and that monitoring renal function could inform cerebral microvascular damage. However, little is known about the relationship

between kidney function and cerebral hemodynamics. In acute stroke patients, less effective dCA in the MCA correlates with lower renal function.[242] In CKD, the increased risk of stroke appears to be mediated by impaired dCA.[243] However, the associations between dCA, $VRCO_2$, and NVC and renal function remain largely unstudied.

Excess dietary salt is a worldwide concern. High dietary salt is a key risk factor for HT, increasing stroke risk, morbidity, and mortality.[244,245] This association is the base for international organisations' daily salt intake recommendations. The World Health Organization and the American Heart Association propose a consumption of no more than 2.0 g/day or 2.3 g/day of sodium (Na^+), respectively, for normal, healthy adults.[246,247] Most European Countries, however, greatly exceed this value.[248] The average intake in Portugal is more than double the recommended consumption.[65] Besides its association with HT, the health consequences of excessive salt intake are still a subject of debate. Individual differences in salt sensitivity may partly be responsible for this controversy.[249] Nevertheless, high salt consumption has been independently linked to an increased risk of stroke, CSVD, and dementia.[94,250,251] Thus, it is critical to understand the impact of dietary salt on CBF regulation. Studies using animal models demonstrated that high salt intake impairs the ability to maintain a constant CBF during decreases in systemic BP and impairs relaxation of the MCA during hypoxia, suggesting altered CA and vasoreactivity.[252,253] However, there is a striking lack of knowledge of the effects of dietary salt on CBF regulation in humans.

Considering all this evidence, important gaps in knowledge remain to be addressed. Studying cerebral hemodynamics in HT patients could add to the current knowledge and help identify predictive biomarkers and viable therapeutic targets for CSVD, one of the greatest public health challenges.

PURPOSES

Main objective

The main objective of this project was to study cerebral blood flow regulation using the TCD, including dynamic cerebral autoregulation, cerebral vasoreactivity to CO_2, and neurovascular coupling, in a cohort of pre-symptomatic HT patients as a possible biomarker for CSVD.

Specific objectives

The following specific aims were established:

1. To investigate TCD markers of cerebrovascular regulation in HT patients without significant CSVD-related impairment, comparing them to those of healthy controls, to identify potential biomarkers for HT-induced CSVD.
2. To assess the potential impact of comorbid DM on HT-induced cerebral microvascular dysfunction in HT patients without significant CSVD-related impairment.
3. To examine the relationship between TCD markers of cerebrovascular regulation and MRI markers of CSVD, considered the gold standard, in HT patients without significant CSVD-related impairment.
4. To explore the association between TCD markers of cerebrovascular regulation and cognitive performance in HT patients without significant CSVD-related impairment, aiming to investigate the potential of the TCD as a tool for predicting cognitive function in HT.
5. To investigate whether markers of renal dysfunction are associated with TCD markers of cerebrovascular regulation in HT patients without significant CSVD-related impairment to assess their potential as predictors of microvascular dysfunction in HT.
6. To examine the relationship between dietary salt consumption and TCD markers of cerebrovascular regulation in HT patients without significant CSVD-related impairment as a potential predictor of microvascular dysfunction in HT.
7. To investigate the relationship between markers of renal dysfunction, dietary salt intake, MRI markers of CSVD, and cognitive performance (as indicators of CSVD) in HT patients without evident CSVD-related impairment.

CHAPTER II – METHODS

Institutional Framework and Foundations of the Research

The present study was conducted at the Neurosonology Unit of the Neurology Department of Centro Hospitalar Universitário de São João (CHUSJ), in collaboration with the Hypertension Unit of Unidade Local de Saúde de Matosinhos (ULSM). The project also benefited from the expertise of Professor Farzaneh Sorond and her team from the Feinberg School of Medicine in Chicago, USA.

As a neurologist with a special interest in dementia, the recognition that CSVD represents a significant contributor to cognitive decline and vascular dementia fueled my interest in investigating this condition.

The genesis of this project was catalyzed by the convergence of several factors. Firstly, during the Neurology residency, my exposure to the Neurosonology Unit´s extensive experience with cerebral vasoregulation studies, including work on genetic forms of CSVD, sparked my interest in extending this knowledge to HT patients, a population at heightened risk for CSVD. Simultaneously, there was a historic collaboration with the ULSM´s Hypertension Unit, with a previous project focusing on the prospective study of systemic and cerebral markers of atherosclerosis in a cohort of HT patients. This synergy between institutions provided an opportunity to expand our investigations to include CSVD in HT and facilitated the integration of expertise, resources, and patient cohorts. Finally, the long-term collaborative relationship between Professor Elsa Azevedo and Professor Farzaneh Sorond enabled the engagement of Professor Farzaneh Sorond's team to contribute expertise in MRI imaging segmentation for the evaluation of CSVD markers.

Study population and clinical evaluation

Hypertensive patients

HT patients were recruited from the ULSM´s Hypertension Unit outpatient clinic, including patients from the original cohort and new HT patients without symptoms of CSVD.

The study had a cross-sectional observational design. Participants' clinical and demographic data were collected, including age, gender, past medical history, and medication. The recruited patients routinely underwent a comprehensive assessment, including a broad laboratory workup and ambulatory blood pressure monitoring (ABPM) (Spacelabs 90207,Redmond, Washington, USA). Additional investigations were conducted as part of the project, encompassing cervical and transcranial Doppler ultrasound examinations (Philips iu22, The Nederlands), cerebral MRI (Siemens Aera 1.5T scanner), and neuropsychological

evaluation. All patients were evaluated by an ophthalmologist, and all had normal binocular visual acuity, allowing for the TCD dynamic testing.

Patients´ vascular comorbidities were summarised into a vascular comorbidity score (VCS), including HT, DM, dyslipidemia, tobacco usage, chronic heart failure, coronary heart disease, arrhythmias, valvulopathy, peripheral artery disease, and nephropathy. These conditions were scored as present (1 point) or absent (0 points) for a score ranging from 0 to 9.[254] The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, and the CKD stage was classified based on eGFR. The albumin-to-creatinine ratio (ACR) from first-morning urine was calculated.

Exclusion criteria were previous stroke or transient ischemic attack or other significant brain pathology (dementia by clinical criteria, brain tumor, traumatic brain injury, previous cerebral infection, or neurodegenerative disease), severe/unstable disease, contraindication for magnetic resonance imaging (MRI), inadequate acoustic temporal bone window, extra- or intracranial artery stenosis >50% and incapability to collaborate or to give informed consent.

Healthy controls

Data from healthy controls of similar age and gender, without vascular risk factors, were obtained from previous studies performed in the Neurosonology Unit and were used for comparison. The data included clinical and demographic characteristics and TCD monitoring data.

Magnetic resonance imaging

Patients who were eligible and consented underwent cerebral MRI. Cortical reconstruction and volumetric segmentation were performed using T1 weighted volumetric sequences collected in the sagittal plane with the Freesurfer image analysis suite (Freesurfer v7.1.0.), documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/). Voxels resolution was 1 x 1 x 1, slices=256, FOV=255, TR=2730, TE=2.5, TI=1000, FA=7. White matter hyperintensity (WMH) volumes were derived from the T2-weighted fluid-attenuated inversion recovery sequences collected in the sagittal plane. Voxels resolution was 1 x 1 x 1, slices=256, FOV=256, TR=5000, TE=336, TI=1800. Briefly, WMH masks were created using the Lesion Segmentation Algorithm (LPA, 1) from the Lesion Segmentation Toolbox for SPM12 in MATLAB R2018a. Following an initial segmentation of the FLAIR image, probability maps were binarised using AFNI (2.3, v21.0.15)

command 3dcalc. The resulting segmentations were quality-checked for sufficient accuracy, and volumes were calculated using Freesurfer (v7.1) command mri_segstats. Volumes were normalized by intracranial volume.

The healthy control group did not undergo MRI imaging.

Neuropsychological examination

An experienced neuropsychologist performed a comprehensive neuropsychological evaluation in a quiet room. The neuropsychological protocol was chosen in conjunction with the Neuropsychologist to focus on the cognitive functions that are most sensitive to vascular cognitive impairment, namely attention, executive functioning, processing speed, and delayed recall.[255,256] The Hospital Anxiety and Depression Scale (HADS) was also included in the evaluation to account for the effects of anxiety and depression on cognitive performance.

The examination included the Mini-Mental State Examination (MMSE), the Montreal Cognitive Assessment (MoCA), verbal fluency (semantic and phonemic), the Auditory-Verbal Learning Test (AVLT), the Stroop Test, the Trail Making Test parts A and B (TMT-A and TMT-B), and the Digit Symbol and Symbol Search tests from the Wechsler Adult Intelligence Scale III (WAIS-III).

Raw test scores were standardized into z-scores for each individual test, and a composite z-score was derived covering attention and executive function, processing speed, and memory. These domains were assessed with the following tests: 1) attention and executive function: TMT-B, Stroop Test part 3 and verbal fluency (semantic, using “animals” and phonemic, using the letters “M”, “P” and “R”); 2) processing speed TMT-A, the Stroop Test parts 1and 2 and the Digit Symbol and the Symbol Search of the WAIS-III; 3) memory: AVLT total score. The total cognitive score was also calculated by adding the scores from the three domains.

The healthy control group did not undergo neuropsychological evaluation.

Transcranial Doppler monitoring protocol

The participants underwent assessment in a dedicated room designed specifically for conducting TCD monitoring studies within the Neurosonology Unit at CHUSJ. They refrained from caffeine, alcohol, exercise, or vasoactive drugs for at least 12 hours before evaluation. 2-MHz TCD probes were secured using a headframe (Doppler BoxX, DWL, Singen, Germany), and CBFV was continuously recorded in the M1 segment of the right MCA and the P2 segment of the left PCA to obtain data from both arterial territories simultaneously. Protocols for dCA,

$VRCO_2$, and NVC were conducted for hypertensive patients as explained in the section "The transcranial Doppler in the study of cerebral hemodynamics.

The controls underwent the same protocols for dCA and $VRCO_2$, but NVC was only performed for the PCA territory using the flickering checkerboard.

We established a 3-month time frame for each patient to perform the TCD, neuropsychological, and MRI evaluations to keep them as temporally close as possible.

CHAPTER III – RESULTS

PARTICIPANT DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

Out of the initial 60 patients enrolled in the study, 3 were excluded due to past lacunar stroke and 1 due to the absence of a suitable acoustic temporal bone window for TCD assessment. Also, 4 patients were enrolled between the analyses for papers 1 and 2. Of the enrolled patients, only 52 attended the neuropsychological evaluation, and 48 consented to undergo cerebral MRI. In addition, data from 6 of the MRIs were excluded from analysis due to insufficient quality. Data from 17 healthy controls of similar age and gender were available for comparison.

The subsequent tables offer detailed insight into the demographic and clinical profiles of the study participants. Table 1 outlines the key characteristics of the patient cohort, including demographic data, clinical parameters, and relevant medical history. Table 2 provides detailed patient systemic and cerebral hemodynamic parameters derived from TCD monitoring. Finally, Table 3 presents data specific to the healthy control group.

The patient cohort exhibited well-controlled HT, as evidenced by the 24-hour ABPM. It is also important to highlight the low educational attainment within the patient group. Seven patients scored below the validated cutoff points for MCI in the MoCA test.

These tables serve as reference points for the subsequent analyses and discussions in the following sections.

Table 1. Patient characterization

Demographics and comorbidities	**Value**	***Laboratory (cont.)***	**Value**
Age, years†	64±10	Triglycerides, mg/dL‡	123±97
Male, n (%)	32 (57)	HbA1C, %‡	
BMI, kg/m2‡	29±5	HT-DM	7.0±1.3
Diabetes Mellitus, n (%)	30 (58)	HT-nDM	5.6±0.6
Dyslipidemia, n (%)	49 (88)	eGFR, ml/min/1.73 m2†	76±24
Tobacco consumption, n (%)	7 (13)	Daily salt intake, g/24h†	12±4
Nephropathy, n (%)		ACR, mg/g‡	18±66
CDK Stage 0–1	18 (32)	***Craniocervical atherosclerosis***	**Value**
CDK Stage 2	27(48)	Plaques (% stenosis), n (%)*	
CDK Stage 3	9(16)	No plaque	21 (38)
CDK Stage 4	2 (4)	≤30%	11 (20)
CDK Stage 5	0 (0)	31-50%	22 (40)
VCS, n (%)		IMT**†	0.7±0.2
1	2 (4)	***ABPM, mmHg‡***	***Value***
2	5 (9)	24-hour systolic BP	132±13
3	23 (41)	24-hour diastolic BP	78±13
4	14 (25)	24-hour pulse pressure	57±14
5	9 (16)	***Cognitive parameters***	***Value***
6	3 (5)	Education, years‡	4±5
HT duration, years‡	17±6	MMSE‡	28±3
Chronic medication	***Value***	MoCA‡	22±5
No. antihypertensives‡	3±2	Cognitive score – total†	-2.4±5.0
ACEI/ARB, n (%)	52 (93)	Cognitive score – EF†	-1.7±2.3
Diuretics, n (%)	44 (79)	Cognitive score - PS†	-0.6±3.0
CCB, n (%)	40 (71)	Cognitive score - memory‡	-0.3±1.0
BB, n (%)	15 (27)	HADS-D†	7±4
Alpha2-agonists, n (%)	4 (7)	HADS-A‡	8±5
Antiplatelets, n (%)	16 (29)	***MRI volumes***	***Value***
Statins, n (%)	44 (79)	Total cortical GM vol./ICV (%)†	27.1±2.2
Laboratory	**Value**	Subcortical GM vol./ICV (%)†	3.5±0.3
Total cholesterol, mg/dL‡	175±56	Total WM volume (%)†	30.0±2.8
LDL cholesterol, mg/dL‡	98±46	WMH vol./ICV (%)‡	0.1±0.6
HDL cholesterol, mg/dL†	45±11		

†Values are presented as mean ± standard deviation. ‡Values are presented as median ± interquartile range; *size of largest plaque; **Average of right and left sides ABPM: ambulatory blood pressure monitoring; ACEI: angiotensin-conversion enzyme inhibitor; ACR: albumin-to-creatinine ratio; ARB: angiotensin receptor blocker; BB: beta-blocker; BMI: body mass index; BP: blood pressure; CCB: calcium channel blocker; CDK: Chronic Kidney Disease; executive function; EF: executive function; eGFR: estimated glomerular filtration rate; GM: grey matter; HADS-D: Hospital Anxiety and Depression Scale - depression; HADS-A: Hospital Anxiety and Depression Scale – anxiety; HbA1C: glycated hemoglobin; HDL: High-density lipoprotein; HT: hypertension; HT-DM: hypertensive diabetic; HT-nDM: hypertensive non-diabetic; ICV: intracranial volume; IMT: intima-media thickness;LDL: Low-density lipoprotein; MMSE: mini-mental state examination; MoCA: Montreal cognitive assessment; PS: processing speed; VCS: vascular comorbidity score; WM: white matter; WMH: white matter hyperintensities.

Table 2. Patient systemic and cerebral hemodynamics

Systemic hemodynamics		
Finapres BP (5 minutes)		
Systolic BP†	136±24	
Diastolic BP†	61±17	
Mean BP SP, mmHg2‡	9±22	
EtCO2, mmHg‡	36±5	
Cerebral hemodynamics	**MCA**	**PCA**
Mean CBFV (cm/s)†	47.1±13.2	31.5±8.3
MFV SP (cm/s²)‡	3.2±5.0	2.5±5.0
CVRi (mmHg·s·cm-1)‡	1.8±0.8	2.7±1.3
Cerebral autoregulation		
VLF gain (%/mmHg)	0.8±0.5†	0.9±0.5‡
LF gain (%/mmHg)	1.0±0.6‡	1.3±0.6†
VLF phase (radians)	1.0±0.4†	0.9±0.4†
LF phase (radians)	0.7±0.3†	0.7±0.5‡
$VRCO_2$ (%/mmHg CO_2)	1.3±0.5†	0.9±0.5‡
Neurovascular coupling†		
N-1 back	-0.8±5.6	-
N-2 back	4.0±5.8	-
Overshoot‖ systolic CBFV (%)	-	22.0±9.4
Gain (%)	-	13.8±7.2
Natural frequency (Hz)	-	0.2±0.0
Attenuation (a.u)	-	0.4±0.3
Rate time (s)	-	0.6±1.7

†Values are presented as mean ± standard deviation. ‡Values are presented as median ± interquartile range; ||Maximal CBFV increase during visual stimulation. a.u.: arbitrary units; CBFV: cerebral blood flow velocity; CVRi: cerebrovascular resistance index; $EtCO_2$: end-tidal carbon dioxide; LF: low frequency; MCA: middle cerebral artery; Mean BP SP: mean blood pressure spectral power; MFV SP: median flow velocity spectral power; PCA: posterior cerebral artery; VLF: very low frequency; $VRCO_2$: vasoreactivity to CO_2.

Table 3. Data from healthy controls

Demographics and comorbidities	**Value**	
Age, years†	60±16	
Male, n (%)	8 (47)	
BMI, kg/m^{2}‡	25±4	
Diabetes Mellitus, n (%)	0 (0)	
VCS, n (%)		
0	17 (100)	
1-6	0 (0)	
Chronic medication		
Antihypertensives, n (%)	0 (0)	
Antiplatelets, n (%)	0 (0)	
Statins, n (%)	0 (0)	
Antidiabetics, n(%)	0 (0)	
Systemic hemodynamics	**Value**	
Finapres BP (5 minutes)		
Systolic BP†	109±25	
Diastolic BP†	50±14	
Mean BP SP, mmHg2‡	7±8	
$EtCO_2$, mmHg‡	38±3	
Cerebral hemodynamics	**MCA**	**PCA**
Mean CBFV (cm/s)†	52.4±16.5	29.1±10.1
MFV SP (cm/s^{2})‡	3.8±4.3	2.3±3.1
Cerebral autoregulation		
VLF gain (%/mmHg)	0.7±0.3†	0.9±0.5‡
LF gain (%/mmHg)	1.4±0.5‡	1.7±0.5†
VLF phase (radians)	1.2±0.4†	1.1±0.4†
LF phase (radians)	0.8±0.2†	0.9±0.3‡
$VRCO_2$ (%/mmHg CO_2)	1.7±0.6†	1.0±0.7‡
Neurovascular coupling†		
Overshoot‖ systolic CBFV (%)	-	31.6±5.7†
Gain (%)	-	17.3±4.8†
Natural frequency (Hz)	-	0.22±0.06‡
Attenuation (a.u)	-	0.4±0.4‡
Rate time (s)	-	0.03±6.81‡

†Values are presented as mean ± standard deviation. ‡Values are presented as median ± interquartile range; ||Maximal CBFV increase during visual stimulation. a.u.: arbitrary units; CBFV: cerebral blood flow velocity; CVRi: cerebrovascular resistance index; $EtCO_2$: end-tidal carbon dioxide; LF: low frequency; MCA: middle cerebral artery; Mean BP SP: mean blood pressure spectral power; MFV SP: median flow velocity spectral power; PCA: posterior cerebral artery; VLF: very low frequency; $VRCO_2$: vasoreactivity to CO_2.

PAPER 1: NEUROVASCULAR COUPLING IS IMPAIRED IN HYPERTENSIVE AND DIABETIC SUBJECTS WITHOUT SYMPTOMATIC CEREBROVASCULAR DISEASE

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Additional evaluations (not included in the paper)

Dynamic cerebral autoregulation: HT patients vs healthy controls

Dynamic cerebral autoregulation: nDM-HT patients vs DM-HT vs healthy controls

Association between cerebrovascular hemodynamics and MRI markers of CSVD in hypertensive patients

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Neurovascular Coupling Is Impaired in Hypertensive and Diabetic Subjects Without Symptomatic Cerebrovascular Disease

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The mechanistic link between hypertension, diabetes and cerebral small vessel disease (CSVD) is still poorly understood. We hypothesized that hypertension and diabetes could impair cerebrovascular regulation prior to irreversibly established cerebrovascular disease. In this study, 52 hypertensive patients [54% males; age 64 ± 11 years; 58% with comorbid diabetes mellitus (DM)] without symptomatic cerebrovascular disease underwent transcranial Doppler (TCD) monitoring in the middle (MCA) and posterior (PCA) cerebral arteries, to assess vasoreactivity to carbon dioxide ($VRCO_2$) and neurovascular coupling (NVC). 1.5T magnetic resonance imaging was also performed and white matter hyperintensity volume was automatically segmented from FLAIR sequences. TCD data from 17 healthy controls were obtained for comparison (47% males; age 60 ± 16 years). Hypertensive patients showed significant impairment of NVC in the PCA, with reduced increment in cerebral blood flow velocity during visual stimulation (22.4 ± 9.2 vs. 31.6 ± 5.7, $p < 0.001$), as well as disturbed NVC time-varying properties, with slower response (lower rate time: 0.00 ± 0.02 vs. 0.03 ± 6.81, $p = 0.001$), and reduced system oscillation (reduced natural frequency: 0.18 ± 0.08 vs. 0.22 ± 0.06, $p < 0.001$), when compared to controls. $VRCO_2$ remained relatively preserved in MCA and PCA. These results were worse in hypertensive diabetic patients, with lower natural frequency ($p = 0.043$) than non-diabetic patients. White matter disease burden did not predict worse NVC. These findings suggest that hypertensive diabetic patients may have a precocious impairment of NVC, already occurring without symptomatic CSVD. Future research is warranted to evaluate whether NVC assessment could be useful as an early, non-invasive, surrogate marker for CSVD.

Keywords: hypertension, diabetes mellitus, neurovascular coupling (NVC), transcranial doppler (TCD), cerebral small vessel disease

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INTRODUCTION

Cerebral small vessel disease (CSVD) has an enormous impact on public health worldwide (GBD 2017 Causes of Death Collaborators, 2018). It accounts for 25% of ischemic strokes and most hemorrhagic strokes and is the second leading cause for cognitive decline (Sudlow and Warlow, 1997; Iadecola et al., 2019).

Hypertension (HT) is the major vascular risk factor (VRF) for CSVD. Alongside HT, diabetes mellitus (DM) is a recognized VRF implicated in CSVD (Brundel et al., 2012; Liu et al., 2018).

While other etiologies for stroke are fairly well-studied, the pathophysiology and causality of CSVD are still poorly understood. Many of its manifestations are clinically silent until the development of clinical consequences, with stroke, cognitive decline, and gait impairment, limiting disease-specific preventive strategies (Pantoni, 2010). Also, CSVD radiological markers and clinical manifestations seem to be dissociated, for reasons not fully explained (Sorond et al., 2011; Jokumsen-Cabral et al., 2019). Biomarkers for the events predating irreversible damage could be key for better clinical management and pre-symptomatic preventive measures.

There is evidence of neurovascular dysfunction in CSVD and it may precede clinical and imaging manifestations (Wardlaw, 2010; Freeze et al., 2018; Castro et al., 2020). Very few studies have investigated neurovascular coupling (NVC) in HT, mostly using imaging modalities. Transcranial Doppler (TCD) is a non-invasive method that allows the monitoring of microvascular hemodynamic functional integrity (Claassen et al., 2016; Malojcic et al., 2017).

We aimed to study cerebrovascular regulation by TCD in hypertensive and diabetic patients without major CSVD-related impairment as a possible surrogate marker for the future development of symptomatic CSVD, to help guide therapies aimed at the cerebral microcirculation and neurovascular unit.

MATERIALS AND METHODS

Study Subjects

A cross-sectional observational study was conducted in a University Hospital. Hypertensive patients were recruited from the hospital's Hypertension Unit. Exclusion criteria were previous stroke or other significant brain pathology (dementia by clinical criteria, brain tumor, traumatic brain injury, previous cerebral infection or neurodegenerative disease), severe/unstable disease, contraindication for magnetic resonance imaging (MRI), inadequate acoustic temporal bone window, extra- or intracranial artery stenosis >50% and incapability to collaborate or to give informed consent. TCD data from healthy controls of similar age and gender were obtained from previous studies performed with the same protocol (Jokumsen-Cabral et al., 2019).

The local ethics committee approved the study protocol, which followed the tenets of the Declaration of Helsinki. Written informed consent was obtained.

Clinical Evaluation

Participant's clinical and demographic data were recorded. Vascular comorbidities were summarized into a vascular comorbidity score (VCS), including HT, DM, dyslipidemia, tobacco usage, chronic heart failure, coronary heart disease, arrhythmias, peripheral artery disease, and nephropathy. These conditions were scored as present (1 point) or absent (0 points), for a score ranging from 0 to 9 (Mossello et al., 2015). All participants underwent cervical and transcranial Doppler ultrasound (Philips iu22, The Nederlands) to exclude hemodynamically significant vessel pathology. The patients underwent routine 24-h ambulatory blood pressure monitoring (Spacelabs 90207, Redmond, Washington, USA). The mini-mental state examination (MMSE) and the Montreal cognitive assessment (MoCA) were used to screen for dementia. The patients were evaluated by an ophthalmologist, and all had normal binocular visual acuity, allowing for the TCD dynamic testing.

Monitoring Protocol

Evaluations were conducted in a dim-lighted, quiet room (~22°C), in a supine position. Cerebral blood flow velocity (CBFV) was continuously recorded in the M1 segment of the right middle cerebral artery (MCA) and the P2 segment of the left posterior cerebral artery (PCA), with 2-MHz TCD probes secured with a headframe (Doppler BoxX, DWL, Singen, Germany), in order to simultaneously obtain data from both arterial territories (Azevedo et al., 2012). Continuous non-invasive arterial blood pressure (BP) was measured with the Finometer (FMS, Amsterdam, The Netherlands). Heart rate was assessed with a three-lead electrocardiogram. End-tidal carbon dioxide ($EtCO_2$) was recorded by capnography (Respsense Nonin, Amsterdam, The Netherlands). Data was synchronized and digitally stored at 400 Hz with Powerlab (AD Instruments, Oxford, UK) for offline analysis. After resting for 20 min, the vasoreactivity to carbon dioxide ($VRCO_2$) and NVC protocols were performed, as described below. CBFV envelopes were continuously registered and analyzed offline.

$VRCO_2$

Participants were monitored through successive 2-min steps of resting, inhalation of a mixture of 5% CO_2 and 95% O_2 mixture ($EtCO_2$ 7–10 mmHg above baseline), resting (room air, until normocapnia) and hyperventilation ($EtCO_2$ 7–10 mmHg below baseline). $VRCO_2$ was calculated as the slope of the relationship between $EtCO_2$ average values plotted against those of relative CBFV achieved at the three stages, expressed as % mean CBFV per mmHg $EtCO_2$ (Madureira et al., 2017).

NVC

NVC was assessed in the PCA territory by a visual paradigm consisting of 10 cycles, each with a 20s resting phase (eyes closed) and 40 s stimulating phase (flickering checkerboard) at 10 Hz (Rosengarten et al., 2001a). The 5s of stable measurement prior to stimulation were used as the baseline (Rosengarten et al., 2001a). All cycles were synchronized and averaged. Peak systolic data was used because it is less prone to artifacts (Rosengarten

et al., 2001b). Maximal systolic CBFV change was obtained to calculate the overshoot parameter as $\frac{maximumCBFV-baselineCBFV}{baselineCBFV} \times 100\%$.[12] The systolic CBFV curve was modeled into a second order linear system to describe the dynamics of NVC response in time according to the equation $G(s) = \frac{K \times (1+Tvs)}{\frac{s^2}{\omega^2}+2\xi*\frac{s}{\omega}+1}$, where "K" stands for gain, "Tv" for rate time, "ω" for natural frequency, and "ξ" for attenuation (Rosengarten et al., 2001a). All the parameters of the equation were determined by the least squares method. The sum of the squared residuals and the $\chi 2$ were also calculated to ensure the goodness of fit into the real measured values, as provided by the lsqnonlin function. Gain describes the relative

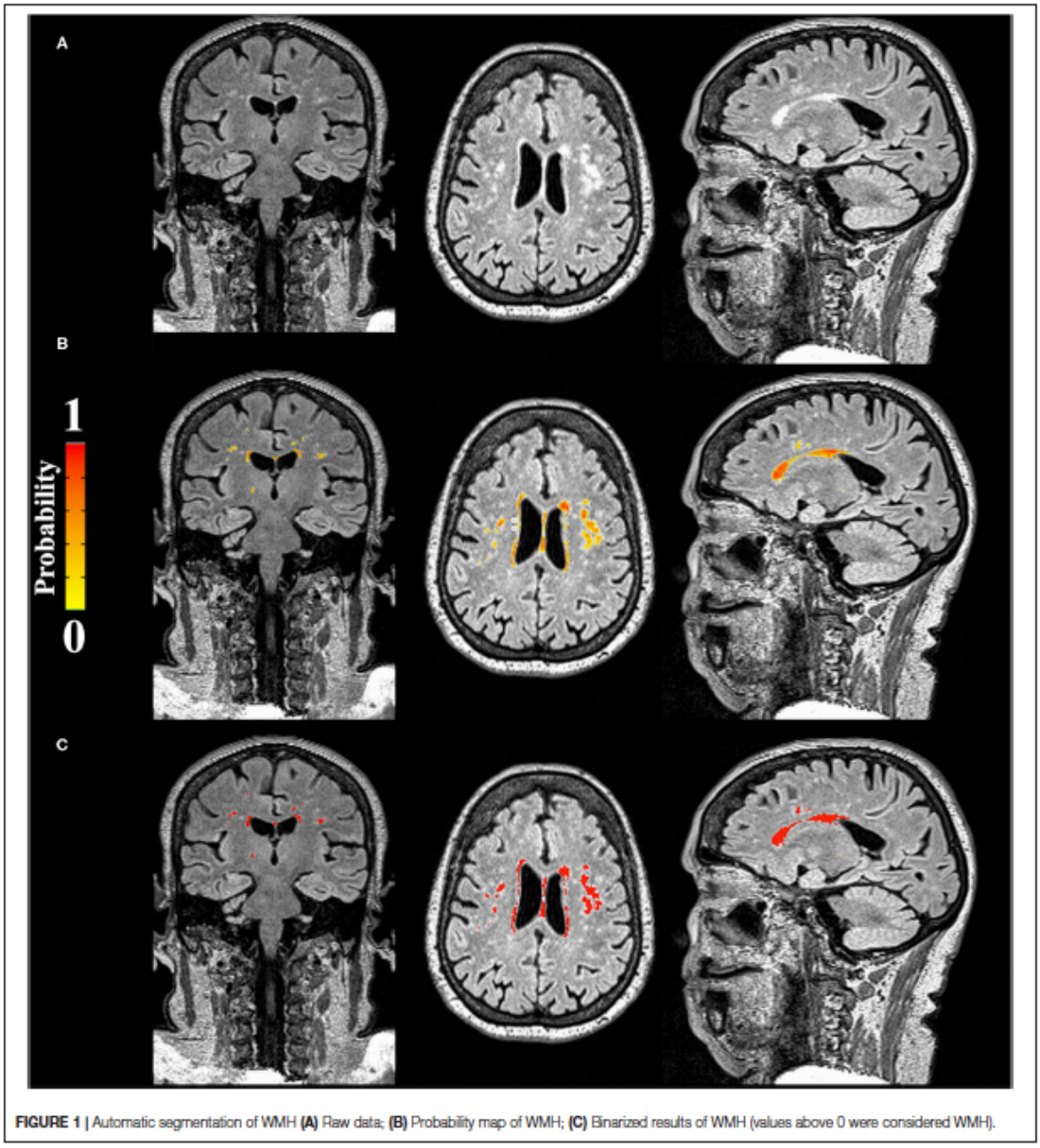

FIGURE 1 | Automatic segmentation of WMH **(A)** Raw data; **(B)** Probability map of WMH; **(C)** Binarized results of WMH (values above 0 were considered WMH).

CBFV difference between rest and steady-state level during visual stimulation; rate time indicates the initial steepness of the CBFV increase; natural frequency represents the oscillatory properties of the system; and attenuation describes dampening and tonus features, such as elastic properties of the vessel wall (Rosengarten et al., 2003). For this specific test, the MCA recordings were used as a control to detect non-specific changes in CBFV during the visual stimulation task.

MRI Imaging

Forty-six patients were eligible and agreed to undergo cerebral MRI (Siemens Aera 1.5T). Of these, data from 6 patients were excluded for lack of quality for the evaluations. White matter hyperintensity (WMH) volumes normalized by intracranial volume were derived from the T2-weighted fluid-attenuated inversion recovery sequences collected in the sagittal plane. Voxels resolution was 1 x 1 x 1, slices = 256, FOV = 256 mm, TR = 5000 ms, TE = 336 ms, TI = 1800 ms (**Figure 1**). Briefly, WMH masks were created using the Lesion Segmentation Algorithm (LPA, 1) from the Lesion Segmentation Toolbox for SPM12 in MATLAB R2018a. Following an initial segmentation of the FLAIR image, probability maps were binarized using AFNI (2,3, v21.0.15) command 3dcalc. Resulting segmentations were quality-checked for sufficient accuracy and volumes were calculated using Freesurfer (v7.1) command mri_segstats.

Additional signs of CSVD were evaluated by an experienced vascular Neurologist to further characterize the CSVD in the patient cohort. Enlarged perivascular spaces (PVS) were defined as small (<0.3 mm) punctate or linear (if perpendicular or longitudinal to the plane of the scan, respectively) hyperintensities on T2 images in the basal ganglia (BG) and centrum semiovale (CS) (Potter et al., 2015). The PVS burden was then stratified into three groups: <11, 11–20 and >20 (Lau et al., 2017). Lacunes were defined as rounded or ovoid lesions >3 mm and <20 mm in diameter in the BG, internal capsule, CS or brainsteam, with CSF density on T2 images (Wardlaw et al., 2013). Cerebral microbleeds were defined as round, hypodense lesions <10 mm on susceptibility weighted imaging collected in the axial plane (slice thickness 2 mm, slices = 256, FOV = 230 mm, TR = 49 ms, TE = 40 ms), according to the guidelines (Greenberg et al., 2009).

Statistical Analysis

Normality was determined using the Shapiro–Wilk test and analysis of skewness. Data with high asymmetry was normalized using logarithmic transformation. Homogeneity of variances was tested for each analysis. Baseline characteristics were compared using the independent sample *t*-test and χ2-test. Mixed ANOVA or ANCOVA (for PCA) were used to compare hemodynamic data. Subgroup analyses were performed using univariate ANOVA, with Bonferroni *post-hoc* tests. The partial eta squared (ηp^2) was used as a measure of effect size: $\eta p^2 > 0.14$ indicates a large effect, $\eta p^2 > 0.06$ indicates a medium effect and $\eta p^2 > 0.01$ indicates a small effect (Cohen, 1998). Age and gender were used as covariates in the ANOVA and ANCOVA comparisons. Supplemental analysis using body mass index (BMI) and VCS as covariates (plus age and gender) were also performed. Patients were dichotomized by the median value of WMH volume to compare the results of $VRCO_2$ and NVC in the PCA between the two WMH burden groups. Subgroups were compared using the independent sample *t*-test.

Values of $p < 0.05$ were considered significant.

RESULTS

Fifty-two hypertensive patients were evaluated and TCD data from 17 healthy controls were used. Baseline characteristics are reported in **Table 1**. **Supplementary File 1** depicts the burden of

TABLE 1 | Demographics and baseline characteristics.

Participant characteristics	Patients	Controls	*p* value*
Age, years (mean ± SD)	64 ± 11	60 ± 16	0.376
Male, n (%)	28 (54)	8 (47)	0.627
BMI, kg/m² (median ± IQR)	29 ± 5	25 ± 4	**<0.001**
Diabetes Mellitus, n (%)	30 (58)	0 (0)	
VCS, n (%)			
0	0 (0)	17 (100)	
1	2 (4)	0 (0)	
2	5 (10)	0 (0)	
3	22 (42)	0 (0)	
4	13 (25)	0 (0)	
5	8 (15)	0 (0)	
6	2 (4)	0 (0)	
HT duration, years (median ± IQR)	17 ± 6	0 (0)	
Chronic medication			
No. antihypertensives (median ± IQR)	3 ± 2	0 (0)	
ACEI/ARB, n (%)	48 (92)	0 (0)	
Diuretics, n (%)	39 (75)	0 (0)	
CCB, n (%)	37 (71)	0 (0)	
BB, n (%)	14 (27)	0 (0)	
Alpha2-agonists, n (%)	4 (8)	0 (0)	
Antiplatelets, n (%)	14 (27)	0 (0)	
Statins, n (%)	40 (77)	0 (0)	
Cognitive parameters (median ± IQR)			
Education, years	4 ± 5		
MMSE	28 ± 3		
MoCA	22 ± 5		
ABPM, mmHg (median ± IQR)			
24-h systolic BP	130 ± 15	-	-
24-h diastolic BP	78 ± 13	-	-
24-h pulse pressure	57 ± 16	-	-
Finapres BP, 5 min (mean ± SD)			
Systolic BP	136 ± 24	109 ± 25	**<0.001**
Diastolic BP	62 ± 17	50 ± 14	**0.035**
Mean BP SP, $mmHg_2$ (median ± IQR)	9 ± 24	7 ± 8	**0.007**
$EtCO_2$, mmHg (median ± IQR)	36 ± 4	38 ± 3	0.553

**Values were obtained using the t-test or the χ2-test. ABPM, ambulatory blood pressure monitoring; BMI, body mass index; BP, blood pressure; $EtCO_2$, end-tidal carbon dioxide; IQR, interquartile range; SD, standard deviation; SP, spectral power; VCS, vascular comorbidity score. Bold values of statistically significant p values (p < 0.05).*

PVS, microbleeds and lacunes in the patient group. Most patients had low burden of PVS in the BG (55.0%) and CS (65.0%). Nine patients (22.5%) presented lacunes and four patients (10%) presented microbleeds.

Cerebral Hemodynamics and $VRCO_2$

Baseline CBFV and CBFV variability (spectral power) were similar in patients and controls (**Table 2**). $VRCO_2$ in either territory (PCA or MCA) was similar between groups and showed no change after adjusting for both VCS and BMI (**Supplementary File 2**).

There were no differences in $VRCO_2$ between hypertensive non-diabetics (HT-nDM), hypertensive diabetics (HT-DM) and controls (**Table 3**). Those results did not change significantly after controlling for BMI or VCS (**Supplementary File 3**).

NVC

NVC in the PCA territory was significantly altered in HT patients, with smaller increases in CBFV during visual

TABLE 2 | Cerebral hemodynamics, $VRCO_2$ and NVC: patients vs. controls, controlling for age and gender.

	Patients		Controls		Artery	Group	Interaction
	MCA	**PCA**	**MCA**	**PCA**	***p* value***	***p* value***	***p* value***
Cerebral hemodynamics							
Mean CBFV (cm/s)	47.6 ± 13.4†	31.4 ± 8.2†	52.4 ± 16.5†	29.1 ± 10.1†	0.044	0.925	0.161
MFV SP (cm/s^2)	3.3 ± 5.0‡	2.5 ± 4.7‡	3.8 ± 4.3‡	2.3 ± 3.1‡	0.976	0.770	0.119
$VRCO_2$ (%/mmHg CO_2)	1.4 ± 0.5†	0.9 ± 0.4‡	1.7 ± 0.6†	1.0 ± 0.7‡	0.107	0.588‖	0.437
Neurovascular coupling							
Overshoot§ systolic CBFV (%)		22.4 ± 9.2†		31.6 ± 5.7†		**<0.001**	
Modeled parameters							
Gain (%)		14.0 ± 7.1†		17.3 ± 4.8†		0.118	
Natural frequency (Hz)		0.18 ± 0.08‡		0.22 ± 0.06‡		**<0.001**	
Attenuation (a.u)		0.4 ± 0.4‡		0.4 ± 0.4‡		0.374	
Rate time (s)		0.00 ± 0.02‡		0.03 ± 6.81‡		**0.001**‖	

**Two-factor mixed-design ANOVA for the interaction between group variable (patients vs. controls) and arterial territory (MCA vs. PCA), controlling for age and gender. For NVC, values were obtained using an ANCOVA. Effect size: rate time $\eta p^2 = 0.174$, natural frequency $\eta p^2 = 0.237$ and overshoot systolic CBFV $\eta p^2 = 0.186$. ‖gender significantly interfered with the model; ‖age significantly interfered with the model; †Values are presented as mean ± standard deviation. ‡Values are presented as median ± interquartile range. §Maximal CBFV increase during visual stimulation. a.u., arbitrary units; CBFV, cerebral blood flow velocity; $VRCO_2$, vasoreactivity to carbon dioxide; MFV SP, median flow velocity spectral power; MCA, middle cerebral artery; PCA, posterior cerebral artery. Bold values of statistically significant p values ($p < 0.05$).*

TABLE 3 | Cerebral hemodynamics, $VRCO_2$ and NVC: controls vs. HT-nDM vs. HT-DM patients, controlled for age and gender.

	HT-nDM		HT-DM		Controls		Group	HT-nDM vs. Controls	HT-DM vs. Controls	HT-nDM vs. HT-DM
	MCA	**PCA**	**MCA**	**PCA**	**MCA**	**PCA**	***p* value***	***p* value***	***p* value***	***p* value***
Cerebral hemodynamics										
Mean CBFV (cm/s)†	47.0 ± 14.1	33.4 ± 8.4	48.0 ± 13.2	30.0 ± 7.8	52.4 ± 16.5	29.1 ± 10.1	0.993			
MFV SP (cm/s^2)‡	3.1 ± 4.7	2.6 ± 5.0	3.7 ± 7.0	2.5 ± 5.0	3.8 ± 4.3	2.3 ± 3.1	0.921			
$VRCO_2$ (%/mmHg CO_2)†	1.4 ± 0.6	1.2 ± 0.7	1.4 ± 0.5	0.8 ± 0.3	1.7 ± 0.6	1.1 ± 0.4	0.676‖			
Neurovascular coupling										
Overshoot§ systolic CBFV (%)†		25.1 ± 8.6		20.7 ± 9.3		31.6 ± 5.7	**<0.001**	0.080	**<0.001**	0.248
Modeled parameters										
Gain (%)†		13.9 ± 5.1		14.1 ± 8.2		17.3 ± 4.8	0.283			
Natural frequency (Hz) †		0.19 ± 0.04		0.16 ± 0.05		0.23 ± 0.06	**<0.001**§	0.052	**<0.001**	**0.043**
Attenuation (a.u)†		0.4 ± 0.3		0.4 ± 0.3		0.5 ± 0.2	0.327‖			
Rate time (s)‡		0.00 ± 0.00		0.00 ± 0.26		0.03 ± 6.81	**0.005**‖	**0.012**	**0.011**	1.000

**Two-factor mixed-design ANOVA for the interaction between group variable (HT-DM vs. HT-nDM vs. controls) and arterial territory (MCA vs. PCA), controlling for age and gender, with Bonferroni post-hoc. For NVC, values were obtained using an ANCOVA. Effect size: rate time $\eta p^2 = 0.174$, natural frequency $\eta p^2 = 0.313$ and overshoot systolic CBFV $\eta p^2 = 0.22$. †Values are presented as mean ± standard deviation. ‡Values are presented as median ± interquartile range. §Maximal CBFV increase during visual stimulation. ‖gender significantly interfered with the model. ‖age significantly interfered with the model. a.u., arbitrary units; CBFV, cerebral blood flow velocity; HT-DM, hypertensive diabetic; HT-nDM, hypertensive non-diabetic; MCA, middle cerebral artery; MFV SP, median flow velocity spectral power; PCA, posterior cerebral artery; $VRCO_2$, vasoreactivity to carbon dioxide. Bold values of statistically significant p values ($p < 0.05$).*

stimulation ($p < 0.001$) and disturbed NVC time-varying properties, with lower natural frequency ($p < 0.001$) and lower rate time ($p = 0.001$) (**Table 2**; **Figure 2**). These results remained similar when adjusting for BMI and VCS, although the overshoot systolic CBFV was over the limit of statistical significance when adjusting for the VCS ($p = 0.065$) (**Supplementary File 2**).

NVC results were worse in HT-DM than HT-nDM (**Table 3**). For the overshoot systolic CBFV and for natural frequency, only patients with both comorbidities showed significant differences when comparing to controls. HT-DM showed lower natural frequency than non-diabetic patients ($p = 0.043$). When controlling for VCS, natural frequency was also worse in both HT-nDM ($p = 0.020$) and HT-DM ($p = 0.002$) when comparing to controls, with the worst results for HT-DM (HT-nDM vs. HT-DM, p=0.011) (**Supplementary File 3**).

Figure 3 represents the evoked systolic CBFV responses in the PCA and MCA during the visual stimulation in one HT subject, to demonstrate the individual NVC responses.

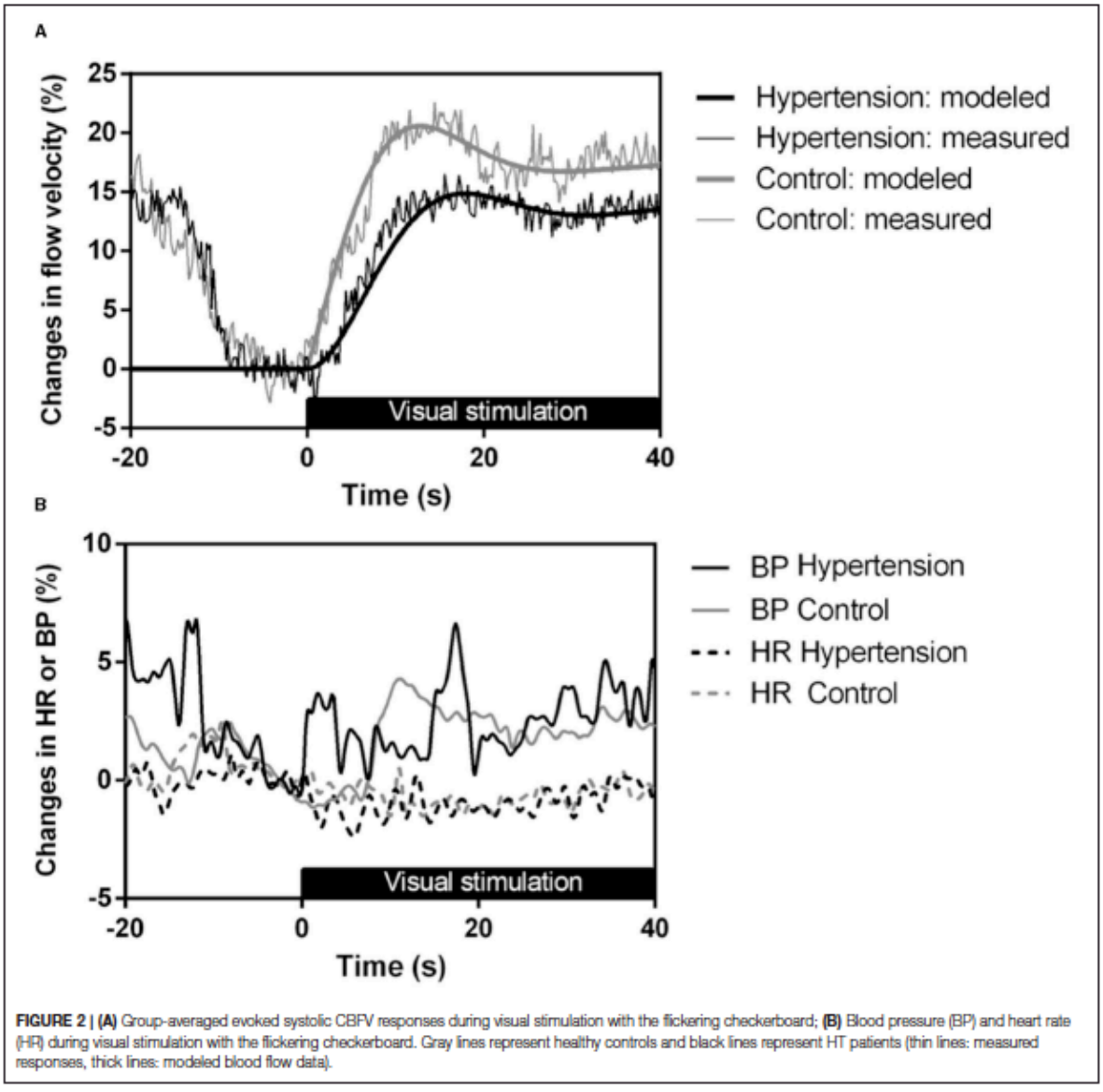

FIGURE 2 | (A) Group-averaged evoked systolic CBFV responses during visual stimulation with the flickering checkerboard; **(B)** Blood pressure (BP) and heart rate (HR) during visual stimulation with the flickering checkerboard. Gray lines represent healthy controls and black lines represent HT patients (thin lines: measured responses, thick lines: modeled blood flow data).

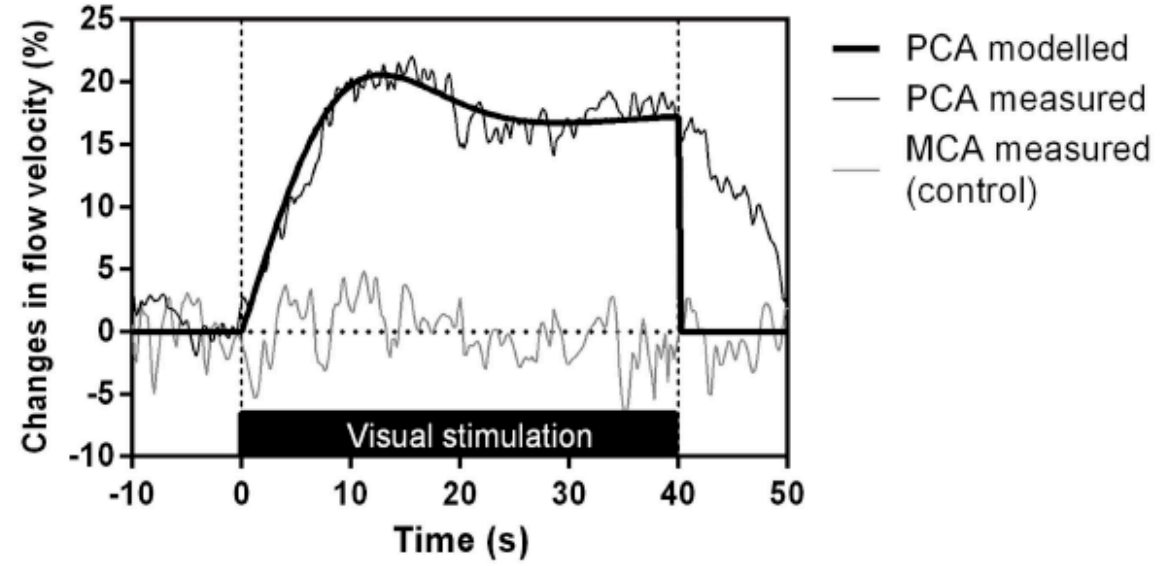

FIGURE 3 | Evoked systolic CBFV responses in the PCA and MCA during visual stimulation with the flickering checkerboard in one HT patient. The black lines represent the PCA response (thin lines: measured responses, thick lines: modeled blood flow data) and the gray lines represent the MCA measured response, used as a control.

TABLE 4 | NVC and $VRCO_2$ in relation to the WMH volume in hypertensive patients.

	WMH volume*					
	MCA			PCA		
	≤ 0.14	> 0.14	*p* value	≤ 0.14	> 0.14	*p* value
$VRCO_2$ (%/mmHg CO_2)	1.6 ± 0.5	1.2 ± 0.5	**0.004**	1.0 ± 0.6‡	0.8 ± 0.5‡	**0.006**
Neurovascular coupling						
Overshoot§ systolic CBFV, (%)	-	-	-	23.4 ± 10.0†	21.8 ± 8.8†	0.573
Gain (%)	-	-	-	11.7 ± 11.1‡	14.6 ± 7.9‡	0.297
Natural frequency (Hz)	-	-	-	0.2 ± 0.0†	0.2±0.1†	0.432
Attenuation (a.u)	-	-	-	0.4 ± 0.3†	0.4 ± 0.2†	0.891
Rate time (s)	-	-	-	0.0 ± 1.2‡	0.0 ± 0.0‡	0.169

**Dichotomized by median values. p values were obtained using the t-test. †Values are presented as mean ± standard deviation; ‡Values are presented as median ± interquartile range. §Maximal CBFV increase during visual stimulation. a.u., arbitrary units; CBFV, cerebral blood flow velocity; MCA, median cerebral artery; PCA, posterior cerebral artery; $VRCO_2$, vasoreactivity to carbon dioxide; WMH, white matter hyperintensities. Bold values of statistically significant p values (p < 0.05).*

Association With White Matter Hyperintensities

As shown in **Table 4**, $VRCO_2$ was lower in the higher burden group in both the MCA ($p = 0.004$) and the PCA ($p = 0.007$). NVC parameters did not differ in both groups.

DISCUSSION

Our study shows that NVC was significantly impaired in hypertensive patients, with reduced CBFV increase and altered time behavior hemodynamic evoked response during visual stimulation. Moreover, NVC tended to be worse in the DM subgroup. $VRCO_2$ remained relatively preserved. These results did not change when adjusting for other vascular risk factors. The novel finding is that the natural frequency seems to be the most sensitive parameter for discriminating abnormal NVC in these patients. Rosengarten and colleagues reported that this parameter had the lowest SD of the modeled parameters, thus having the potential for better differentiating between normal and abnormal NVC, but its capacity to identify vascular response dysfunction in disease settings needed to be studied (Rosengarten et al., 2001a). Our results seem to point toward natural frequency as a possible marker for NVC dysfunction in hypertension and diabetes.

Overall, the white matter disease (WMD) burden was low in the patient cohort. The NVC results were similar between

higher and lower WMD burden groups, while higher burden patients showed worse $VRCO_2$. It has been recently reported that reduced $VRCO_2$ precedes the development of WMH (Sam et al., 2016), which could help explain the difference between the disease burden groups. Similar NVC with higher and lower WMH volume has been previously reported (Sorond et al., 2013). Although WMH volume predicts an increased risk of stroke and cognitive decline, the clinical expression seems to vary (Sorond et al., 2011). Moreover, silent markers of CSVD are frequently detected on the MRI of older individuals without cognitive impairment (Vernooij et al., 2009; Debette and Markus, 2010). Thus, besides macrostructural changes, other modalities reflecting microstructural integrity and function, such as TCD dynamic studies, may provide additional information to further stratify patients at risk.

Neurovascular Unit at the Core of Target Brain Damage in Hypertension

Our results showed a significantly reduced PCA CBFV magnitude response and altered time behavior of reactive hyperemia during visual stimulation in HT patients, especially when diabetes was an added comorbidity. These findings indicate disturbed NVC in these patients' PCA cortical territory, independently of established WMD, as was observed by other groups (Birns et al., 2009; Purkayastha et al., 2014). The decreased CBFV during visual stimulation reflects less robust functional hyperemia, causing failure to meet the metabolic demands and neuronal damage (Iadecola et al., 2019).

The reduced overshoot systolic CBFV in the patient group was not accompanied by a reduction in the modeled parameter gain. This could reflect lack of statistical power to detect the differences between the groups, since the mean gain was lower in the patient group. In addition, it has been demonstrated that the overshoot of systolic CBFV is significantly influenced by not only gain, but also rate time and attenuation (Rosengarten et al., 2001a). Hence, the parameter gain and the overshoot systolic CBFV may not be perfectly matched.

These results are in line with studies on genetically determined CSVD. CADASIL patients demonstrated less robust functional hyperemia in the PCA during visual stimulation, with changes in time dynamics, very similar to our findings (Jokumsen-Cabral et al., 2019). Comparable NVC dysfunction in the posterior circulation was shown in Fabry patients (Azevedo et al., 2012; Castro et al., 2020). Both diseases are characterized by abnormal material deposition in the vessel walls (Baudrimont et al., 1993; Rombach et al., 2010). Interestingly, amyloid deposition also seems to cause NVC impairment (Iadecola and Davisson, 2008; Brickman et al., 2015), and HT appears to have a role in promoting amyloid deposition, thus working synergistically to worsen CSVD and cognitive decline (Iadecola and Davisson, 2008).

Chronic HT leads to structural (mal)adaptations in the cerebral circulation, with remodeling of the cerebral arteries and arterioles. This remodeling involves smooth muscle cell hypertrophy and hyperplasia, increased deposition of extracellular matrix components and degenerated smooth muscle (lipohyalinosis) and fibrinoid necrosis, leading to arterial stiffening and loss of elasticity (Iadecola and Gottesman, 2019). These changes in the proximal resistance arteries cause substantial burden on the vulnerable downstream microcirculation, promoting pressure-induced oxidative stress to the endothelial cells and neuroinflammation (Ungvari et al., 2021). In experimental models, HT results in impairment of endothelium mediated neurovascular coupling responses, in part resulting from this oxidative stress and neuroinflammation (Iadecola and Gottesman, 2019; Ungvari et al., 2021). Hence, the structural changes induced by HT play an important role in the loss of functional integrity of the neurovascular unit. Natural frequency is assumed to represent the tonus and the speed of the system (Rosengarten et al., 2003), which would be altered by the increased rigidity of the vessels and endothelial dysfunction, and our results show that this parameter was the most sensitive in differentiating patients from controls.

Less effective NVC in the MCA territory has been associated with significant cognition, balance, and walking velocity changes in the elderly (Sorond et al., 2011; Purkayastha et al., 2014). Despite the presence and burden of WMD, normal NVC was associated with preserved walking speed, while slower walking is one of the earliest manifestations of CSVD. Further prospective work could use NVC for predicting symptomatic CSVD. Curiously, cocoa and deferoxamine have been demonstrated to reverse some of these changes suggesting the possibility for pharmacological modulation of the neurovascular function (Sorond et al., 2013, 2015).

Additional Contribute From Diabetes Mellitus

Comorbid diabetes associated with increased cerebrovascular dysfunction. NVC was worse in this subgroup of hypertensive patients, particularly in its oscillatory properties (natural frequency), in accordance to previous studies in early type 1 DM (Rosengarten et al., 2002). This might signify higher rigidity of the small arteries due to the accumulation of advanced glycated by-products. In fact, type 2 DM patients have particularly high incidence of lacunar stroke (van Harten et al., 2006; Brundel et al., 2014). Diabetes induced chronic vascular changes include not only macrovascular disorders, such as cardiovascular and cerebrovascular large vessel disease, but also microvascular disorders, with nephropathy, retinopathy and neuropathy (Chawla et al., 2016). Furthermore, studies have implicated DM as a risk factor for cognitive impairment, which may be related to CSVD. However, the mechanism by which cognitive decline occurs and whether it can be explained by dysfunction of the neurovascular unit remains to be elucidated (Mogi and Horiuchi, 2011). The sympathetic nervous system seems to attenuate the cerebrovascular response to hypercapnia, suggesting a direct effect on the cerebral vasculature (Jordan et al., 2000). NVC is also affected by autonomic dysfunction (Azevedo et al., 2011). Thus, differences in DM vs. nDM hypertensive patients could be related to DM associated dysautonomia in CSVD.

Overall, our study further supports cerebrovascular dynamics dysfunction as a major player in explaining the relationship between increased VRF burden and CSVD manifestations, independently of macroscopic white matter lesions.

Limitations

We acknowledge several methodological limitations. Due to the cross-section design, our study cannot provide evidence to support cerebrovascular dysfunction as an early predictor of CSVD. However, these patients had well-controlled HT, based on average ABPM values, with no clinical manifestations of cerebrovascular disease. All the patients were referred to the Hypertension Unit due to severe or difficult to manage HT, and we do not know the duration of the untreated disease, which could impact the degree of microvascular dysfunction.

Although the patient group's sample size is relatively large for hemodynamic physiology studies, this is an exploratory study and must be validated in larger, multicenter cohorts. Also, the control group is relatively small, not exactly age- and gender-matched, and the differences in comorbidities between the two groups can be potential confounders to the observed differences. Besides HT and DM, already discussed, other vascular comorbidities have been associated with the development of CSVD. Dyslipidemia plays an important role in the development of large vessel disease and stroke, but its role in CSVD is still controversial (Tsai et al., 2018). However, animal studies have demonstrated cerebral autoregulation impairment with hyperlipidemia and a relationship between hyperlipidemia and the development of CSVD (Ayata et al., 2013; Kraft et al., 2017). Obesity has been demonstrated to have an impact in the development of CSVD (Yamashiro et al., 2014), and it has been shown to affect cerebral vasoreactivity (Selim et al., 2008). Smoking appears to worsen the effects of hypertension in the cerebral microvasculature (Hara et al., 2019), and there is impaired neurovascular coupling in the PCA of young chronic smokers (Olah et al., 2008). Chronic heart failure can affect cerebral autoregulation, reduce cerebral blood flow (CBF) and has been shown to affect NVC in the PCA (Aires et al., 2020; Ovsenik et al., 2021). The prevalence of CSVD is higher in patients with coronary artery disease (Berry et al., 2019), and it has been identified as an independent risk factor for vascular dementia (Gorelick et al., 2011). It was recently demonstrated that aTrial fibrillation reduces cerebral autoregulation and impairs neurovascular coupling responses to visual stimulation (Junejo et al., 2020). There is evidence that peripheral artery disease is associated with white matter disease and the development of vascular dementia (Bots et al., 1993; Gorelick et al., 2011). In patients with ischemic stroke, impaired renal function correlated with worse dCA and associated with an increased risk of hemorrhagic transformation (Castro et al., 2018). However, in addition to adjusting for age and gender, adjusting for the vascular comorbidities did not modify the results significantly.

Hypertension affects cerebral small vessels heterogeneously, and MRI seems the logical choice for its superior spatial resolution. However, MRI $VRCO_2$ protocols are more prone to failure (Moreton et al., 2018), expensive and not as standardized as TCD (Malojcic et al., 2017). Moreover, TCD offers extraordinary time resolution (~5 ms) for studying the time behavior of CBFV activation in downstream cortical microvasculature. There are also inherent limitations for continuous blood flow monitoring by TCD, namely, providing a measure of blood flow velocity and not flow. However, the former is an adequate surrogate for the latter as long as the insonated artery diameter remains constant. Since data were acquired in a supine position and relied on spontaneous measurements, changes in artery diameter are not anticipated for NVC. Nevertheless, it has been demonstrated that with increasing partial pressure of arterial CO_2 there is noticeable increase in vessel diameter, which could lead to underestimation of cerebral blood flow in the $VRCO_2$ protocol (Coverdale et al., 2014).

We did not report the presence of cortical microinfarcts (CMI) in this study, despite having been found in cohorts of hypertensive patients (although not consistently) and despite their importance in cognitive decline (van Veluw et al., 2017). We did not detect CMI upon visual inspection of the MRI scans. However, the protocols for detecting CMI are mostly validated in 3T MRI scanners (Coverdale et al., 2014), and our 1.5T scan protocol is underpowered for their detection.

In conclusion, neurovascular coupling, and more specifically the modeled parameter natural frequency, seems to be particularly affected in hypertension and diabetes and could be useful as an early biomarker for microvascular dysfunction, future irreversible vascular damage, and cognitive decline. Additionally, our study supports TCD dynamic tests as useful tools for better understating microvascular damage associated with these diseases, but future research is warranted to confirm these results.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article may be made available by the authors upon request, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comissão de Ética da U. L. S. Matosinhos, Hospital de Pedro Hispano, EPE, Unidade Local de Saúde de Matosinhos, Portugal. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AM: conception and design, data collection, literature search, drafting the manuscript, and critical revision of the manuscript. PC: conception and design, data collection, critical revision of the manuscript, and supervision. GP, CF, and JP: data collection and critical revision of the manuscript. FS, AM, and JH: critical revision of the manuscript. EA: conception and design, critical revision of the manuscript, and supervision. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2021.728007/full#supplementary-material

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Supplementary Material

Supplementary Figures and Tables

Supplementary file 1. Imaging characteristics of the patient group

Imaging characteristics	N (%)
Basal ganglia PVS	
<10	22 (55)
10-20	8 (20)
>20	10 (25)
Centrum semiovale PVS	
<10	26 (65)
10-20	7 (17.5)
>20	7 (17.5)
Lacunes	
Present	9 (22.5)
Absent	31 (77.5)
Microbleeds	
Present	4 (10.0)
Absent	36 (90.0)
Strictly deep	2 (5.0)
Strictly lobar	1 (2.5)
Mixed location	1 (2.5

PVS: perivascular space

Supplementary file 2. Cerebral hemodynamics, $VRCO_2$ and NVC: patients versus controls (controlling for age, gender and BMI or age, gender and VCS)

	Age + Gender + BMI			Age + Gender + VCS		
	Artery	**Group**	**Int.**	**Artery**	**Group**	**Int.**
	p value*	*p* value*	*p* value*	*p* value*	*p* value*	*p* value*
Cerebral hemodynamics						
Mean CBFV (cm/s)	0.210	0.718	0.171	0.070	0.396	0.146
MFV SP (cm/s^2)	0.745	0.333	0.472	0.878	0.935	0.107
$VRCO_2$ (%/mmHg CO_2)	**0.008**	0.840‖	0.908	0.144	0.581‖	0.141
Neurovascular coupling						
Overshoot† systolic CBFV (%)		**0.002**			0.065	
Modeled parameters						
Gain (%)		0.117			0.926	
Natural frequency (Hz)		**0.001**			**0.046**	
Attenuation (a.u)		0.312			0.210‖	
Rate time (s)		**0.012**‖			**0.047**‖	

*Two-factor mixed-design ANOVA for the interaction between group variable (patients vs controls) and arteria territory (MCA vs PCA), controlling for age and gender, age, gender and BMI or age, gender and VCS. For NVC, value: were obtained using an ANCOVA. Effect size: BMI - rate time $\eta p^2 = 0.109$, natural frequency $\eta p^2=0.176$ and overshoo systolic CBFV $\eta p^2= 0.140$; VCS - rate time $\eta p^2 = 0.068$ and natural frequency $\eta p^2=0.069$. †Maximal CBFV increase during visual stimulation. §gender significantly interfered with the model. ||age significantly interfered with the model. a.u. arbitrary units; BMI: body mass index; CBFV: cerebral blood flow velocity; Int: interaction; MCA: middle cerebral artery MFV SP: median flow velocity spectral power; PCA: posterior cerebral artery; VCS: vascular comorbidities score; $VRCO_2$ vasoreactivity to carbon dioxide.

Supplementary file 3. Cerebral hemodynamics, VRCO2 and NVC: controls versus HT-nDM versus HT-DM patients (controlling for age and gender, age, gender and BMI or age, gender and VCS)

	Age + Gender +BMI				Age + Gender + VCS			
	Group	**HT-nDM vs controls**	**HT-DM vs controls**	**HT-nDM vs HT-DM**	**Group**	**HT-nDM vs controls**	**HT-DM vs controls**	**HT-nDM vs HT-DM**
	p value*	*p* value*	*p* value*	*p* value*	*p* value*	*p* value*	*p* value*	
Cerebral hemodynamics								
Mean CBFV (cm/s)	0.936				0.565			
MFV SP (cm/s²)	0.621				0.987			
$VRCO_2$ (%/mmHg CO2)	0.910‖				0.860‖			
Neurovascular coupling								
Overshoot† systolic CBFV (%)	**0.002**	0.126	**0.002**	**0.249**	**0.022**	0.071	**0.018**	0.118
Modeled parameters								
Gain (%)	0.287				0.558			
Natural frequency (Hz)	**<0.001**‖	0.090	**<0.001**	0.067	**0.002**‖	**0.020**	**0.002**	**0.011**
Attenuation (a.u)	0.323‖				0.333‖			
Rate time (s)	**0.043**‖	0.054	0.104	1.000	0.142‖			

*Two-factor mixed-design ANOVA for the interaction between group variable (HT-nDM vs HT-DM vs controls) and arterial territory (MCA vs PCA), controlling for age, gender and BMI or age, gender and VCS, with Bonferroni *post-hoc*. For NVC, values were obtained using an ANCOVA, with Bonferroni *post-hoc*. Effect size: BMI - natural frequency ηp^2=0.249 and overshoot systolic CBFV ηp^2= 0.180; VCS - natural frequency ηp^2=0.198 and overshoot systolic CBFV ηp^2= 0.119. †Maximal CBFV increase during visual stimulation. §gender significantly interfered with the model. ‖age significantly interfered with the model. a.u.: arbitrary units; BMI: body mass index; CBFV: cerebral blood flow velocity; MCA: middle cerebral artery; MFV SP: median flow velocity spectral power; PCA: posterior cerebral artery; VCS: vascular comorbidities score; $VRCO_2$: vasoreactivity to carbon dioxide.

PAPER 1: ADDITIONAL EVALUATIONS (NOT INCLUDED IN THE PAPER)

Dynamic cerebral autoregulation: HT patients vs healthy controls

Dynamic cerebral autoregulation: nDM-HT patients vs DM-HT vs healthy controls

Association between cerebrovascular hemodynamics and MRI markers of CSVD in hypertensive patients

ADDITIONAL EVALUATIONS

METHODS

dCA calculations

For each heartbeat, systolic, diastolic, and mean cerebral blood flow velocity (CBFV) and blood pressure (BP) were calculated in the dedicated software (MATLAB, MathWorks, Inc., US). by transfer function analysis (TFA) of the parameters coherence, gain, and phase from beat-to-beat spontaneous oscillations of BP to CBFV, in compliance with standard recommendations: interpolation at 10Hz, window length of 102s, Hanning anti-leakage, 50% superposition, and triangular filtering.[1] As TFA is a linear model-based method, signals with low coherence between BP and CBFV were excluded. The minimum coherence value was determined by calculating the 95% confidence limit based on degrees of freedom.[1] Values were reported in very low (VLF: 0.02–0.07 Hz) and low (LF: 0.07–0.20 Hz) frequency bands. dCA seems to operate at lower frequency bands,[1] and phase is the more reliable parameter.[2]

Magnetic resonance imaging

Eligible patients who agreed to undergo cerebral MRI were scanned with a Siemens Aera 1.5T scanner. Cortical reconstruction and volumetric segmentation were performed from T1 weighted volumetric sequences collected in the sagittal plane with the Freesurfer image analysis suite (Freesurfer v7.1.0.), documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/). Voxels resolution was 1 £ 1 £ 1, slices=256, FOV=255, TR=2730, TE=2.5, TI=1000, FA=7. Cortical grey matter (GM), subcortical GM, and total white matter (WM) volumes were obtained. White matter hyperintensity (WMH) volumes were derived from the T2-weighted fluid-attenuated inversion recovery sequences collected in the sagittal plane. Voxels resolution was 1 £ 1 £ 1, slices=256, FOV=256, TR=5000, TE=336, TI=1800. Briefly, WMH masks were created using the Lesion Segmentation Algorithm (LPA, 1) from the Lesion Segmentation Toolbox for SPM12 in MATLAB R2018a. Following an initial segmentation of the FLAIR image, probability maps were binarised using AFNI (2.3, v21.0.15) command 3dcalc. The resulting segmentations were quality-checked for sufficient accuracy, and volumes were calculated using the Freesurfer (v7.1) command mri_segstats. Volumes were normalized by intracranial volume.

Statistical Analysis

Normality was determined using the Shapiro–Wilk test and analysis of skewness. Data with high asymmetry was normalised using logarithmic transformation. The homogeneity of variances was tested for each analysis.

Mixed ANOVA with one between-subjects factor (group) and one within-subjects factor (artery) was used to compare cerebral hemodynamic data, controlling for age. Subgroup analysis was performed, and univariate ANOVA was used to compare hypertensive non-diabetic patients (HT-nDM), hypertensive diabetic patients (HT-DM) and controls, with Bonferroni post-hoc tests. Supplemental analyses using body mass index (BMI) and the VCS as covariates (including age and gender in both analyses) were also performed.

Pearson's correlation coefficient (r) was used to assess whether a relationship existed between the TCD and the MRI variables and to identify which demographic or clinical parameters should be included

as covariates in regression analyses. Heat maps were created to depict the correlations. No linear regression analyses were conducted since no significant correlations existed between the variables of interest. Values of $p < 0.05$ were deemed significant.

RESULTS

Dynamic cerebral autoregulation: HT patients vs healthy controls

Additional Table 1. depicts the results of the dCA indexes in patients and controls. The main autoregulatory parameter phase was similar between groups. Nevertheless, hypertensive patients showed a lower gain in both arterial territories in the LF range (p=0.040). As shown in Additional Table 2, the results were the same after controlling for age and VCS (p=0.004). However, the difference in LF gain lost significance when adjusting for age and BMI (p=0.052).

Dynamic cerebral autoregulation: nDM-HT patients vs DM-HT vs healthy controls

We further explored the differences between hypertensive non-diabetic patients (HT-nDM), hypertensive diabetic patients (HT-DM) and controls. Age was similar between HT-DM and HT-nDM (66±9 vs 62±12, p=0.165). There were no differences in gender (p=0.350) or ABPM (24-hour BP, HT-DM vs HT-nDM: 136±16/76±8mmHg vs 131±10/79±7mmHg; p>0.05) between the two patient groups.

As shown in Additional Table 3, there were no differences in gain or phase parameters between DM and non-DM hypertensive patients within the frequency ranges of autoregulation. The results were not altered when controlling for BMI or VCS, in addition to age (Additional Table 4).

Association between cerebrovascular hemodynamics and MRI markers of CSVD in hypertensive patients

MRI data was available for 48 patients, although six were excluded from the analysis due to poor imaging quality.

Fig 1 depicts the heat maps for the correlations between the TCD hemodynamic data (dCA, $VRCO_2$, and NVC) and MRI variables. There were no significant correlations between TCD hemodynamic data and cortical and subcortical grey matter volumes, total white matter volume, or WMH volume.

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ADDITIONAL TABLE 1 | dCA: patients vs. controls

	Patients		Controls		Artery	Group	Interaction
	MCA	PCA	MCA	PCA	*p* value*	*p* value*	*p* value*
Cerebral autoregulation							
VLF gain (%/mmHg)	0.8±0.5	1.0±0.6	0.7±0.3	0.9±0.5	**0.008**	0.898	0.480
LF gain (%/mmHg)	1.1±0.5	1.3±0.6	1.4±0.5	1.7±0.5	0.104	**0.040**	**0.041**
VLF phase (radians)	1.0±0.4	1.0±0.4	1.2±0.4	1.1±0.4	0.930	0.548	0.579
LF phase (radians)	0.7±0.3	0.8±0.4	0.8±0.2	0.9±0.3	0.958	0.325	0.559

**Two-factor mixed-design ANOVA for the interaction between group variable (patients vs controls) and arterial territory (MCA vs PCA), controlling for age and gender. Values are presented as mean ± standard deviation. LF: low frequency; MCA: middle cerebral artery; PCA: posterior cerebral artery; VLF: very low frequency.*

ADDITIONAL TABLE 2 | dCA: patients vs. controls (controlling for age, gender, BMI, and VCS)

	Age + gender			Age + gender + BMI			Age + gender + VCS		
	Artery	Group	Int.	Artery	Group	Int.	Artery	Group	Int.
	p value*	*p* value*	*p* value*	*p* value*	*p* value*	*p* value*	*p* value*	*p* value*	*p* value*
Cerebral autoregulation									
VLF gain (%/mmHg)	**0.008**	0.898	0.480	0.240	0.985	0.319	**0.013**	0.557	0.345
LF gain (%/mmHg)	0.104	**0.040**	**0.041**	0.325	0.052	0.052	0.212	**0.004**	**0.004**
VLF phase (radians)	0.930	0.548	0.579	0.496	0.367	0.400	0.954	0.813	0.909
LF phase (radians)	0.958	0.325	0.559	0.899	0.519	0.536	0.981	0.641	0.642

**Two-factor mixed-design ANOVA for the interaction between group variable (patients vs controls) and arterial territory (MCA vs PCA), controlling for age and gender, age, gender and BMI or age, gender and VCS. BMI: body mass index; Int: interaction; LF: low frequency; VCS: vascular comorbidities score; VLF: very low frequency.*

ADDITIONAL TABLE 3 | dCA: HT-nDM vs. HT-DM patients vs. controls

	HT-nDM		HT-DM		Controls		Artery	Group	Int
	MCA	PCA	MCA	PCA	MCA	PCA	*p* value*	*p* value*	*p* value*
Cerebral autoregulation									
VLF gain (%/mmHg)	0.7±0.4	0.8±0.4	0.9±0.5	1.1±0.7	0.7±0.3	1.1±0.6	**0.009**	0.379	0.662
LF gain (%/mmHg)	1.0±0.3	1.1±0.4	1.2±0.4	1.4± 0.5	1.6±0.6	1.9±0.5	0.578	0.119	0.130
VLF phase (radians)	1.0±0.4	1.0±0.4	1.1±0.5	0.9±0.4	1.1±0.6	1.0±0.5	0.731	0.462	0.421
LF phase (radians)	0.7±0.3	0.6±0.2	0.6±0.3	0.6±0.4	0.7±0.2	0.7±0.1	0.970	0.735	0.758

**Two-factor mixed-design ANOVA for the interaction between group variable (HT-nDM vs HT-DM vs controls) and arterial territory (MCA vs PCA), controlling for age and gender. Values are presented as mean ± standard deviation; HT-DM: hypertensive diabetic; HT-nDM: hypertensive non-diabetic; Int: interaction; LF: low frequency; MCA: middle cerebral artery; PCA: posterior cerebral artery; VLF: very low frequency.*

ADDITIONAL TABLE 4 | dCA: HT-nDM vs. HT-DM patients vs. controls (controlling for age, gender, BMI and VCS)

	Age + gender			Age + gender +BMI			Age + gender + VCS		
	Artery	Group	Int.	Artery	Group	Int.	Artery	Group	Int.
	p value*	*p* value*	*p* value*	*p* value*	*p* value*	*p* value*	*p* value*	*p* value*	*p* value*
Cerebral autoregulation									
VLF gain (%/mmHg)	**0.009**	0.379	0.662	0.250	0.402	0.552	**0.025**	0.075	0.626
LF gain (%/mmHg)	0.578	0.119	0.130	0.476	0.186	0.205	0.922	0.092	0.111
VLF phase (radians)	0.731	0.462	0.421	0.315	0.312	0.289	0.523	0.386	0.378
LF phase (radians)	0.970	0.735	0.758	0.799	0.675	0.727	0.805	0.653	0.711

**Two-factor mixed-design ANOVA for the interaction between group variable (patients vs controls) and arterial territory (MCA vs PCA), controlling for age and gender, age, gender, and BMI or age, gender, and VCS. BMI: body mass index; HT-DM: hypertensive diabetic; HT-nDM: hypertensive non-diabetic; Int: interaction; LF: low frequency; MCA: middle cerebral artery; PCA: posterior cerebral artery; VCS: vascular comorbidities score; VLF: very low frequency.*

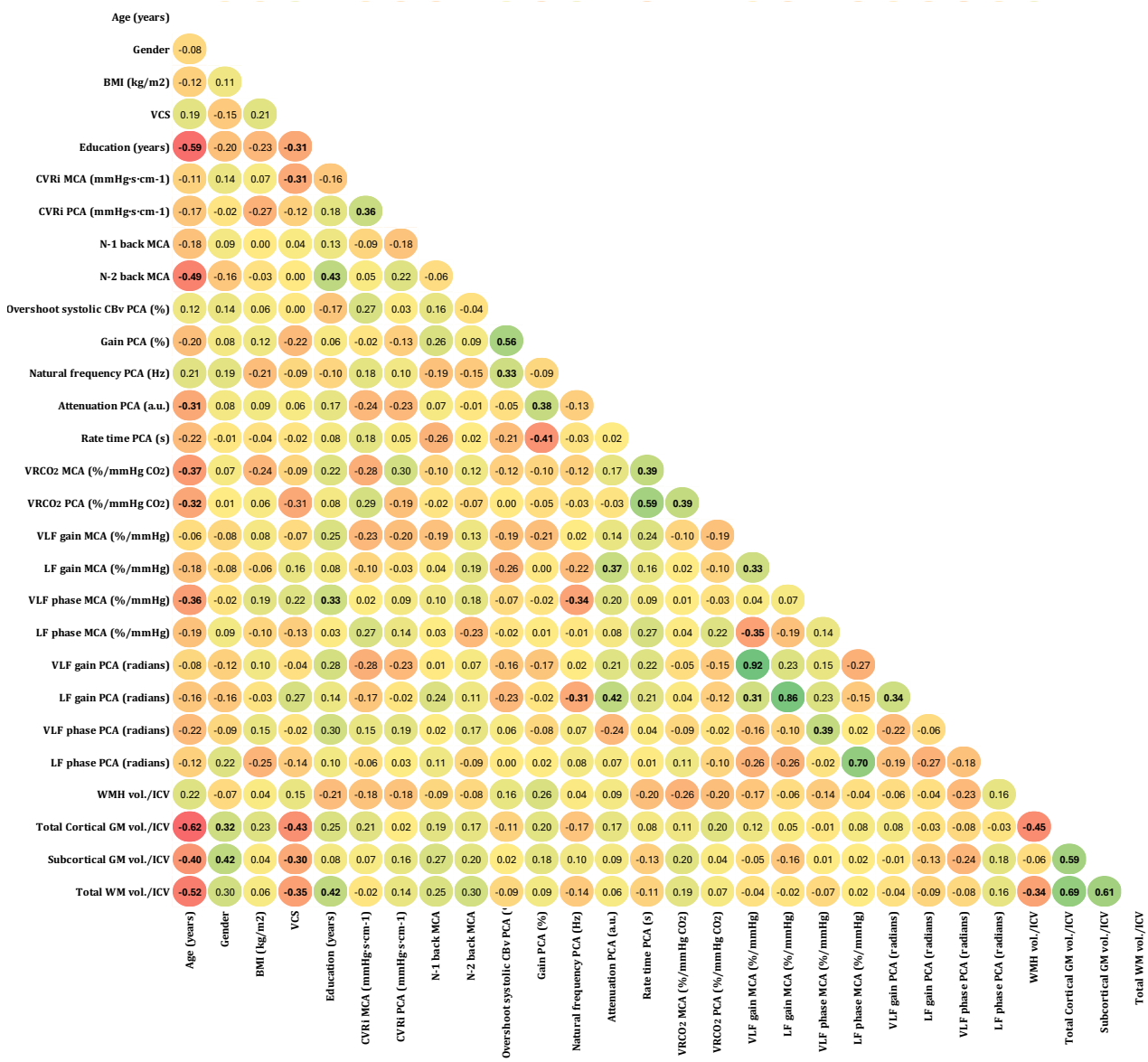

Figure 1. Heatmap for the correlations between TCD and MRI variables. Age, gender, VCS, BMI, education, and CVRi were also studied. Pearson's correlation coefficient (r) is displayed within each circle, with bold values denoting statistically significant correlations ($p<0.05$). Negative correlations are depicted in red, while positive correlations are in green. The intensity of color shading reflects the magnitude of correlation strength. a.u.: arbitrary units; BMI: body mass index; CBFV: cerebral blood flow velocity; CVRi: cerebrovascular resistance index; GM: grey matter; ICV: intracranial volume; LF: low frequency; MCA: middle cerebral artery; MRI: magnetic resonance imaging; PCA: posterior cerebral artery; TCD: Transcranial Doppler; VCS: vascular comorbidities score; VLF: very low frequency; $VRCO_2$: vasoreactivity to CO_2; WM: white matter; WMH: white matter hyperintensities.

PAPER 2: COULD SALT INTAKE DIRECTLY AFFECT THE CEREBRAL MICROVASCULATURE IN HYPERTENSION?

Reference:

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Could salt intake directly affect the cerebral microvasculature in hypertension?

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Objectives: Excess dietary salt and chronic kidney disease (CKD) are acknowledged stroke risk factors. The development of small vessel disease, similarly affecting the cerebral and renal microvasculatures, may be an important mechanistic link underlying this interaction. Therefore, we aimed to evaluate if the dietary salt intake and markers of CKD (estimated glomerular filtration rate, albuminuria) relate to transcranial Doppler (TCD) markers of cerebral small vessel disease (CSVD) in hypertensive patients. *Materials and methods:* Fifty-six hypertensive patients (57% with diabetes) underwent TCD monitoring in the middle (MCA) and posterior (PCA) cerebral arteries for evaluating neurovascular coupling (NVC), dynamic cerebral autoregulation (dCA), and vasoreactivity to carbon dioxide ($VRCO_2$). We investigated the relation between renal parameters and TCD studies using Pearson's correlation coefficient and linear regression analyses. *Results:* There were no associations between dCA, $VRCO_2$, NVC, and renal function tests. However, there was a negative association between the daily salt intake and the natural frequency during visual stimulation (r^2=0.101, ß=-0.340, p=0.035), indicative of increased rigidity of the cerebral resistance vessels that react to cognitive activation. *Conclusions:* In this cross-sectional study, we found an association between excess dietary salt consumption and CSVD in hypertensive patients. Future research is needed to evaluate whether the natural frequency could be an early, non-invasive, surrogate marker for microvascular dysfunction in hypertension.

Keywords: Neurovascular coupling—Vasoreactivity—Dynamic cerebral autoregulation—Cerebral small vessel disease—Hypertension—Diabetes mellitus—Salt Intake

Introduction

Cerebral small vessel disease (CSVD) is a significant cause of stroke and dementia,[1,2] as well as gait impairment and falls in the elderly.[3] However, its pathophysiology is still poorly understood, and no specific treatment strategies exist.

There is growing evidence of the increased prevalence of cerebrovascular disease in patients with chronic kidney disease (CKD), defined by a reduction of the estimated

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glomerular filtration rate (eGFR) or by the presence of proteinuria. These patients have a higher prevalence of stroke, though it is unclear whether this suggests a causative relation or is due to the shared traditional vascular risk factors (VRF).[4] Nevertheless, stroke severity and probability of poor outcome seem higher with comorbid CKD.[5] In addition, studies suggest that renal dysfunction increases the risk of cognitive impairment.[6,7] This cerebro-renal correlation may derive from small vessel disease (SVD), common to both organs.[8] Hence, renal dysfunction could provide information about cerebral small vessel dysfunction in at-risk patients.

On the other hand, increased salt intake leads to increased blood pressure, especially in CKD, increasing stroke risk, morbidity, and mortality.[9-11] In addition, studies have suggested a possible contributory role of dietary salt in developing cerebral SVD (CSVD).[12]

Markers of CSVD on magnetic resonance imaging (MRI) have been extensively studied, including white matter lesions, enlarged perivascular spaces, lacunar stroke, microbleeds and brain atrophy. However, for reasons not fully explained yet, there seems to be a dissociation between radiological markers and the clinical manifestations of CSVD.[13,14] Hence, combining MRI studies with cerebral hemodynamic measures may allow new insights into CSVD development and progression.

Microvascular hemodynamic functional integrity can be studied non-invasively by transcranial Doppler (TCD) monitoring, including dynamic cerebral autoregulation (dCA), vasoreactivity to carbon dioxide ($VRCO_2$) and neurovascular coupling (NVC).

We aimed to evaluate if markers of renal dysfunction and dietary salt consumption relate to TCD markers of cerebral microvascular dysfunction in hypertensive and diabetic patients, to explore possible tools for studying CSVD. In addition, we also evaluated the relation of these renal parameters to cognitive function and MRI markers, as these are usually considered the gold standard for studying CSVD.

Methods

Population

We conducted a cross-sectional observational study in a University Hospital. Patients with hypertension (HT), with and without diabetes mellitus (DM), were recruited from the hospital's Hypertension Unit. Demographic and clinical data were collected. Exclusion criteria were as follows: previous clinical stroke or other significant brain pathology (brain tumour, traumatic brain injury, previous central nervous system infection or symptomatic neurodegenerative disease affecting the brain), absence of adequate temporal bone window for TCD, significant extra/intracranial artery stenosis (>50%), severe/unstable medical condition (including severe valvulopathy and unstable arrhythmias) and incapability to cooperate with the evaluations or to give informed consent. The local ethics committee approved the study protocol, and the tenets of the Declaration of Helsinki were followed. In addition, written informed consent was obtained from all subjects.

Clinical evaluation

Participants' age, gender, past medical history, and medication were collected. Routine blood and urine analyses were performed. Vascular comorbidities were summarised into a vascular comorbidity score (VCS), including HT, DM, dyslipidemia, tobacco usage, chronic heart failure, coronary heart disease, arrhythmias, valvulopathy, peripheral artery disease and nephropathy. These conditions were scored as present (1 point) or absent (0 points) for a score ranging from 0 to 9.[15] The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, and the CKD stage was classified based on eGFR. The albumin-to-creatinine ratio (ACR) from first-morning urine was calculated. Cervical and transcranial Doppler ultrasound examinations (Philips iu22, The Nederlands) were performed to exclude artery stenosis (>50%). The patients underwent routine 24-h ambulatory blood pressure monitoring (Spacelabs 90207, Redmond, Washington, USA).

Daily salt intake

Twenty-four-hour urine was collected from each patient as described by Polonia *et al.*, and 24-hour Na^+ excretion (UNa^+) was used to determine the daily sodium intake.[10] The estimated daily salt intake was calculated as 1mmol/24h sodium=0.05844 g/day salt. Whenever possible, a second 24 h valid urinary collection was performed, and the average of the measurements was used. Two measures were available for 61% of the samples.

Monitoring protocol

Evaluations were conducted in a dim-lighted, quiet room at around 22°C in supine. Subjects refrained from caffeine, alcohol, exercise, or vasoactive drugs for at least 12 h before evaluation. Cerebral blood flow velocity (CBFV) was continuously recorded in the M1 segment of the right middle cerebral artery (MCA) and the P2 segment of the left posterior cerebral artery (PCA) with 2-MHz TCD probes secured with a headframe (Doppler BoxX, DWL, Singen, Germany), to obtain data from both arterial territories.[16] Continuous non-invasive arterial blood pressure was measured with Finometer (FMS, Amsterdam, The Netherlands). Heart rate was assessed with a three-lead electrocardiogram. End-tidal carbon dioxide ($EtCO_2$) was recorded by capnography (Respsense Nonin, Amsterdam, The Netherlands). Data was synchronised and digitally stored at 400Hz with Powerlab (AD Instruments, Oxford, UK) for offline analysis. After

resting for 20 min, a 5-min period of resting data was stored to calculate dCA indexes, followed by the vasoreactivity to carbon dioxide ($VRCO_2$) and NVC protocols.

dCA calculations

For each heartbeat, systolic, diastolic, and mean cerebral blood flow velocity (CBFV) and blood pressure (BP) were calculated in the dedicated software (MATLAB, MathWorks, Inc., US). dCA was assessed by transfer function analysis (TFA) of the parameters coherence, gain, and phase from beat-to-beat spontaneous oscillations of BP to CBFV, in compliance with standard recommendations: interpolation at 10Hz, window length of 102s, Hanning anti-leakage, 50% superposition, and triangular filtering.[17] As TFA is a linear model-based method, signals with low coherence between BP and CBFV were excluded. The minimum coherence value was determined by calculating the 95% confidence limit based on degrees of freedom.[17] Lower gain (lower amplitude of the BP oscillations) and higher phase (higher time shift of the response) between oscillations of BP and CBFV indicate a more effective dCA. Higher gain (less effective dampening capability to oscillations in BP) and lower phase in the very low (VLF: 0.02–0.07 Hz) and low (LF: 0.07–0.20 Hz) frequency bands are associated with impaired dCA.[17] Phase is the most reliable parameter.[18]

Vasoreactivity protocol

Participants were monitored through successive 2- min steps of resting, inhalation of a mixture of 5% CO_2 and 95% 0_2 (until hypercapnia steady-state $EtCO_2$ level of 7–10mmHg above baseline), resting (room air, until returning to normocapnia) and hyperventilation ($EtCO_2$ 7–10mmHg below baseline). VR to CO_2 ($VRCO_2$) was calculated as the slope of the relationship between the average values of $EtCO_2$ plotted against those of relative CBFV achieved at the three stages, expressed as % mean CBFV per mmHg $EtCO_2$.[19]

Neurovascular coupling (NVC) protocol

NVC in the right MCA was assessed with the N-Back (1-Back and 2-Back) tasks, normalised to a control task (Identify "X"), as described by Sorond *et al.*[14] Briefly, a sequence of single letters was displayed on the ceiling while in the supine position. The patients were instructed to press the mouse button each time a letter was repeated (1-Back), and then each time a letter was repeated every other letter (2-Back). The control task (pressing the mouse button each time the letter X was on screen) was performed before each N-Back task. NVC was calculated as the ratio of the relative CBFV increase during the N-Back (CBFVNB) compared with the control task Identify "X" (CBFVIDX) using the following formula: $\frac{(CBFVNB - CBFVIDX)}{CBFVIDX} \times 100\%$. The 2-Back Task results are the most reliable since they induce a higher CBFV rise and potentially represent the NVC more accurately.

NVC in the PCA was assessed by a visual paradigm consisting of ten cycles, each with a resting phase of 20 seconds (eyes closed) and a stimulus phase (flickering checkerboard) at 10 Hz for 40 seconds.[20] All cycles were synchronised and averaged. Peak systolic data was used because it is less prone to artefacts.[21] Maximal systolic CBFV change was obtained to calculate the overshoot parameter as $\frac{(maximum\ CBFV\ -\ baseline\ CBFV)}{baseline\ CBFV} \times 100\%$.[22] The systolic CBFV curve was also modelled to describe the dynamics of NVC response in time according to the second-order linear equation $G(s) = \frac{K \times (1+Tvs)}{\frac{s^2}{\omega^2}+2\times\xi\frac{s}{\omega}+1}$, where "K" stands for gain, "Tv" for rate time, "ω" for natural frequency, and "ξ" for attenuation.[20] Gain describes the relative CBFV difference between the resting stage and the steady-state level during visual stimulation, while rate time indicates the initial steepness of the CBFV increase. Natural frequency represents the oscillatory properties of the system, and attenuation describes dampening and tonus features, such as the elastic properties of the wall vessel.[23]

Magnetic resonance imaging

Eligible patients who agreed to undergo cerebral MRI were scanned with a Siemens Aera 1.5T scanner. Cortical reconstruction and volumetric segmentation were performed from T1 weighted volumetric sequences collected in the sagittal plane with the Freesurfer image analysis suite (Freesurfer v7.1.0.), documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/). Voxels resolution was 1 × 1 × 1, slices=256, FOV=255, TR=2730, TE=2.5, TI=1000, FA=7. White matter hyperintensity (WMH) volumes were derived from the T2-weighted fluid-attenuated inversion recovery sequences collected in the sagittal plane. Voxels resolution was 1 × 1 × 1, slices=256, FOV=256, TR=5000, TE=336, TI=1800. Briefly, WMH masks were created using the Lesion Segmentation Algorithm (LPA, 1) from the Lesion Segmentation Toolbox for SPM12 in MATLAB R2018a. Following an initial segmentation of the FLAIR image, probability maps were binarised using AFNI (2.3, v21.0.15) command 3dcalc. The resulting segmentations were quality-checked for sufficient accuracy, and volumes were calculated using Freesurfer (v7.1) command mri_segstats. Volumes were normalised by intracranial volume.

Neuropsychological examination

A comprehensive neuropsychological evaluation was performed in a quiet room by an experienced Neuropsychologist. The examination included the Mini-Mental State Examination (MMSE), the Montreal Cognitive Assessment (MoCA), verbal fluency (semantic and phonemic), the Auditory-Verbal Learning Test (AVLT), the Stroop Test, the Trail Making Test parts A and B (TMT-A

and TMT-B) and the Digit Symbol and Symbol Search tests from the Wechsler Adult Intelligence Scale III (WAIS-III).

Raw test scores were standardised into z-scores per test, and a composite z-score of tests covering attention and executive function, processing speed and memory was used since these are most sensitive for vascular disease.[24,25] These domains were assessed with the following tests: 1) attention and executive function: TMT-B, Stroop Test part 3 and verbal fluency (semantic, using "animals" and phonemic, using the letters "M", "P" and "R"); 2) processing speed TMT-A, the Stroop Test parts 1 and 2 and the Digit Symbol and the Symbol Search of the WAIS-III; 3) memory: AVLT total score. The total cognitive score was also calculated by adding the scores from the three different domains.

Statistical analysis

Data analysis was performed in SPSS version 26. Normality was determined using the Shapiro–Wilk test and analysis of skewness. The Pearson's correlation coefficient (r) was used to assess whether a relationship existed between the renal parameters (eGFR, ACR or 24h UNa+) and the TCD variables and to identify which demographic or clinical parameters should be included as covariates. Heat maps were created to depict the correlations. Linear regression analyses were then conducted for the significant correlations. Similarly, linear regression analyses were performed for the relation between renal parameters and cognitive and MRI data whenever the Pearson's correlations were significant. Values of $p<0.05$ were considered significant. Due to the study's exploratory nature, no mathematical corrections were made for multiple comparisons.

Results

Fifty-six hypertensive patients were evaluated, 32 with comorbid diabetes. Demographics and comorbidities are summarised in Table 1. Patients were predominantly in the lower stages of nephropathy, and most had at least three vascular risk factors. The patients had well-controlled hypertension, as demonstrated by the 24-hour ambulatory blood pressure monitoring.

Daily salt intake was 12g on average. Our patients were on stable anti-hypertensive medication for at least one month before the urine sample collection to avoid any influence on sodium excretion as an indicator of salt intake.[26,27]

Hemodynamic data are depicted in Table 2. We established a 3-month time limit for performing the TCD, neuropsychological and MRI evaluations to keep them as temporally close as possible: 29% of patients underwent these evaluations within ten days, 34% within 30 days, 27% within 60 days and 9% within 82 days.

Table 1. *Demographics and comorbidities.*

Demographics and comorbidities	Value
Age, years†	64±10
Male, n (%)	32 (57)
BMI, kg/m2†	29±5
Diabetes Mellitus, n (%)	32 (57)
Dyslipidaemia, n (%)	49 (88)
Tobacco consumption, n (%)	7 (13)
Nephropathy, n (%)	
CDK Stage 0–1	18 (32)
CDK Stage 2	27 (48)
CDK Stage 3	9 (16)
CDK Stage 4	2 (4)
CDK Stage 5	0 (0)
VCS, n (%)	
1	2 (4)
2	5 (9)
3	23 (41)
4	14 (25)
5	9 (16)
6	3 (5)
HT duration, years†	17±6
Chronic medication	Value
No. antihypertensives†	4±3
ACEI/ARB, n (%)	52 (93)
Diuretics, n (%)	44 (79)
CCB, n (%)	40 (71)
BB, n (%)	15 (27)
Alpha2-agonists, n (%)	4 (7)
Antiplatelets, n (%)	16 (29)
Statins, n (%)	44 (79)
Laboratory	Value
Total cholesterol, mg/dL†	175±56
LDL cholesterol, mg/dL†	98±46
HDL cholesterol, mg/dL†	45±11
Triglycerides, mg/dL‡	123±97
HbA1C, %†	
HT-DM	7.0±1.3
HT-nDM	5.6±0.6
eGFR, ml/min/1.73 m²†	76±24
Daily salt intake, g/24h†	12±4
ACR, mg/g‡	18±51
ABPM, mmHg†	Value
24-hour systolic BP	132±13
24-hour diastolic BP	78±13
24-hour pulse pressure	57±14
Cognitive parameters	Value
Education, years†	4±5
MMSE†	28±3
MoCA†	22±5
Cognitive score – total†	-2.4±5.0
Cognitive score – EF†	-1.7±2.3
Cognitive score – PS†	-0.6±3.0
Cognitive score – memory†	-0.3±1.0

†Values are presented as mean ± standard deviation. ‡Values are presented as median ± interquartile range; ABPM: ambulatory blood pressure monitoring; ACEI: angiotensin-conversion enzyme inhibitor; ACR: albumin-to-creatinine ratio; ARB: angiotensin receptor blocker; BB: beta-blocker; BMI: body mass index; BP: blood pressure; CCB: calcium channel blocker; CDK: Chronic Kidney Disease; executive function; eGFR: estimated glomerular filtration rate; HbA1C: glycated haemoglobin; HDL: High-density lipoprotein; HT: hypertension; HT-DM: hypertensive diabetic; HT-nDM: hypertensive non-diabetic; LDL: Low-density lipoprotein; MMSE: mini-mental state examination; MoCA: Montreal cognitive assessment; PS: processing speed; VCS: vascular comorbidity score.

Table 2. *Systemic and cerebral hemodynamics.*

Systemic hemodynamics		
Finapres BP (5 minutes)†		
Systolic BP	136±24	
Diastolic BP	61±17	
Mean BP SP, mmHg2‡	9±22	
EtCO2, mmHg†	36±5	
Cerebral hemodynamics	MCA	PCA
Mean CBFV (cm/s)	47.1±13.2†	31.5±8.3†
MFV SP (cm/s^2)	3.2±5.0	2.5±5.0‡
Cerebral autoregulation		
VLF gain (%/mmHg)	0.8±0.5†	0.9±0.5‡
LF gain (%/mmHg)	1.0±0.6‡	1.3±0.6†
VLF phase (radians)	1.0±0.4†	0.9±0.4†
LF phase (radians)	0.7±0.3†	0.7±0.5‡
$VRCO_2$ (%/mmHg CO_2)	1.3±0.5†	0.9±0.5‡
Neurovascular coupling†		
N-1 back	-0.8±5.6	-
N-2 back	4.0±5.8	-
Overshoot‖ systolic CBFV (%)	-	22.0±9.4
Gain (%)	-	13.8±7.2
Natural frequency (Hz)	-	0.2±0.0
Attenuation (a.u)	-	0.4±0.3
Rate time (s)	-	0.6±1.7

†Values are presented as mean ± standard deviation. ‡Values are presented as median ± interquartile range; ‖Maximal CBFV increase during visual stimulation. a.u.: arbitrary units; CBFV: cerebral blood flow velocity; $EtCO_2$: end-tidal carbon dioxide; LF: low frequency; MCA: middle cerebral artery; Mean BP SP: mean blood pressure spectral power; MFV SP: median flow velocity spectral power; PCA: posterior cerebral artery; VLF: very low frequency; $VRCO_2$: vasoreactivity to CO_2.

TCD dynamic tests and salt consumption

Fig. 1 depicts the heat maps for the correlations between the TCD hemodynamic tests (dCA, $VRCO_2$ and NVC) and the renal parameters. There was a negative correlation between the daily salt intake and the $VRCO_2$ in the MCA ($r = -0.414$, $p = 0.007$) and the natural frequency in the PCA ($r = -0.318$, $p = 0.035$). There were no other correlations between TCD dynamic tests and the daily salt intake. As shown in table 3, the relationship between $VRCO_2$ in the MCA and daily salt intake lost significance when adjusted for other significant covariates (namely age and BMI). However, the daily salt intake significantly predicted this cohort's natural frequency during visual stimulation.

There was no correlation between 24-hour systolic and diastolic BP and TCD dynamic tests, except for a positive correlation between systolic BP and LF phase in the PCA ($r = 0.290$, $p = 0.048$) (Supplement Table 1).

TCD hemodynamic tests and renal function

As shown in Fig. 1, there was no correlation between the TCD hemodynamic tests and renal function tests (eGFR and ACR). Hence, no regression models were created.

Renal function, salt intake, MRI volumes, and cognitive measures

MRI data was available for 48 patients, although six patients were excluded from analysis for lack of quality for the evaluations. Fifty-two patients agreed to undergo cognitive evaluation. Fig. 2 depicts the heat maps for the correlations between the renal, MRI and cognitive variables. As shown in Fig. 2, there were no correlations between grey matter volumes, normal white matter volume and white matter hyperintensity volume and renal function. The subcortical grey matter volume negatively correlated with the daily salt intake ($r = -0.316$, $p = 0.044$). As for the cognitive scores, the daily salt intake negatively correlated with the MoCA test ($r = -0.340$, $p = 0.016$), the total cognitive score ($r = -0.338$, $p = 0.033$) and executive function ($r = -0.369$, $p = 0.019$). Likewise, the ACR negatively correlated with executive function ($r = -0.355$, $p = 0.037$). However, the relationship between daily salt intake and ACR and the MRI and cognitive measures lost significance when adjusted for other significant covariates (table 4). There were no correlations between the cognitive measures and GFR.

Discussion

In this pilot study of hypertensive patients not yet symptomatic for cerebrovascular disease, higher daily salt intake was associated with an altered hemodynamic evoked response to visual stimulation in the posterior cerebral artery, namely with a worse natural frequency. Conversely, daily salt intake was not associated with dCA or $VRCO_2$, nor was there an association between renal function, as measured by the eGFR and the ACR, and dCA, $VRCO_2$ or NVC in both territories.

The natural frequency is assumed to represent the tonus and the system's speed, that is, the oscillatory properties of the vessel.[20] Our results support that excess dietary salt may particularly affect this aspect of the microvasculature. On the other hand, as shown in our previous work, the natural frequency appears to be the most sensitive parameter for discriminating abnormal NVC in hypertensive patients.[28] Rosengarten and colleagues hypothesised that this parameter could differentiate between normal and abnormal NVC since it had the lowest SD of the modelled parameters.[20] Hence, the other modelled parameters may lack the sensitivity to demonstrate NVC impairment in the presence of excess dietary salt consumption in hypertensive patients.

Excess dietary salt is a pressing public health concern, with many adverse health effects. It is a significant cause of hypertension, and it is independently associated with stroke, cardiovascular disease, renal dysfunction and cognitive decline.[11,29–32] Furthermore, salt stimulates the

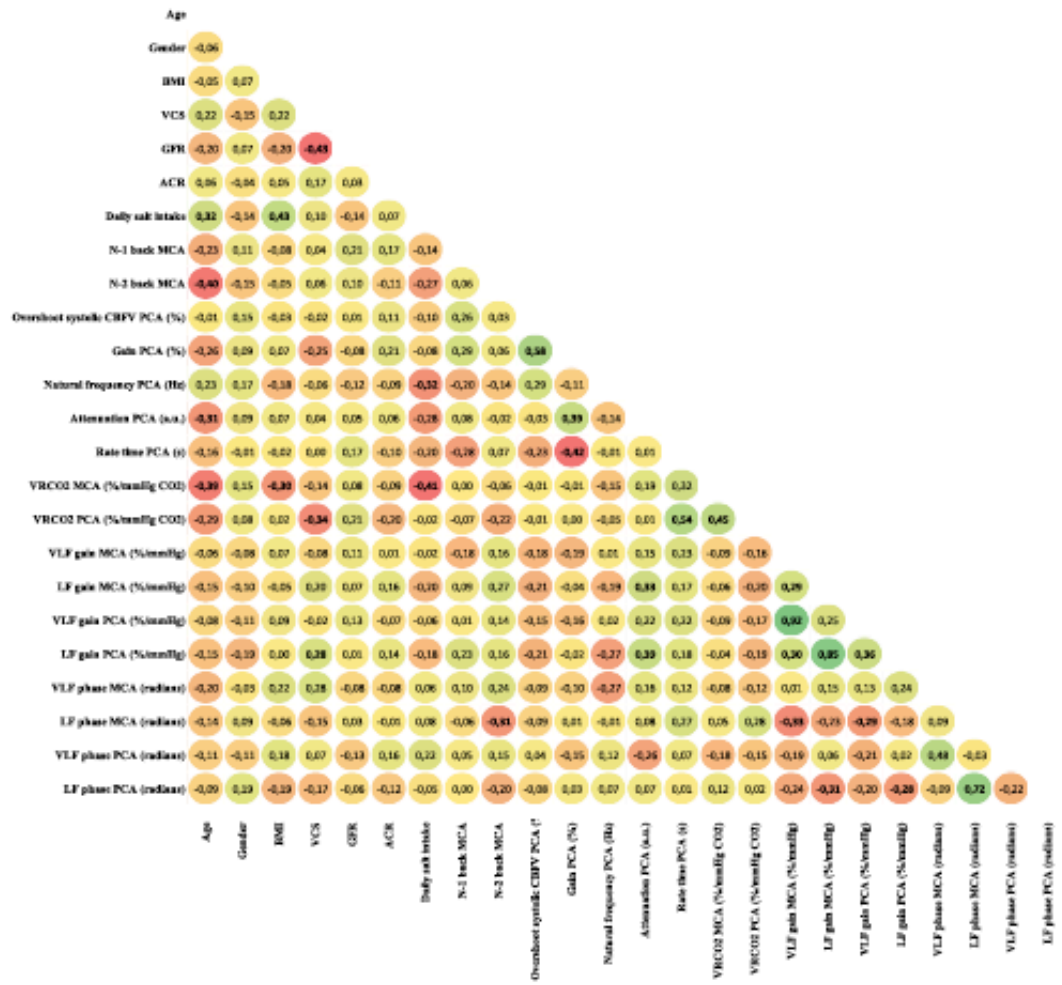

Fig. 1. *Heat map for the correlations between eGFR, ACR, daily salt intake, and TCD variables. Age, gender, VCS and BMI were also studied. ACR: albumin-creatinin ratio; a.u.: arbitrary units; BMI: body mass index; eGFR: estimated glomerular filtration rate; CBFV: cerebral blood flow velocity; LF: low frequency; MCA: middle cerebral artery; PCA: posterior cerebral artery; TCD: Transcranial Doppler; VCS: vascular comorbidities score; VLF: very low frequency; VRCO2: vasoreactivity to CO2.*

production of reactive oxygen species and inflammatory cytokines, with a potential direct toxic effect on the endothelium of cerebral small vessels.[33,34] We found no association between 24-hour BP and TCD dynamic tests. Our results could point to a direct effect of salt on the cerebral microvasculature, in addition to its impact on HT.

Despite the World Health Organization (WHO) recommendation of less than 5g of salt consumption per day, most European Countries exceed this value, ranging from 7 to 13 g per day.[35] Portugal is no exception, with an average intake of more than double the recommended.[26,36] Thus, it is critical to understand the impact of dietary salt on cerebral blood flow (CBF) regulation. However, despite this evidence, there is a striking lack of knowledge on the effects of dietary salt on CBF regulation in humans.

Studies using animal models demonstrated that high salt intake impairs the ability to maintain a constant CBF during decreases in systemic BP[37] and impairs relaxation of the MCA during hypoxia, suggesting altered cerebral autoregulation and vasoreactivity.[38] However, recent work by Migdal KU *et al.* showed no effects of a single meal or ten days of high dietary Na^+ intake on $VRCO_2$ in healthy young adults.[39,40] The same authors found that a high salt meal acutely caused a modest reduction in dCA during supine spontaneous fluctuations in blood pressure in healthy young adults, but there was no effect of ten days of high dietary sodium in dCA.[40] To our knowledge, no other studies have looked at the impact of dietary salt on TCD hemodynamic tests in either healthy or hypertensive individuals. Both the single meal and the ten days of high salt intake are acute changes when considering a lifetime of excess salt consumption, despite the latter being used as a chronic condition in the mentioned studies. This could explain the lack of significant impact of excess salt

Table 3. *Association between the daily salt intake and TCD hemodynamic tests.*

	Daily salt intake							
	MCA				PCA			
		Linear regression models				Linear regression models		
	Pearson's *r*	ß	R^2	*p*	Pearson's *r*	ß	R^2	*p*
Cerebral autoregulation								
VLF gain (%/mmHg)	-0.017	-	-	-	-0.063	-	-	-
LF gain (%/mmHg)	-0.195	-	-	-	-0.177	-	-	-
VLF phase (radians)	-0.060	-	-	-	0.218	-	-	-
LF phase (radians)	0.075	-	-	-	-0.045	-	-	-
$VRCO_2$ (%/mmHg CO_2)	-0.414	-0.321	0.203	0.092*	-0.017	-	-	-
Neurovascular coupling								
N-1 back	-0.135	-	-	-				
N-2 back	-0.273	-	-	-				
Overshoot† systolic CBFV (%)	-	-	-	-	-0.098	-	-	-
Gain (%)	-	-	-	-	-0.076	-	-	-
Natural frequency (Hz)	-	-	-	-	-0.318	-0.340	0.101	0.035
Attenuation (a.u)	-	-	-	-	-0.283	-	-	-
Rate time (s)	-	-	-	-	-0.195	-	-	-

*Age and BMI significantly influenced the model ($p<0.05$). †Maximal CBFV during visual stimulation. a.u.: arbitrary units; BMI: body mass index; LF: low frequency; CBFV: cerebral blood flow velocity; MCA: middle cerebral artery; PCA: posterior cerebral artery; TCD: Transcranial Doppler; VLF: very low frequency; $VRCO_2$: vasoreactivity to CO_2. Bold values represent correlations or regressions with significant *p* values.

consumption in dCA and $VRCO_2$ observed in the cited paper.

The kidney and the brain have similar autoregulation mechanisms, anatomically and functionally.[8] Both are target organs to microvascular damage due to systemic risk factors, such as HT, DM, dyslipidemia, obesity, and smoking[4]. Hence, it seems reasonable that renal function tests could be surrogate markers for cerebral microvascular dysfunction. Studies have shown that subjects with lower eGFR have a higher volume of white matter lesions.[41,42] In patients with acute ischemic stroke, worse dCA correlated with lower eGFR and was associated with an increased risk of hemorrhagic transformation of ischemic stroke[5]. However, our study showed no robust association between TCD hemodynamic tests and eGFR. Studies using MRI have shown conflicting results regarding the association between eGFR and CBF.[43-45]

Albuminuria results from endothelial damage to the kidney and is increasingly considered a marker of generalised endothelial dysfunction. It is a marker of pressure-induced injury of the "strain vessels", vessels with high vascular tone and exposure to higher pressures, such as the pre-glomerular vessels and cerebral perforating arteries.[46] Besides being a sign of early kidney disease, it has emerged as an independent predictor of cardiovascular morbidity and mortality in HT and DM.[47] It has been suggested that white matter disease and cognitive decline could be associated with microalbuminuria.[45,48-50] Albuminuria has also been associated with cortical thinning and WMH burden in cognitively normal patients with VRF.[46,51] However, the Rotterdam Study found no association between ACR and CBF after adjusting for the VRF.[43] Likewise, we did not find an association between the microvascular function tests and ACR.

Lower grey matter volume and higher white matter mean diffusivity seem to be associated with higher mortality in CSVD.[52] Terminal CKD is associated with increased white matter disease and cerebral atrophy.[53] White matter changes in normal-appearing white matter contribute to and may precede WMH,[54] and these changes are not usually depicted in the neuroimaging studies of CSVD, thus limiting its capacity to reveal white matter disease burden fully. TCD hemodynamic tests could further add to the MRI markers of CSVD and could be valuable in studying earlier steps of cerebral endothelial dysfunction in these patients.

Our results show no association between eGFR and ACR and MRI markers or cognitive function, perhaps because our patients had early-stage CKD predominantly, further arguing in favour of the value of TCD studies in early stages of vascular disease.

Limitations

We acknowledge several methodological limitations. First, this study has a small sample size, although it follows the average sample size for hemodynamic physiology studies using TCD dynamic tests. Due to their observational and exploratory nature, these results must be regarded as preliminary. Further research is needed to confirm if dietary salt intake is directly related to small vessel dysfunction in hypertensive patients and if the

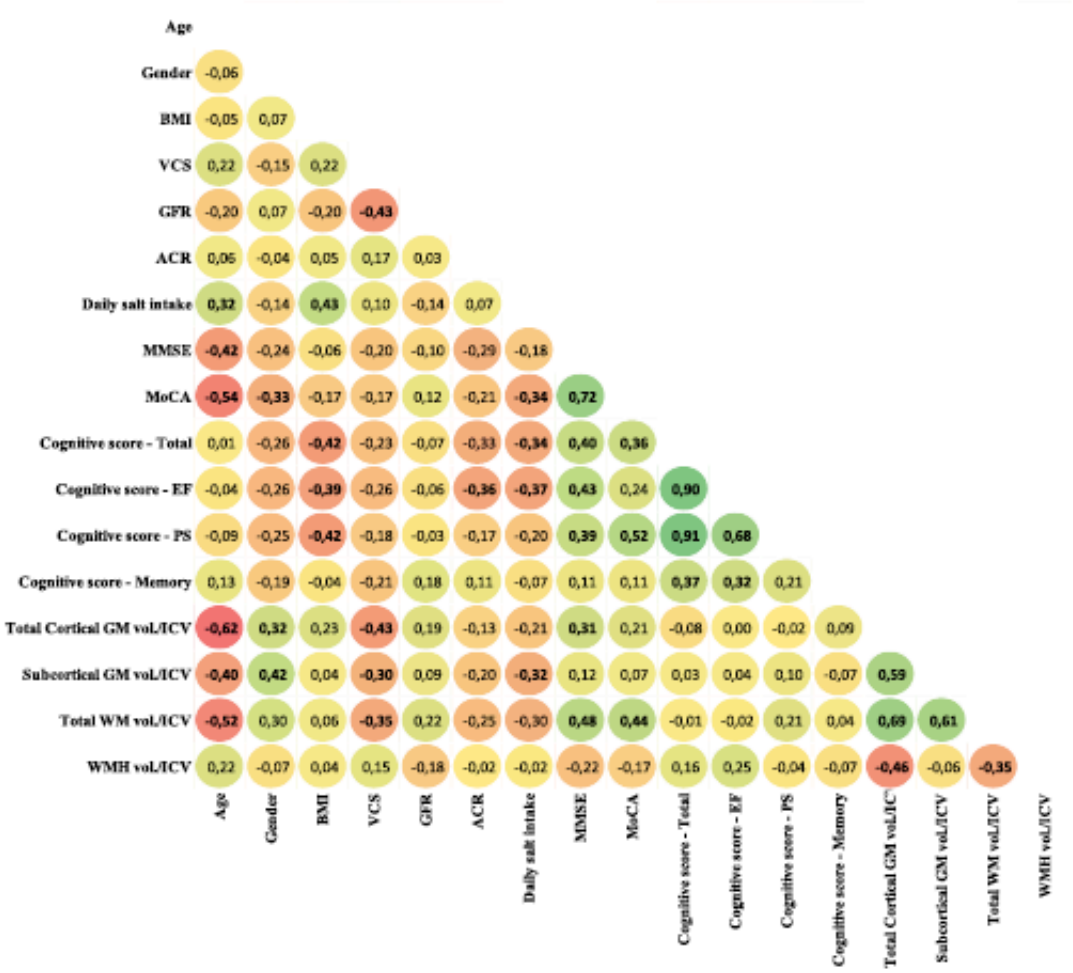

Fig. 2. *Heat map for the correlations between eGFR, ACR, daily salt intake, and cognitive and MRI variables. Age, gender, VCS and BMI were also studied. ACR: albumin-creatinin ratio; BMI: body mass index; EF: executive function; eGFR: estimated glomerular filtration rate; GM: grey matter; ICV: intracranial volume; MMSE: mini-mental state examination; MoCA: Montreal cognitive assessment; PS: processing speed; VCS: vascular comorbidities score; WM: white matter; WMH: white matter hyperintensities.*

natural frequency is a sensitive parameter to detect it. In addition, since the dietary salt intake was derived from an estimation based on 24h urine assessments, it may not reflect the lifelong effect of dietary salt on the cerebral vasculature.

We must also mention the inherent limitation of continuous blood flow monitoring by TCD since it measures blood flow velocity and not flow. However, the former is an adequate surrogate for the latter if the insonated artery diameter remains constant. Since data were acquired in a supine position and relied on spontaneous measurements, changes in artery diameter are not anticipated for dCA and NVC. However, increasing partial pressure of arterial CO_2 leads to a noticeable increase in vessel diameter, which could lead to underestimating cerebral blood flow in the $VRCO_2$ protocol.[55]

In conclusion, in this cross-sectional study, we found an observational association between high dietary salt intake and cerebral microvascular dysfunction, namely in the oscillatory properties of the cerebral small vessels. This

Table 4. *Association between renal function and sodium daily intake and cognitive and MRI data.*

	ACR				Daily salt intake			
		Linear regression models				Linear regression models		
	Pearson's r	ß	R^2	*p**	Pearson's r	ß	R^2	*p**
Cognitive parameters								
MMSE	-0.289	-	-	-	-0.180	-	-	-
MoCA	-0.206	-	-	-	-0.340	-0.223	0.447	0.063‡
Cognitive score - Total	-0.325	-	-	-	-0.338	-0.143	0.241	0.387†
Cognitive score – EF	-0.355	-0.308	0.254	0.054†	-0.369	-0.218	0.213	0.199†
Cognitive score – PS	-0.166	-	-	-	-0.201	-	-	-
Cognitive score – memory	-0.110	-	-	-	-0.072	-	-	-
MRI volumes								
Total Cortical GM vol./ICV	-0.128	-	-	-	-0.209	-	-	-
Subcortical GM vol./ICV	-0.203	-	-	-	-0.316	-0.128	0.345	0.399†#
Total WM vol./ICV	-0.245	-	-	-	-0.296	-	-	-
WMH vol./ICV	-0.016	-	-	-	-0.021	-	-	-

*p-value for the variable of interest. †BMI significantly influenced the model ($p<0.05$). ‡Age and gender significantly influenced the model ($p<0.05$). #VCS significantly influenced the model ($p<0.05$). ACR: albumin-to-creatinine ratio; BMI: body mass index; MMSE: Mini-mental state examination; MoCA: Montreal cognitive score; EF: executive function; PS: processing speed; ICV: intracranial volume; GM: grey matter; VCS: vascular cognitive score; WM: white matter; WMH: white matter hyperintensities. Bold values represent significant correlations.

change was detected in hypertensive patients asymptomatic for cerebrovascular disease. Since CSVD is a major, growing threat to cerebral health, studying specific risk factors while focusing on preventive strategies is paramount. Furthermore, the neurovascular coupling and, more specifically, the modelled parameter natural frequency seem particularly affected and should be studied as potential early, non-invasive surrogate markers for microvascular dysfunction in patients at risk.

Institutions

This work was performed in the Faculty of Medicine of the University of Porto, Portugal. The patients were recruited form the Hypertension Unit of Unidade Local de Saúde de Matosinhos, Portugal.

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Declarations of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jstrokecerebrovasdis.2022.106632.

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Supplement Table 1. Correlation between TCD hemodynamic tests and 24-hour BP

	24-hour BP							
	Systolic BP				Diastolic BP			
	MCA		PCA		MCA		PCA	
	Pearson's r	p^*	Pearson's r	p^*	Pearson's r	p^*	Pearson's r	p^*
Cerebral autoregulation								
VLF gain (%/mmHg)	0.050	0.739	0.045	0.762	-0.006	0.966	-0.029	0.849
LF gain (%/mmHg)	0.044	0.767	0.067	0.655	0.140	0.349	0.105	0.484
VLF phase (radians)	-0.082	0.626	-0.196	0.282	0.037	0.825	0.132	0.471
LF phase (radians)	0.243	0.104	**0.290**	**0.048**	0.128	0.370	0.090	0.550
$VRCO_2$ (%/mmHg CO_2)	-0.045	0.785	-0.047	0.775	0.095	0.565	0.093	0.574
Neurovascular coupling								
N1-back test (%)	0.044	0.790	-	-	0.212	0.173	-	-
N2-back test (%)	-0.081	0.624	-	-	0.156	0.316	-	-
Overshoot† systolic CBFV (%)	-	-	0.005	0.142	-	-	0.009	0.951
Gain (%)	-	-	-0.163	0.302	-	-	-0.043	0.781
Natural frequency (Hz)	-	-	-0.106	0.505	-	-	-0.121	0.428
Attenuation (a.u)	-	-	-0.039	0.805	-	-	0.050	0.747
Rate time (s)	-	-	0.016	0.922	-	-	0.168	0.276

*p-value for the Pearson's correlation. $p<0.05$ was considered significant. † Maximal CBFV increase during visual stimulation. a.u.: arbitrary units; BP: blood pressure; LF: low frequency; MCA: middle cerebral artery; PCA: posterior cerebral artery; VLF: very low frequency; $VRCO_2$: vasoreactivity to CO_2.

PAPER 3: CEREBRAL BLOOD FLOW REGULATION AND COGNITIVE PERFORMANCE IN HYPERTENSION

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Research Techniques:	Neurosonology, CBF measurement, Transcranial Doppler

Title

Cerebral Blood Flow Regulation and Cognitive Performance in Hypertension

Short title

Cerebral Perfusion and Cognition in Hypertension

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The Ethics Committee from Unidade Local de Saúde de Matosinhos approved the study protocol (approval number 082/CE/JAS).

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Abstract

We examined the relation between transcranial Doppler (TCD) markers of cerebral blood flow regulation and cognitive performance in hypertension (HT) patients to evaluate the predictive value of these markers for cognitive decline. We assessed dynamic cerebral autoregulation (dCA), vasoreactivity to carbon dioxide, and neurovascular coupling (NVC) in the middle (MCA) and posterior (PCA) cerebral arteries of 52 patients. Neuropsychological evaluation included the Montreal Cognitive Assessment and tests covering attention, executive function, processing speed, and memory. Notably, reduced rate time in the PCA significantly predicted better processing speed (p=0.003). Furthermore, reduced overshoot systolic cerebral blood velocity in the PCA and reduced phase in the VLF range in the MCA (p=0.021 and p=0.017, respectively) significantly predicted better memory. Intriguingly, enhanced dCA in the MCA predicted poorer memory performance, while reduced NVC in the PCA predicted both superior processing speed and memory performance. These findings suggest that HT-induced changes in cerebral hemodynamics impact cognitive performance. Further research should verify these observations and elucidate whether these changes represent adaptive responses or neurovascular inefficiency. TCD markers might provide insights into HT-related cognitive decline.

Keywords

Cerebral small vessel disease; Cognitive impairment; Dynamic cerebral autoregulation; Hypertension; Neurovascular coupling.

INTRODUCTION

Cerebral small vessel disease (CSVD) is the most common pathological neurological process,[1] and stands as a principal contributor to global mortality and morbidity.[2] It is the main cause of vascular cognitive impairment and dementia and ranks second as a cause for cognitive decline.[3,4] Furthermore, CSVD plays a significant role in the emergence and progression of Alzheimer's disease.[4,5] Despite its prevalence, our understanding of CSVD remains limited, with pathological manifestations often going unnoticed until substantial damage has occurred.

Hypertension (HT) is the most important risk factor for CSVD.[6] Chronic blood pressure (BP) elevation leads to structural (mal)adaptations in the cerebral circulation, with arterial stiffening, vascular occlusions, and changes in vessels' mechanical and hemodynamic properties.[6,7] Evidence suggests early cerebrovascular and cognitive dysfunction in HT before reaching diagnostic BP levels.[8] There is evidence of impaired cognition in the high-normotensive range in midlife,[9] and long-term BP variability from healthy young adulthood is associated with worse verbal memory and psychomotor speed in midlife.[10] Nevertheless, HT-related cognitive decline is diagnosed late, when there is significant impairment and patients already present criteria for mild cognitive impairment or dementia.

There is an unmet need for the evaluation and follow-up of patients at risk for microvascular disease. CSVD imaging markers and symptoms take years to develop, and this window of ongoing damage could be an opportunity

for halting the pathological cascade if detected early. The American Heart Association/American Stroke Association recommends further research to identify individuals at higher risk for silent CSVD, to allow cost-effective use of targeted neuroimaging screening, and to study therapeutic measures to reduce the incidence of adverse outcomes, including cognitive decline.[11]

The transcranial Doppler (TCD) is a relatively inexpensive and noninvasive tool for measuring cerebral blood velocity (CBv) as a perfusion correlate.[12] Unlike MRI and PET-based studies, it provides high temporal resolution to assess fast changes in flow parameters resulting from neural activity. Three fundamental mechanisms drive the cerebral blood flow (CBF) adaptations to meet the brain's metabolic demand: cerebral autoregulation, cerebral vasoreactivity, and neurovascular coupling. A comprehensive evaluation of cerebrovascular function should include assessing all these mechanisms. In the absence of significant arterial disease, the CBv measured at the base of the brain reflects resistance in distal microvessels.[13,14] Microvessel disease is the main driver of vascular cognitive impairment, but there is remarkable heterogeneity in cognitive symptoms among patients with similar burden of CSVD markers on brain imaging.[15,16] Hence, TCD studies could be cost-effective tools for detecting dysfunctions in the integrated responses regulating CBF in at-risk individuals.

Our group previously studied TCD markers of CBF regulation in HT patients, including dynamic cerebral autoregulation (dCA), vasoreactivity to CO_2 ($VRCO_2$), and neurovascular coupling (NVC). We observed reduced NVC in the posterior cerebral artery (PCA) of HT patients compared to healthy controls, particularly the natural frequency parameter, indicating increased rigidity of the cerebral resistance vessels.[17] Additionally, we investigated the influence of renal function and dietary salt consumption in these TCD metrics. Notably, dietary salt consumption emerged as an independent predictor of microvascular dysfunction in HT, with the natural frequency parameter in the PCA territory again showing promise as a surrogate marker for CBF dysregulation in HT.[18]

Few studies have examined CBF regulation and cognitive function in HT.[19-21] Building on prior research, we hypothesized that TCD markers of CBF regulation may serve as predictors of cognitive performance in HT. This study aimed to assess the potential connection between dCA, $VRCO_2$, NVC, and cognitive performance in HT patients to explore the usefulness of TCD markers as tools for predicting HT-induced cognitive impairment.

METHODS

Study Population

This was a cross-sectional observational study. Patients with HT were enrolled in the Hypertension Unit. Included patients had a confirmed diagnosis of HT for a minimum duration of one year and an absence of neurological clinical manifestations associated with the disease. The following exclusion criteria were defined: past stroke or other major brain conditions (clinically defined dementia, brain neoplasia, brain trauma, prior brain infection, or degenerative neurological disease), serious/unstable illnesses, unsuitable acoustic temporal bone window, stenosis in extra- or intracranial arteries exceeding 50%, and inability to cooperate or provide informed consent. The research adhered to the principles of the Declaration of Helsinki, and the protocol was approved by

the Ethics Committee from Unidade Local de Saúde de Matosinhos (approval number 082/CE/JAS). Participants provided written consent.

Clinical evaluation

Age, gender, educational level, health background, body mass index (BMI), and medications were documented. Routine blood and urine tests were conducted. Vascular comorbid conditions were compiled into a vascular comorbidity score (VCS): HT, DM, dyslipidemia, tobacco consumption, chronic heart failure, coronary artery disease, arrhythmia, valve disorders, nephropathy, and peripheral vascular disease. Each comorbidity was marked as either present (1 point) or absent (0 points), leading to a score ranging from 0 to 9.[17,18] Arterial stenosis above 50% or other significant vascular abnormalities were ruled out through cervical and transcranial Doppler ultrasonography (Philips iu22, The Netherlands). Per their regular protocol, patients underwent 24-hour ambulatory blood pressure monitoring (ABPM) (Spacelabs 90207, Redmond, Washington, USA).

Monitoring Protocol

Assessments took place in a dimly lit, silent room (around 22°C), while supine. CBv was continuously monitored in the right middle cerebral artery (MCA, M1 segment) and left posterior cerebral artery (PCA, P2 segment), with 2-MHz TCD probes held in place with a head mount (Doppler BoxX, DWL, Singen, Germany), enabling simultaneous data collection from both arterial territories.[22] The Finometer (FMS, Amsterdam, The Netherlands) was used for continuous noninvasive measurement of arterial BP. Heart rate was tracked using a three-lead electrocardiogram. End-tidal carbon dioxide (EtCO2) was captured via capnography (Respsense Nonin, Amsterdam, The Netherlands). Following a 20-minute rest, tests for $VRCO_2$ and NVC were performed (as detailed below). CBv envelopes were continuously recorded and later analyzed. Data was synchronized and saved digitally at a rate of 400Hz using Powerlab (AD Instruments, Oxford, UK) for subsequent review.

Dynamic cerebral autoregulation

Systolic, diastolic, and mean CBv and BP were calculated for each heartbeat using specialized software (MATLAB, MathWorks, Inc., US). The cerebrovascular resistance index (CVRi) was determined as the ratio of mean BP to mean CBv.[23] Through transfer function analysis (TFA), gain, phase, and coherence were determined by analyzing the spontaneous beat-by-beat fluctuations of BP to CBv, adhering to standard recommendations.[24] Since TFA employs a linear model approach, signals with low coherence were rejected. The lowest coherence threshold was set by determining the 95% confidence boundary based on degrees of freedom.[24] Measurements were presented in very low (VLF: 0.02–0.07 Hz) and low (LF: 0.07–0.20 Hz) frequency ranges as dCA operates at lower frequencies.[24] Phase appears to be the more dependable metric, as studies have shown discordant findings in phase and gain parameters, with changes in $EtCO_2$ leading to highly consistent changes in phase, but less consistent changes in gain.[25] Differences in gain may reflect a methodological consequence of the difference in BP levels *per se* and not increased dCA efficiency.

Vasoreactivity to CO_2

Participants underwent sequential 2-minute intervals of rest while breathing ambient air (normocapnia), breathing CO_2-enriched air (5% CO_2, until a stable hypercapnia $EtCO_2$ level of 7–10mmHg above normocapnia), resting again until reaching normocapnia, and hyperventilation (until $EtCO_2$ 7–10mmHg below normocapnia). $VRCO_2$ was calculated as the correlation gradient between average $EtCO_2$ values and corresponding relative CBv values observed across the three phases, denoted as % average CBv for each mmHg of $EtCO_2$.[26]

Neurovascular coupling

NVC in the right MCA was evaluated using the N-Back (1-Back and 2-Back) tasks, standardized to a reference task (Spot "X").[27] In this task, a series of individual letters were projected on the ceiling while participants lay supine. They were directed to click the mouse each time they saw a repeating letter (1-Back) and, subsequently, whenever a letter recurred every alternate letter (2-Back). Before each N-Back exercise, the reference task was conducted. NVC was determined by the proportion of the relative rise in CBv during the N-Back (CBvNB) relative to the reference task (CBvIDX) with the equation: $\frac{(CBvNB - CBvIDX)}{CBvIDX} \times 100\%$. The outcomes from the 2-Back task are deemed the most reliable as they lead to a greater rise in CBv and potentially better depict the NVC mechanism.[26-29]

NVC in the PCA was assessed using the flickering checkerboard method, which involved ten cycles, each containing a 20-second resting period (eyes shut) and a 40-second 10Hz stimulus phase.[30] All cycles were synchronized and averaged. Peak systolic readings were used due to their lower susceptibility to artifacts.[31] The maximum change in systolic CBv was derived to compute the overshoot metric: $\frac{maximum\ CBv - baseline\ CBv}{baseline\ CBv} \times 100\%$.[32] The systolic CBv curve was also modeled to outline the NVC dynamics over time according to the second-order linear formula $G(s) = \frac{K \times (1 + Tvs)}{\frac{s2}{\omega 2} + 2\xi * \frac{s}{\omega} + 1}$, where "K" symbolizes gain, "Tv" denotes rate time, "ω" indicates the natural frequency, and "ξ" represents attenuation.[30] Gain portrays the relative CBv shift between the resting and steady states during visual activation, rate time indicates the initial rapid rise in CBv, natural frequency reflects the system´s oscillatory properties, and attenuation describes the dampening and tonus features (the elastic properties of the vessel wall).[33]

Neuropsychological examination

An experienced Neuropsychologist performed a comprehensive neuropsychological evaluation in a tranquil setting. To screen for cognitive decline, the Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MoCA) were administered. We utilized age- and education-adjusted criteria for the MoCA test, identifying patients who fell below the validated cutoff points for mild cognitive impairment (MCI), defined as scores 1.5 standard deviations below the mean of the normative sample for the Portuguese population.[34] The remaining assessment consisted of verbal fluency (both semantic and phonemic), the Auditory-Verbal Learning

Test (AVLT), the Stroop Test, the Trail Making Test parts A and B (TMT-A and TMT-B), and the Digit Symbol and Symbol Search tests from the Wechsler Adult Intelligence Scale III (WAIS-III), as described in previous work.[18] Raw test results were normalized into z-scores for each test. A composite z-score for tests focusing on attention, executive function, processing speed (PS), and memory were employed since these most indicate vascular disease.[35] The domains were assessed as such:[18] 1) attention and executive function: TMT-B, Stroop Test part 3 and verbal fluency (semantic, using "animals" and phonemic, using the letters "M", "P" and "R"); 2) PS: TMT-A, the Stroop Test parts 1 and 2 and the Digit Symbol and the Symbol Search of the WAIS-III; 3) memory: AVLT total score. The resulting total cognitive score was also calculated.

Statistical Analysis

Normality was assessed through the Shapiro–Wilk test and analysis of skewness. Data exhibiting pronounced skewness underwent normalization via logarithmic transformation. Each analysis tested the homogeneity of variances. Pearson's correlation coefficient (r) was used to determine any associations between the TCD and cognitive metrics and to identify demographic or clinical parameters to be incorporated as covariates for each regression analysis. Correlations were presented using heatmaps. Linear regression studies were conducted on significant correlations, with the cognitive variables as the dependent variables and including all the significant covariates. Regression diagnostics were run using the analysis of residuals to test model assumptions and the variation inflation coefficient (VIF) to detect collinearity in regression models. A p-value lower than 0.05 was deemed statistically significant. Due to the study's exploratory nature, no mathematical corrections were made for multiple comparisons.[36,37] Data analysis was performed using SPSS version 26.

RESULTS

Demographics

Sixty patients were initially enrolled, but 3 patients were excluded for past lacunar stroke, 1 for lacking a suitable acoustic temporal bone window for TCD assessment, and 4 patients did not attend the Neuropsychological evaluation. A total of 52 HT patients were assessed. Demographics and comorbid conditions are detailed in Table 1. These patients exhibited well-controlled HT, as evidenced by the 24-hour ABPM. Notably, the educational level of the patients was low. Seven patients scored below the validated cutoff points for MCI in the MoCA test. We established a 3-month time frame to conduct the TCD and neuropsychological tests to ensure they were carried out in close temporal proximity.

Association between cerebrovascular hemodynamics and cognitive performance in hypertensive patients

Table 1 depicts the results of the cognitive evaluation. Table 2 depicts the TCD hemodynamic data for this cohort. Fig. 1 depicts the heat maps for the correlations between the TCD hemodynamic data (dCA, $VRCO_2$, and NVC) and the cognitive variables. Table 3 represents the final regression models for the significant correlations.

The MoCA scores significantly correlated with phase in the VLF range in the MCA (r=0.327, p=0.039), the N2-NBT in the MCA (r=0.383, p=0.015), the education level (r=0.728, p<0.001), age (r=-0.536, p<0.001), and gender (r=-0.326, p=0.018). However, the associations between the MoCA scores and the TCD variables lost significance when all covariables were included in the regression model (p=0.538 and p=0.356 for phase in the VLF range in the MCA and N2-NBT in the MCA, respectively). There were no other significant correlations for dCA.

The PS significantly correlated with the rate time (r=-0.357, p=0.022), the education level (r=0.490, p<0.001), and the BMI (r=-0.415, p=0.003). Reduced rate time significantly predicted better PS (p=0.003) after adjusting for the significant covariables. Fig. 2 depicts the scatterplot for rate time against PS. The final model explained 44.6% of the variability of the PS. A regression equation was defined to predict PS from the independent variables: $PS = 9.631 - 20.896 \times rate\ time + 13.412 \times education - 0.173 \times BMI$.

Memory significantly correlated with the overshoot systolic CBv in the PCA (r=-3.00, p=0.041) and phase in the VLF range in the MCA (r=-0.342, p=0.031). Reduced overshoot systolic CBv in the PCA and reduced phase in the VLF range in the MCA (p=0.021 and p=0.017, respectively) significantly predicted better memory. Fig. 2 depicts the scatterplots for the overshoot systolic CBv in the PCA against memory and for phase in the VLF range in the MCA against memory. The final model explained 25.0% of the variability of memory. A regression equation was defined to predict memory from the independent variables: $memory = 0.747 - 0.682 \times VLF\ phase\ in\ the\ MCA - 0.028 \times overshoot\ systolic\ CBv\ in\ the\ PCA$.

There were no significant correlations between the MMSE, the total cognitive score, attention and executive function, and the hemodynamic variables.

DISCUSSION

In this cohort of HT patients, we found that reduced dCA in the MCA predicted better memory performance. In addition, reduced NVC in the PCA predicted better processing speed and memory performance. There were no significant associations between TCD markers of CBF regulation and overall cognition, namely the MMSE, the MoCA test, and the total cognitive score.

Processing speed mostly reflects structures supplied by the anterior circulation, and impairments in this function are among the earliest and most noticeable cognitive signs of CSVD.[38,39] Conversely, memory was tested using the AVLT total score, which has been shown to correlate to several temporal structures, including the hippocampus, the rostral medial temporal cortex and temporal pole, and the caudal middle frontal gyrus.[40] These structures receive blood from both the carotid and the vertebrobasilar systems.[41,42]

HT induces chronic hemodynamic changes, and several authors have shown intact or improved dCA in HT compared to normotensive subjects, suggesting permanent remodeling of the cerebral vasculature to accommodate the higher BP.[17,43-45] We have also observed this phenomenon in this cohort of HT patients compared to healthy controls, with similar phase parameters in both groups (p>0.05) but lower gain in the LF range in both arterial territories in the HT group (p=0.038, unpublished data). Additionally, we

have previously found reduced NVC in the PCA in this cohort of HT patients compared to controls despite preserved dCA, unrelated to the white matter damage.[17] In healthy subjects, reduced dCA in the MCA has been observed during a cognitive challenge, irrespective of oxygenation.[46] Other groups have shown attenuated dCA responses during visual stimulation in healthy volunteers.[47,48] This could reflect an evolutionary adaptation in which NVC mechanisms would increase CBF to the active brain regions independently of the hemodynamic status, allowing decision-making in potentially harmful situations. The HT-induced changes to the dCA, while improving the dampening capacity of the increased BP, may disrupt the hemodynamic adaptations that occur during a cognitive challenge. These chronic changes to the microvessel hemodynamics could account, at least partially, for the early cognitive impairment observed in the disease,[4,6,8,49] and are worth studying.

The unanticipated association of reduced NVC in the PCA with better memory performance and processing speed could also reflect a remodeling of the functional hyperemia responses, either compensatory or maladaptive, especially affecting the posterior circulation. Studies on NVC in HT are scarce, but reduced NVC responses have been demonstrated in the posterior circulation in other forms of CSVD.[50-53] In this regard, Jennings *et al.*, observed that parietal CBF was positively associated with verbal working memory in HT patients, but the inverse was true in normotensive patients. HT patients who performed poorly also showed less prefrontal cortex rCBF activation.[54] Another group observed major functional reorganization in cognitive processing in uncomplicated HT irrespective of macrostructural damage, with significant hyperactivation of several brain areas during an executive function task, mainly in the medial parieto-occipital cortex.[55] This could suggest the development of compensatory changes, with the involvement of additional areas in processing, even before recognizable tissue damage,[8] but could also reflect nonspecific cortical activation from disruption of neural networks. Another group has shown unaltered NVC in the MCA of middle-aged, well-controlled HT patients compared to healthy controls.[19] Our findings suggest improved cognitive function in tasks dependent on the frontal lobe, despite reduced NVC in the PCA area. This may indicate additional engagement of processing areas in posterior regions for these tasks with compromised cognition, leading to overcompensation of the NVC response in the PCA. Conversely, patients exhibiting better cognitive performance may not yet display overactivation of the posterior regions, with more efficient NVC in the PCA. The overall preservation of cognitive function in most patients may also indicate compensatory mechanisms contributing to cognitive maintenance within this population, supporting our hypothesis that TCD parameters may be reliable markers for early changes in the relationship between cerebral hemodynamics and cognitive performance.

Several groups have observed a posterior-to-anterior shift with aging (PASA) in cortical activity, usually understood as compensatory and contributing to the maintenance of cognitive performance.[56-58] However, Morcom *et al.* demonstrated that this increased prefrontal activation reflects reduced efficiency or specificity rather than compensation.[59] It is possible that these results translate to a less efficient neural function requiring greater hyperemia for the same level of processing in frontal regions,[58,60-62] which aligns

with our hypothesis that the NVC response in the PCA may be an indicator of cognitive performance. Using functional Near-Infrared Spectroscopy (fNIRS), Vermeij *et al.* found that healthy older adults use both hemispheres during an N-back task, even at low working-memory load (1-Back), compared to healthy young adults, indicating an effort to compensate for age-related decline in performance.[29] Alternative networks have also been demonstrated in patients with AD.[63-65] Perhaps similar modifications occur in the HT brain, caused by the subcortical small vessel damage, often more pronounced in the frontal white matter.[66] This phenomenon could contribute to accelerated brain aging and cognitive decline. More studies are needed to interrogate the long-term effects of HT on microvascular and brain network functioning.

Our sample had a low educational level and MoCA test scores despite only a small proportion scoring below the threshold for MCI when adjusting for age and literacy. This prompts consideration of the potential contribution of the educational level to our results. Several studies have shown a reduced risk of dementia with increased educational attainment.[67-69] Higher education has been linked to increased cognitive reserve, a concept describing the modulation of the brain's resilience to age- and pathology-related changes before clinical symptoms manifest.[70] It has been demonstrated that higher cognitive reserve may improve the efficiency of the brain networks involved in episodic memory maintenance in the face of advancing brain pathology.[71] In a fMRI study of older adults with high levels of WMH, higher education was associated with additional recruitment of brain networks not expressed by younger adults during a working memory task.[72] It stands to reason that changes in vascular hemodynamics could contribute to the cognitive reserve and explain some of the resiliency observed in patients with markers of CSVD. Our results align with this hypothesis since a higher educational level contributed to the better processing speed observed with reduced NVC in the PCA.

Regarding $VRCO_2$, we found no relation to cognitive performance in this cohort. We found no other studies on the relationship between $VRCO_2$ and cognition in HT patients. A study of older adults at increased risk of cognitive decline showed that whole-brain $VRCO_2$ was positively associated with cognitive performance.[73] In DM patients, baseline $VRCO_2$ did not predict cognitive decline after four years.[74] In AD patients, $VRCO_2$ was reduced compared to controls and significantly predicted the MMSE in these patients.[75,76] However, this could reflect reduced metabolism from brain atrophy instead of loss of vasomotor reserve, as studies in MCI patients show $VRCO_2$ similar to controls.[76,77] The role of $VRCO_2$ as a biomarker for cognitive decline in HT remains to be elucidated.

Our study has several important limitations. As noted, the cross-sectional design prevents inferences about the causal relationship between altered cerebral hemodynamics and cognitive performance. Also, the small sample size may have prevented the detection of significant associations. The limited scope of testing conducted on visuospatial and visuoconstruction abilities may have hindered the discovery of significant connections between hemodynamic changes in the PCA and cognitive performance related to parieto-occipital functions. The low educational level, a prevalent sociocultural trait in the Portuguese population,[78-81] and the presence of patients with scores below the cutoff for MCI are also noteworthy, as

previously discussed. Future larger, longitudinal studies are required to better understand the relationships described in our study, considering the influence of the cognitive status and sociocultural variables, and our data must be considered exploratory. In addition, the use of blood flow velocity as a surrogate for blood flow must be mentioned as a possible limitation, although since data was acquired in supine and relied on spontaneous measurements, changes in artery diameter are not anticipated for the dCA and NVC protocols. However, increasing partial pressure of arterial CO_2 leads to an increase in vessel diameter, which can lead to an underestimation of cerebral blood flow in the $VRCO_2$ protocol.[82]

In conclusion, our results suggest that changes in cerebral hemodynamics may impact cognitive performance in chronic HT and that TCD markers of CBF regulation, particularly the NVC in the PCA, could serve as valuable tools for predicting cognitive decline in this population. Future research is needed to further explore whether these changes are adaptive or if they occur due to reduced efficiency or specificity of the neurovascular networks. Additionally, investigating TCD studies as markers for early detection of cerebrovascular regulation changes associated with HT could offer valuable insights into potential therapeutic strategies for HT-induced cognitive decline.

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Author contribution statement

1A - Conception and design, 1B - Data collection and analysis, 1C - Literature search;

2A - Drafting the manuscript, 2B - Critical revision of the manuscript;

3. Supervision

4. Final approval

Ana Monteiro: 1A, 1B, 1C, 2A, 2B, 4

Pedro Castro: 1A, 1B, 2B, 3, 4

Gilberto Pereira, Carmen Ferreira, Mariana Lobo and Jorge Polónia: 1B, 2B, 4

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Disclosure/conflict of interest

The authors declare no conflicts of interest.

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Titles and legends to figures

Fig. 1 Heatmap for the correlations between TCD and cognitive variables. Age, gender, VCS, BMI and CVRi were also studied. The Pearson's correlation coefficient (r) is displayed within each circle, with bold values denoting statistically significant correlations ($p<0.05$). Negative correlations are depicted in red, while positive correlations are in green. The intensity of color shading reflects the magnitude of correlation strength. BMI: body mass index; CBv: cerebral blood velocity; CVRi: cerebrovascular resistance index; LF: low frequency; MCA: middle cerebral artery; PCA: posterior cerebral artery; TCD: Transcranial Doppler; VCS: vascular comorbidities score; VLF: very low frequency; $VRCO_2$: vasoreactivity to CO_2.

Fig. 2 Scatterplots for the dependent variables against the independent variables of interest. **A**: Rate time and processing speed; **B**: VLF phase in the MCA and memory; **C**: Overshoot systolic CBv in the PCA and memory. CBv: cerebral blood velocity; MCA: anterior cerebral artery; PCA: posterior cerebral artery; PS: processing speed; VLF: very low frequency.

Table1. Clinical characterization and cognitive evaluation

Demographics and comorbidities	**Value**	***Chronic medication***	**Value**
Age, years†	64±10	No. antihypertensives‡	3±2
Male, n (%)	28 (54)	ACEI/ARB, n (%)	48 (94)
BMI, kg/m2‡	29±5	Diuretics, n (%)	40 (77)
Diabetes Mellitus, n (%)	30 (58)	CCB, n (%)	38 (73)
Dyslipidemia, n (%)	45 (87)	BB, n (%)	15 (29)
Tobacco consumption, n (%)	6 (12)	Alpha2-agonists, n (%)	4 (8)
Nephropathy, n (%)		Antiplatelets, n (%)	14 (27)
CDK Stage 0–1	16 (31)	Statins, n (%)	40 (77)
CDK Stage 2	26 (51)	***ABPM, mmHg‡***	***Value***
CDK Stage 3	8 (16)	24-hour systolic BP	130±15
CDK Stage 4	1 (2)	24-hour diastolic BP	75±13
CDK Stage 5	0 (0)	24-hour pulse pressure	57±13
VCS, n (%)		***Cognitive parameters***	***Value***
1	2 (4)	Education, years‡	4±5
2	5 (10)	MMSE‡	28±3
3	21 (40)	MoCA‡	22±5
4	13 (25)	Cognitive score – total†	-2.4±5.0
5	8 (15)	Cognitive score – EF†	-1.7±2.3
6	3 (6)	Cognitive score - PS†	-0.6±3.0
HT duration, years‡	17±6	Cognitive score - memory‡	-0.3±1.0

†Values are presented as mean ± standard deviation. ‡Values are presented as median ± interquartile range; ABPM: ambulatory blood pressure monitoring; ACEI: angiotensin-conversion enzyme inhibitor; ARB: angiotensin receptor blocker; BB: beta-blocker; BMI: body mass index; BP: blood pressure; CCB: calcium channel blocker; CDK: Chronic Kidney Disease; executive function; EF: executive function; MMSE: mini-mental state examination; MoCA: Montreal cognitive assessment; PS: processing speed; VCS: vascular comorbidity score.

Table 2. Systemic and cerebral hemodynamics

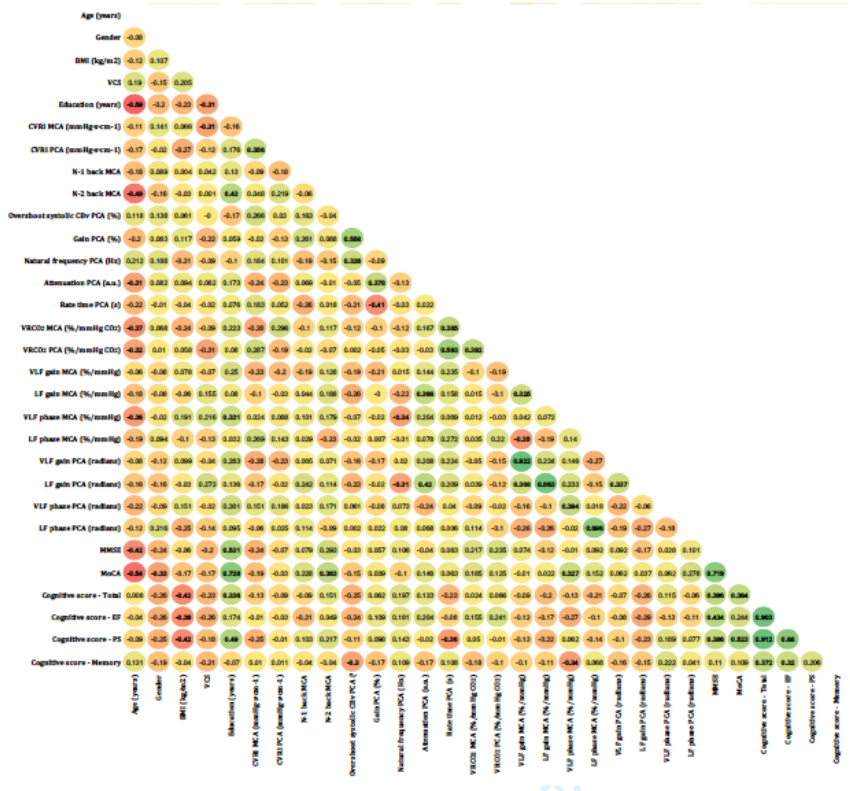
Systemic hemodynamics		
Finapres BP (5 minutes)		
Systolic BP†	135±24	
Diastolic BP†	61±17	
Mean BP SP, mmHg2‡	8±24	
EtCO2, mmHg‡	36±5	
Cerebral hemodynamics	**MCA**	**PCA**
Mean CBv (cm/s)†	48.0±13.2	32.1±8.0
MFV SP (cm/s^2)‡	3.2±5.2	2.5±5.1
CVRi (mmHg·s·cm-1)‡	1.8±0.8	2.6±1.0
Cerebral autoregulation		
VLF gain (%/mmHg)	0.8±0.5†	0.9±0.5‡
LF gain (%/mmHg)	1.0±0.6‡	1.2±0.6‡
VLF phase (radians)	1.0±0.4†	0.9±0.4†
LF phase (radians)	0.7±0.3†	0.7±0.5‡
$VRCO_2$ (%/mmHg CO_2)	1.4±0.5†	0.9±0.4‡
Neurovascular coupling†		
N-1 back	-0.7±5.5	-
N-2 back	3.4±4.3	-
Overshoot‖ systolic CBv (%)	-	22.3±9.0
Gain (%)	-	13.9±7.2
Natural frequency (Hz)	-	0.2±0.1
Attenuation (a.u)	-	0.4±0.3
Rate time (s)	-	0.6±1.8

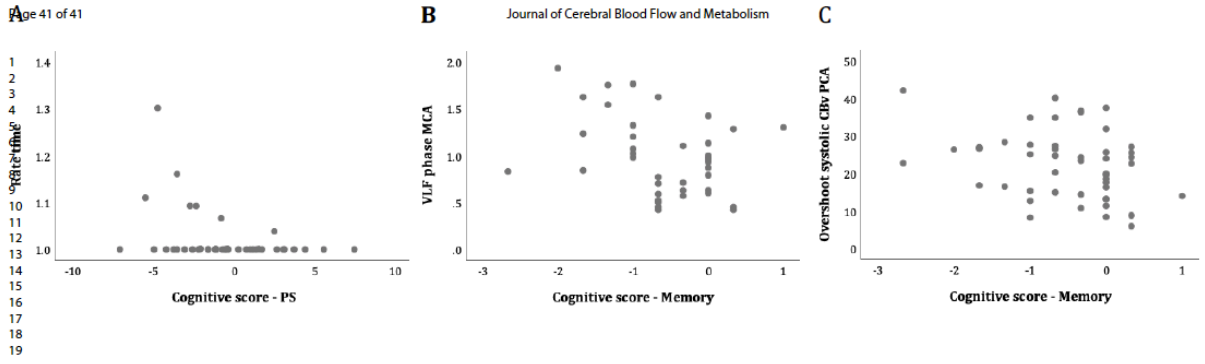
†Values are presented as mean ± standard deviation. ‡Values are presented as median ± interquartile range; ‖Maximal CBv increase during visual stimulation. a.u.: arbitrary units; CBv: cerebral blood velocity; $EtCO_2$: end-tidal carbon dioxide; LF: low frequency; MCA: middle cerebral artery; Mean BP SP: mean blood pressure spectral power; MFV SP: median flow velocity spectral power; PCA: posterior cerebral artery; VLF: very low frequency; $VRCO_2$: vasoreactivity to CO_2.

Table 3. Final regression models for the associations between cognitive performance (dependent variables) and TCD hemodynamic tests

MoCA			
	Estimated ß	*Standard Error*	*p-value**
Intercept	64.204	30.176	0.043
VLF phase MCA	0.781	1.252	0.538
N-2 back MCA	-0.133	0.142	0.356
Education	25.124	6.519	**0.001**
Age	-0.120	0.059	0.053
Gender	-65.042	24.770	**0.014**
R^2 = 0.690; aR^2 = 0.630; Model p<0.001			
Cognitive Score — processing speed			
	Estimated ß	*Standard Error*	*p-value**
Intercept	9.631	8.998	0.291
Rate time PCA	-20.896	6.583	**0.003**
Education	13.412	4.095	**0.002**
BMI	-0.173	0.079	**0.034**
R^2 = 0.446; aR^2 = 0.401; Model *p*<0.001			
Cognitive Score — memory			
	Estimated ß	*Standard Error*	*p-value**
Intercept	0.747	0.403	0.073
VLF phase MCA	-0.682	0.272	**0.017**
Overshoot systolic CBv† PCA	-0.028	0.012	**0.021**
R^2 = 0.250; aR^2 = 0.206; Model *p*=0.007			

**p-value for the variable of interest. †Maximal CBv during visual stimulation. R^2: Coefficient of determination (% of variability explained by the model). aR^2: adjusted R^2 (corrected goodness-of-fit, adjusting for predictors not significant in the model); BMI: body mass index; CBv: cerebral blood velocity; MCA: middle cerebral artery; MoCA: Montreal cognitive score; PCA: posterior cerebral artery; Bold values represent significant correlations.*





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Body: 22-Apr-2024

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PAPER 3: ADDITIONAL EVALUATIONS (NOT INCLUDED IN THE PAPER)

Association between cerebrovascular hemodynamics and cognitive performance in hypertensive patients: adjusted for depression and anxiety symptoms.

ADDITIONAL EVALUATIONS

METHODS

Neuropsychological examination

As a part of the comprehensive neuropsychological evaluation, the Hospital Anxiety and Depression Scale (HADS) was also performed to account for the effects of depression and anxiety on cognitive performance. As an oversight, these data were not included in the analysis for the paper submitted for publication. Since the investigator feels this data is essential and could potentially alter the results, the analysis was rerun, including the results for HADS-depression (HADS-D) and HADS-anxiety (HADS-A) (see Table 1. Patient characterization in the "Methods" section of the dissertation).

Statistical Analysis

The analysis was performed as described in paper 3.

RESULTS

Association between cerebrovascular hemodynamics and cognitive performance in hypertensive patients

Fig. 1 depicts the heat maps for the correlations between the TCD hemodynamic data (dCA, $VRCO_2$, and NVC) and the cognitive variables. Table 1 represents the final regression models for the significant correlations.

The MoCA scores significantly correlated with VLF phase in the MCA (r=0.327, p=0.039), the N2-NBT in the MCA (r=0.383, p=0.015), education (r=0.728, p<0.001), age (r=-0.536, p<0.001), gender (r=-0.326, p=0.018) and HADS-A (r=-0.361, p=0.009). However, the associations between the MoCA scores and the TCD variables lost significance when all covariables were included in the regression model (p=0.551 and p=0.309 for the VLF phase in the MCA and N2-NBT in the MCA, respectively). There were no other significant correlations for dCA.

The PS significantly correlated with the rate time (r=-0.357, p=0.022), education (r=0.490, p<0.001), HADS-D (r=-0.426, p=0.002), HADS-A (r=-0.491, p<0.001), and the BMI (r=-0.415, p=0.003). Reduced rate time significantly predicted better PS (p=0.032) after adjusting for the significant covariables. Since BMI, HADS-D, and HADS-A lost significance after adjusting for all covariables (p=0.133, p=0.434, and p=0.449), a new model was run with only the significant covariables. The final model explained 37% of the PS variability. A regression equation was defined to predict PS from the independent variables: $PS = 0.899 - 20.411 \times rate\ time + 15.935 \times education$.

Memory significantly correlated with the overshoot systolic CBFV in the PCA (r=-3.00, p=0.041) and VLF phase in the MCA (r=-0.342, p=0.031). Reduced overshoot systolic CBFV in the PCA and reduced VLF phase in the MCA significantly predicted better memory (p=0.021 and p=0.017, respectively). The final model explained 25% of the variability of memory. A regression equation was defined to predict memory from the independent variables: $memory = 0.747 - 0.682 \times VLF\ phase\ in\ the\ MCA - 0.028 \times overshoot\ systolic\ CBFV\ in\ the\ PCA$.

There were no significant correlations between the MMSE, the total cognitive score, attention, and executive function, and the hemodynamic variables.

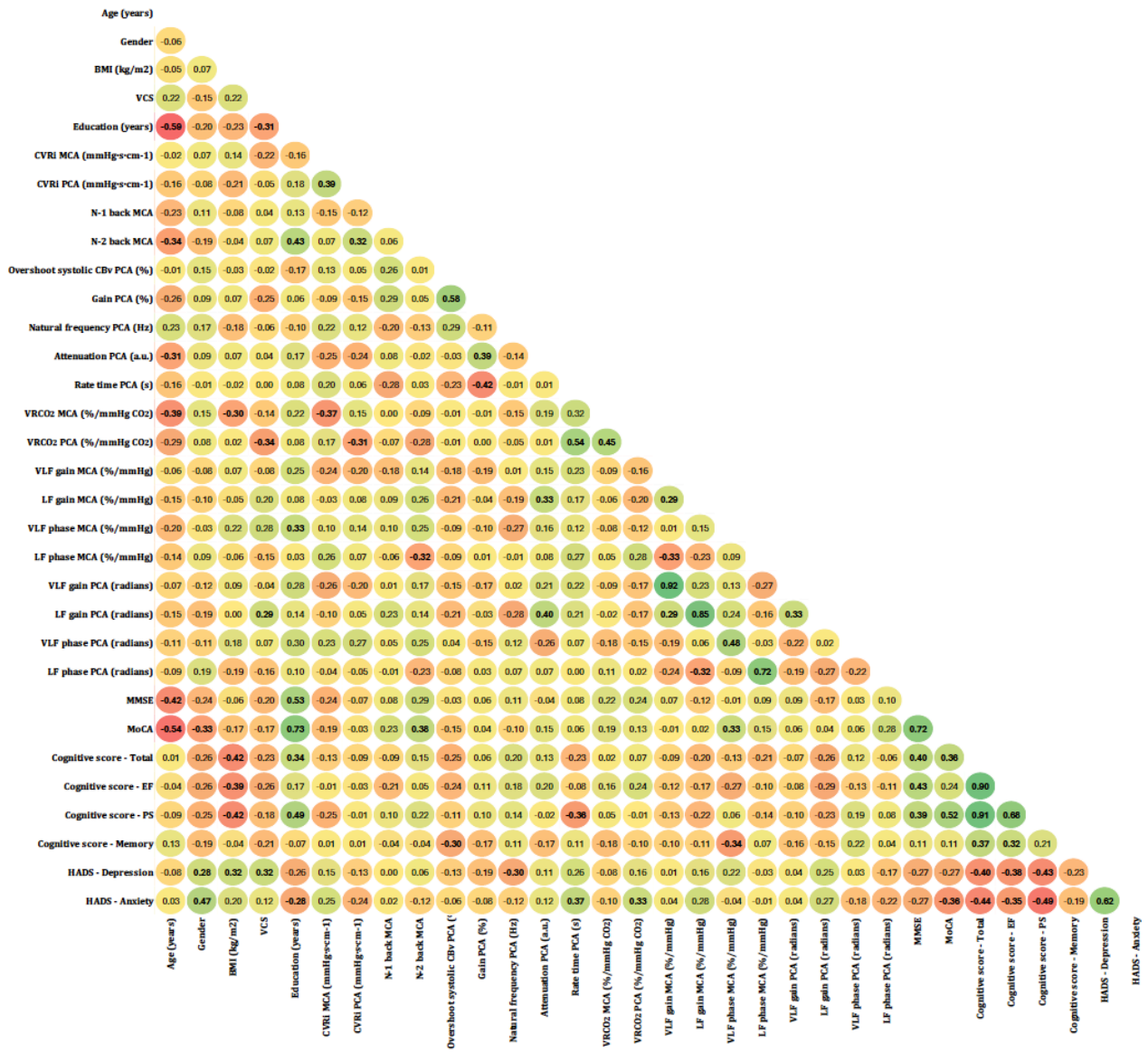

Figure 1. Heatmap for the correlations between TCD and MRI variables. Age, gender, VCS, BMI, education, HADS-D, HADS-A, and CVRi were also studied. Pearson's correlation coefficient (r) is displayed within each circle, with bold values denoting statistically significant correlations ($p<0.05$). Negative correlations are depicted in red, while positive correlations are in green. The intensity of color shading reflects the magnitude of correlation strength. a.u.: arbitrary units; BMI: body mass index; CBFV: cerebral blood flow velocity; CVRi: cerebrovascular resistance index; EF: executive function; HADS-D: Hospital Anxiety and Depression Scale – depression; HADS-A: Hospital Anxiety and Depression Scale – anxiety; LF: low frequency; MCA: middle cerebral artery; PCA: posterior cerebral artery; PS: processing speed; TCD: Transcranial Doppler; VCS: vascular comorbidities score; VLF: very low frequency; $VRCO_2$: vasoreactivity to CO_2.

Table 1. Final regression models for the associations between cognitive performance (dependent variables) and TCD hemodynamic tests

MoCA			
	Estimated ß	*Standard Error*	*p-value**
Intercept	65.856	30.325	0.040
VLF phase MCA	0.760	1.256	0.551
N-2 back MCA	-0.149	0.143	0.309
Education	22.871	6.988	**0.003**
HADS-A	-0.115	0.126	0.369
Age	-0.120	0.059	0.053
Gender	-62.180	25.045	**0.020**
R^2 = 0.700; aR^2 = 0.628; Model p<0.001			
Cognitive Score — processing speed			
	Estimated ß	*Standard Error*	*p-value**
Intercept	0.899	8.473	0.916
Rate time PCA	-20.411	6.904	**0.005**
Education	15.935	4.125	**<0.001**
R^2 = 0.374; aR^2 = 0.341; Model *p*<0.001			
Cognitive Score — memory			
	Estimated ß	*Standard Error*	*p-value**
Intercept	0.747	0.403	0.073
VLF phase MCA	-0.682	0.272	**0.017**
Overshoot systolic CBFV† PCA	-0.028	0.012	**0.021**
R^2 = 0.250; aR^2 = 0.206; Model *p*=0.007			

**p-value for the variable of interest. †Maximal CBFV during visual stimulation. R^2: Coefficient of determination (% of variability explained by the model). aR^2: adjusted R^2 (corrected goodness-of-fit, adjusting for predictors not significant in the model); BMI: body mass index; CBFV: cerebral blood flow velocity; MCA: middle cerebral artery; MoCA: Montreal cognitive assessment; PCA: posterior cerebral artery; Bold values represent significant correlations (p<0.05).*

CHAPTER IV – DISCUSSION

This research project investigated the potential utility of transcranial Doppler markers of microvascular dysfunction as possible tools for studying cerebral small vessel disease in hypertensive patients. We selected to study HT due to its status as the foremost modifiable risk factor for CSVD, a condition with an undeniable impact on public health, yet lacking preventive and interventional measures. Given the widespread occurrence and chronic nature of CSVD, we aimed to employ a methodology that would be readily implementable, sensitive, cost-effective, and safe for long-term application. The TCD appeared optimal due to its non-invasive nature, affordability, remarkable temporal resolution, and versatility across various circumstances. We focused our research on HT patients who do not exhibit any symptoms, as silent CSVD represents the most prevalent manifestation of the disease, and for which cost-effective diagnostic and monitoring tools are still wanting.

To fulfil these objectives, we sequentially studied dynamic cerebral autoregulation, vasoreactivity to CO_2 and neurovascular coupling within a cohort of HT patients asymptomatic for CSVD. The purpose was to obtain a comprehensive overview of CBF regulation in this patient population. We collected simultaneous data from the right MCA and the left PCA. To assess dCA, we leveraged spontaneous fluctuations in BP, thereby obviating the need for patient cooperation and avoiding the confounding effects of pharmacological manipulation. $VRCO_2$ measurements involved the consecutive application of hypercapnic and hypocapnic stimuli interspersed with rest periods. The NVC protocol encompassed an executive function task to evaluate the MCA and a visual paradigm to assess the PCA. In addition to quantifying the maximal visually evoked systolic change in CBFV, we analysed the modelled CBFV curve to provide a more nuanced characterisation of its dynamics and enhance sensitivity in detecting disruptions in NVC within the PCA. Furthermore, the patients underwent cognitive and imaging assessments, considered the gold standard for CSVD diagnosis, and we compared the TCD results to those of a cohort of healthy subjects previously studied in our laboratory. We also investigated aspects of renal function and quantified daily salt intake, as renal disease and excessive salt consumption are prevalent in HT populations, aiming to further contribute to understanding the factors implicated in CSVD progression.

Main findings

First, we demonstrated that NVC in the PCA is impaired in HT patients compared to controls and that comorbid DM further compromises functional hyperemia in this territory. In addition, we found that the natural frequency parameter is particularly sensitive for identifying abnormal NVC in these patients. These results were independent of WMH burden

and suggest that NVC in the PCA, particularly the modeled parameter natural frequency, is a potential surrogate marker for microvascular dysfunction in HT patients.

Secondly, we found evidence that increased salt intake is significantly associated with worse natural frequency in the PCA territory in HT patients. Again, these results were dissociated from the MRI markers of CSVD, and there was no association between daily salt intake and cognitive performance. These results further indicate that the natural frequency may be a sensitive marker for small vessel disease in the brain and that increased salt intake may contribute to CSVD independently of its effect on blood pressure.

Finally, we found that enhanced dCA in the MCA predicted poorer memory performance, while reduced NVC in the PCA predicted superior processing speed and memory performance. These effects on cognition may indicate hemodynamic and neural modifications occurring within brain networks affected by HT.

CSVD remains an understudied condition. Prospective studies have shown that MRI markers of CSVD are highly prevalent in the general population and significantly increase the risk of stroke, dementia and death.[257] However, guidelines for managing incidental findings of CSVD are still lacking, likely representing missed opportunities to prevent complications.

CSVD related to HT is a progressive disease despite multiple pharmacological interventions that can successfully reduce BP. There is limited evidence that the reduction of BP alone significantly reduces the progression of CSVD (as discussed in "Therapeutic considerations").

One possible explanation could relate to the long-term effects of HT on cognition, even before reaching BP levels diagnostic for HT. Worse cognitive performance has been demonstrated in prehypertensive young adults during sleep deprivation compared to normotensives.[258] Long-term BP variability from healthy young adulthood is associated with worse verbal memory and psychomotor speed in midlife.[202] There is evidence of impaired cognition in the high-normotensive range in midlife, an effect lost in late life.[201] In fact, higher BP seems protective against cognitive decline in the elderly.[259-261] In a study with midlife prehypertensive adults, frontostriatal regional CBF (rCBF) and cognitive performance significantly predicted subsequent BP elevations over 2 years.[262] Hence, high BP per se may not cause cognitive decline, and long-term brain modifications may occur that precede or simultaneously occur with the systemic BP elevations and detrimental microvascular and cognitive modifications associated with HT.

Dynamic cerebral autoregulation in hypertension

The differences in risk profile with BP variations with age may reflect changes in the autoregulatory curve. The curve is right-shifted in chronic hypertension, lowering the threshold for ischemia.[261] Cerebral autoregulation is impaired in older adults, which could explain the higher sensitivity to lowering BP.[263] Also, dCA is more efficient in counteracting acute BP elevations than hypotension.[15]

In our study, HT patients showed a lower gain in the LF range when compared to controls, reflecting a higher dampening capacity to counteract the magnitude of BP oscillations. This could be an adaptive response to chronically higher systemic BP. As reviewed in Chapter I, HT causes structural and functional changes in cerebral arteriolar walls. An increase in carotid distensibility has been observed in aggressively treated, previously uncontrolled HT patients and correlates to an increase in CBFV, which may help dampen oscillations in systemic BP.[264] Other authors have shown better attenuation of BP fluctuations in HT subjects when compared to normotensive subjects, not explained by cardiac output or cerebrovascular resistance, favouring the hypothesis of permanent remodelling of the cerebrovasculature to accommodate the higher pressures.[205,215,265] Nevertheless, recent studies in chronic HT found impaired dCA compared to healthy controls, suggesting that other non-pressure-dependent mechanisms may contribute to the dysfunction in long-term HT.[210,266] Differences in the results from dCA studies in HT may also be related to the failure to account for age of onset of HT (midlife vs late-life). In mouse models, HT induced in aged mice resulted in impaired CA, whereas in young mice, the autoregulated range expanded, indicating an adaptative response. Moreover, in aged mice, HT was associated with increased BBB disruption, neuroinflammation, and cognitive decline.[51] We found no differences in phase parameters when comparing patients and controls, which is consistent with the results from previous studies.[205,264] These results must be cautiously interpreted since phase is the most robust dCA parameter. Differences in gain could reflect a methodological consequence of the difference in BP levels per se and not increased dCA efficiency.[267] Moreover, HT patients showed higher BP variability, probably due to diminished baroreflex sensitivity, which can also influence dCA measurements.[268] Also noteworthy, our results lost significance when adjusting for BMI.

We found no correlation between dCA parameters and MRI markers of CSVD in our cohort, which may not be surprising considering we found preserved dCA in our patient cohort, which is consistent with findings from other authors.[215] Another group found no correlation between WMH and dCA even when dCA is impaired.[210] A study found no association between dCA and brain atrophy in healthy and diabetic older adults.[269] Interestingly, preserved dCA has been demonstrated in patients with MCI and dementia due to AD.[270] Studies have shown cerebral

hypoperfusion and reduced cortical thickness in HT patients,[271,272] but there are no other studies on the association between dCA and brain volume. Our data suggests that dCA may not be a major contributing factor to WMH burden and brain atrophy in HT patients. Additional investigations are warranted to clarify the role of dCA in CSVD in HT.

The role of cerebral vasoreactivity in hypertension-induced CSVD

$VRCO_2$ remained intact in our cohort of well-controlled HT patients. This contrasts with the findings from Serrador *et al.*, where $VRCO_2$ was impaired even with intact dCA.[205] $VRCO_2$ has been shown to be independent of BP levels but significantly affected by the duration of HT.[215] Animal models have also suggested this relationship, indicating that the duration of HT is important for the loss of vasodilatory capacity.[273] However, our cohort of HT patients with even longer disease duration exhibited no $VRCO_2$ impairment. Similar to findings from other cohorts,[215,274,275] there was no correlation between $VRCO_2$ and WMH. It has been previously reported that reduced $VRCO_2$ is present in areas of NAWM that progress to WMH in the following year, suggesting that $VRCO_2$ precedes the development of WMH.[226] A reduced $VRCO_2$ in NAWM is associated with greater disruption of WM integrity.[226,276] Impaired $VRCO_2$ has been demonstrated in patients with first-ever symptomatic ischemic stroke and was worse in patients with multiple infarctions.[274,275] In patients with cerebral autosomal dominant arteriopathy with subcortical microinfarcts and leukoencephalopathy (CADASIL), a genetic form of CSVD, worse $VRCO_2$ is associated with an increasing number of lacunes.[277] $VRCO_2$ impairment has also been demonstrated in patients with different manifestations of CSVD (lacunar stroke, vascular dementia, and vascular parkinsonism) as compared to controls, with a significant decline over time, independently of the radiological progression of CSVD.[224] These data could indicate that impaired $VRCO_2$ precedes but may be a relatively late occurrence in the cascade that leads to CSVD. Our findings support this notion, as our cohort of patients had low WMH volume despite a long disease duration, although the lack of MRI studies in the control group is a significant limitation. Additional research is necessary to gain a deeper understanding of the potential impact and timing of $VRCO_2$ dysfunction in the development of CSVD. Moreover, longer prospective studies would be valuable in determining whether sustainable control over HT from early adulthood contributes to maintaining cerebrovascular reactivity instead of focusing solely on establishing adequate control during the months preceding the evaluations.

Neurovascular coupling as a biomarker and therapeutic target in CSVD

Another explanation for the lack of benefit of antihypertensive agents in reducing CSVD-associated cognitive decline may be a lack of effect on the increased BBB permeability. Mamo *et al*. demonstrated that candesartan and ursodeoxycholic acid did not prevent BBB dysfunction and cognitive decline in HT mice despite preventing BP elevation.[278] As previsoully highlighted, there is evidence of increased BBB dysfunction with increasing CSVD severity in multiple brain regions. It is detectable in NAWM as well as lesional tissue,[154,279,280] and it is worst near WMH.[281] Studies have shown the presence of BBB damage even in patients with mild, adequately controlled HT and with minor clinical and MRI signs of CSVD.[282] BBB dysfunction is also an early biomarker of cognitive decline.[46] Therefore, NGVU dysfunction should be studied as an early biomarker and therapeutic target for HT-induced CSVD. As the NGVU is a major contributor to the NVC,[191,283] studying the latter may provide a reasonable surrogate marker.

Our patients exhibited reduced NVC in the PCA before developing symptoms of CSVD and independently of WMH burden and cortical thickness. Studies on NVC in HT are scarce. Jennings *et al.* assessed rCBF responses by PET and found that HT patients had reduced rCBF in the parietal cortex when performing verbal and spatial working memory tasks. However, there were no differences in rCBF from controls in the prefrontal cortex.[284] A pilot randomized controlled trial demonstrated enhanced NVC in the MCA of borderline HT patients after 20 weeks of supplementation with long-chain omega-3 polyunsaturated fatty acids.[285] To our knowledge, no other studies of cerebral NVC in HT subjects have been reported. In the retinal vasculature, functional hyperemia was impaired in young HT subjects.[286] A recent study also showed decreased NVC responses in the retina of older healthy adults compared to young adults. Studying NVC in the retinal vasculature could also be a viable biomarker and should be explored concomitantly with cerebral NVC in future studies.[287]

NVC dysfunction has been demonstrated in other forms of CSVD. There is altered NVC in the visual cortex of patients with symptomatic sporadic cerebral amyloid angiopathy (CAA), a disease that causes loss of small vessel integrity due to the ß-amyloid deposition.[288,289] CAA patients have lower BOLD fMRI responses with normal visual evoked potentials, suggesting a decoupling of the vascular and neuronal responses rather than a decrease in metabolic activity.[289] In hereditary forms of CAA, both symptomatic and presymptomatic patients showed reduced BOLD signal changes in the occipital lobe after visual stimulation.[227] In patients with Fabry disease, an X-linked condition also resulting in cerebral microvascular disease, maximal CBFV increase during visual stimulation was significantly lower than in controls.[158]

Another novel finding of our work is that the natural frequency is the most sensitive parameter for discriminating abnormal NVC in HT. The natural frequency, believed to characterize the tonus and the system's speed, provides insight into the oscillatory properties of the vessel.[194] Rosengarten *et al.* hypothesised that this parameter was the most likely to differentiate between normal and abnormal NVC. Nevertheless, its capacity for discriminating microvascular dysfunction in disease states remained to be determined.[194] Reduced oscillatory properties in the PCA territory were subsequently demonstrated in Fabry disease patients.[169] More recently, less robust functional hyperemia during visual stimulation was demonstrated in patients with CADASIL. These patients presented lower rate time and natural frequency, suggesting delayed hemodynamic adaptation and loss of elasticity in the posterior vascular territory.[290] Thus, impairment of the NVC appears to be an early occurrence in multiple diseases where CSVD is the pathological hallmark.

Evidence shows that the NGVU and the NVC, as a surrogate for the NGVU, can be therapeutic targets in CSVD. NVC responses in the brain involve, at least in part, the release of NO from the endothelial cells in the microvasculature.[291] In recent work, NOS inhibitors significantly decreased the peak NVC response and rapidity of the initial rise in the NVC response in healthy subjects, depending on whether the inhibition was non-selective or selective.[292,293] Angiotensin II (Ang II), a critical mediator in HT, has emerged as the perpetrator of NVC dysfunction. Chronic subpressor infusion of Ang II disrupts NVC even before the development of HT in animal models,[294] and it has been suggested that Ang II and not HT is the cause of NVC disruption.[295] Ang II has vasoconstrictor and remodelling effects on cerebral arteries, induces proinflammatory responses and increases oxidative stress.[296] Angiotensin-converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARB) have been studied for the prevention of cerebrovascular consequences of HT, but the effects on brain atrophy and cognitive decline have been less than ideal. In a mouse model of HT, functional hyperemia was progressively impaired in 20 and 40-week-old mice and treatment of 40-week-old mice with losartan for 10 weeks did not improve NVC despite reversing capillary blood flow velocity increase to control levels.[297] Targeting Ang II receptors in earlier phases of the disease, using NVC as a guide, could elicit better results. It has been proposed that the aldosterone breakthrough, induced by ACEi and ARB, could explain this lack of effect on NVC. Combining an ACEi and a mineralocorticoid receptor antagonist improved NVC in a mouse model.[298]

Impaired NVC has also been demonstrated in AD due to amyloid-β (Aβ) deposition in the NGVU.[299-301] The NGVU dysfunction contributes to the early, pre-plaque cognitive impairment and the subsequent progression of the disease.[299] Intriguingly, nicotinamide adenine

dinucleotide phosphate (NADPH) oxidase-derived oxidative stress mediates Aβ-induced cerebrovascular dysfunction, a mechanism shared with Ang II.[302] More recently, impaired NVC was demonstrated in the hippocampus of a mouse model of AD, and was reversed using antioxidant treatment.[303] HT increases the risk of AD and accelerates disease progression.[44,299,304] Evidence suggests that HT promotes Aβ deposition, working synergistically to worsen CSVD and cognitive decline.[44,299] HT, aging and AD share a pathway for microvascular dysfunction: NADPH oxidase-derived oxidative stress.[305] HT is increasingly recognised as a subclinical inflammatory condition. In mouse models of Ang II-induced HT, treatment with the anti-inflammatory interleukin-10 (IL-10) prevented neurovascular uncoupling.[306] The pro-inflammatory IL-17A is upregulated in HT patients, and serum levels are correlated with BP.[307] The effects of Ang II on NVC may even be mediated by IL-17A.[308]

Pericytes have gained interest for their role in regulating CBF and maintaining the BBB, as discussed in previous sections. Pericyte dysfunction is associated with reduced functional hyperaemia, cerebral hypoperfusion and hypoxia, leading to WM disease.[309] Pericytes have a vital role in modulating the NVC processes,[310] and their loss may be an important element of CSVD and neurodegenerative conditions. Depletion of pericytes causes BBB breakdown, severe loss of blood flow, rapid neuronal loss and degeneration.[311] BOLD functional imaging signals might reflect capillary dilation by pericytes.[312] In stroke, cerebral ischaemia causes pericyte death in rigor after constricting, causing long-lasting decrease in blood flow.[312] Both DM and HT have been associated with pericyte disfunction,[62,313] and pericyte loss is an early feature of diabetic retinopathy.[314] Interestingly, the pericyte may be a target for the ANS in blood flow regulation,[315] and a role of the ANS in controlling functional hyperemia has been suggested, as demonstrated by NVC impairment in the PCA in familial amyloidotic polyneuropathy.[18] As such, the pericyte may be a target for therapies aiming to improve CBF regulation and the efficacy of such therapies could be monitored by assessing changes in NVC.

Diabetes mellitus additional contribution to CBF dysregulation

Our study demonstrated increased cerebrovascular dysfunction in HT patients with comorbid DM. This subgroup had worse NVC, particularly the oscillatory properties of the vessel, represented by the natural frequency. Another group has demonstrated decreased functional hyperemia during mental activation in DM adults using TCD.[316] The same group conducted a randomised controlled trial using a TCD NVC protocol as an outcome measure and reported improved NVC in the MCA of DM patients after resveratrol consumption.[317] To our knowledge, no other studies reported NVC using TCD in this patient population.

NVC dysfunction during visual stimulation has been demonstrated in children with type 1 DM and pregnant women with gestational diabetes.[318,319] Studies of NVC in DM patients have mainly used ALS and fMRI. Impaired BOLD response to a visual paradigm was observed in intact brain tissue of DM patients.[320] NVC changes in specific brain regions have been demonstrated in DM, contributing to cognitive decline.[321,322] Canna *et al*. observed reduced NVC in the default mode network (DMN) and increased NVC in frontal regions relating to attention networks (namely the dorsal (DAN) and the salience-ventral (SVAN) attention networks) in DM patients.[323] Disrupted DMN has been consistently found in Alzheimer's disease,[324-326] and may reflect common disease mechanisms. The increased NVC in the DAN and SVAN, which are involved in executive and emotional processing and visual processing, were associated with better cognitive performance and could reflect the development of compensatory circuits.[323]

NVC disruption has also been demonstrated in the retina in early DM, even before there are signs of diabetic retinopathy (DR), and worsens as the DR progresses.[327] Reduced NVC occurs independently of electroretinogram deficits or capillary loss, indicating that the NVC abnormality predates neuronal dysfunction and contributes to reduced blood flow.[328,329]

Sustained hyperglycemia leads to neuroinflammation and increased oxidative stress, leading to BBB damage and neuronal dysfunction.[330] BBB dysfunction has been shown to precede the neurodegeneration and cognitive decline in diabetic mice, suggesting causation,[331] and treatment with a BBB protective agent prevents cognitive decline.[332] The accumulation of advanced glycation end-products (AGEs) in the small arteries contributes to increased wall rigidity and could explain the worse natural frequency we observed in this subgroup. AGEs-induced glycation of basement membrane components reduces NO production by endothelial cells and impairs vasodilation.[333] AGEs also increase inflammation and oxidative stress via AGE receptor activation.[334]

These data suggest that, as with HT, NVC impairment is an early occurrence in DM and is a viable biomarker for treatment development.

We found no differences in dCA or $VRCO_2$ between the HT-nDM and HT-DM subgroups. Other studies have shown no differences in dCA between DM patients and healthy controls.[335] Our results could reflect the small sample size for identifying differences between subgroups. However, it could also indicate that HT-induced dCA adaptations may resist the influence of other risk factors, at least in well-controlled DM (as was the case in our cohort, HbA1c 6.2±1.3%, mean±SD, data not published). Kim *et al.* described impaired dCA in DM patients compared to healthy controls.[336] The severity of dCA dysfunction was higher in DM patients with microvascular complications (DM+) than in DM without (DM-). However, most (if not all)

patients were HT, and these comparisons were identified using ANOVA, not controlling for the presence of HT or other risk factors as contributors to the result. Nevertheless, a regression analysis was performed, including SBP and DBP, in which only HbA1c and DM duration contributed to the differences in phase. A possible explanation is that the influence of HT and DM in dCA may differ whether HT predates or follows the development of DM. Moreover, their patients had worse controlled DM (HbA1c 7.2±0.8% and 8.0±1.1%, mean±SD, in DM+ and DM-, respectively), which could contribute to the differences from our results. In type 1 DM, cardiovascular autonomic neuropathy (CAN) was associated with impaired dCA, irrespective of HbA1c and BP. Type I DM patients without CAN had preserved dCA, similar to the control group, despite similar disease duration as the type I DM patients with CAN.[337] This offers a mechanism for the differences observed and suggests a role of dysautonomia in the microvascular damage of DM.

Studies on $VRCO_2$ in DM have elicited conflicting results. Some studies have observed no differences in $VRCO_2$ between DM and controls,[335,338,339] whereas others demonstrated impaired $VRCO_2$ in DM with long disease duration or microangiopathic complications.[340-343] Impaired $VRCO_2$ was been demonstrated in DM without HT relative to controls.[344,345] Betterman *et al*. showed impaired $VRCO_2$ in DM even after controlling for age and HT.[346] In another study, however, impaired $VRCO_2$ in DM patients was associated with HT, WMH, and higher HbA1c.[347] Elevated serum inflammatory markers were associated with the subsequent decrease in vasoregulation and cognition in DM patients after a 2-year follow-up,[343] again suggesting a role of inflammation and oxidative stress in this decline, as with HT. Taken together, these results point to a contribution of DM to impaired $VRCO_2$, and our study was probably underpowered to detect differences between the HT-nDM and HT-DM groups. Additional studies are needed to uncover further the role of impaired dCA and $VRCO_2$ in DM-associated CSVD.

The potential role of dietary salt in microvascular dysfunction

In our cohort of HT patients, higher daily salt intake was associated with an altered hemodynamic evoked response to visual stimulation in the PCA with worse natural frequency, while BP did not influence microvascular hemodynamics.

A high-Na^+ diet has long been associated with HT, especially in salt-sensitive individuals. Salt sensitivity of BP (SSBP) refers to changes in BP that mirror fluctuations in dietary salt intake. Research of SSBP in rodents is dichotomised, using salt-sensitive and salt-resistant strains to study the effects of salt on BP and cerebral hemodynamics. However, human research is more complex due to the continuous, normally distributed nature of SSBP in

individuals.[249] Defining individual levels of Na^+ sensitivity relies on arbitrarily determined thresholds for the magnitude of BP changes. Furthermore, physiological factors can potentially influence the accurate assessment of how BP responds to variations in Na^+ consumption.[249]

Excessive salt consumption is not only associated with high BP, but also poses an independent risk for stroke, dementia and WM damage.[94,348,349] Studies have shown that the detrimental effects of salt on vascular health are observed in individuals with prehypertension and in healthy individuals.[350-353] Animal research has demonstrated that an excessive salt intake directly affects the vasculature by promoting microvascular rarefaction and disrupting the balance between vasoconstrictors and vasodilators, leading to impaired vasorelaxation.[354] Moreover, elevated Na^+ levels stimulate the production of ROS and inflammatory cytokines, which may impact the cerebral small vessel endothelium directly.[355,356] High Na^+ impairs endothelial function even in normotensive salt-resistant individuals, supporting the direct role of salt in microvascular disease.[357] Human and animal research have demonstrated increased arterial stiffness with high salt consumption, independent of BP.[358] There is also evidence of effects on the immune system and gut microbiome, and salt may trigger autoimmune disease.[354]

Rodent research demonstrated that chronic exposure to a high salt diet impairs cerebral autoregulation during decreases in systemic BP.[252] Flow-induced dilation of the MCA was reduced in rats exposed to chronically high dietary salt, and this effect was reversed using a superoxide scavenger, indicating that ROS play a role in this process.[359] Moreover, even short-term intake of high salt levels has been linked to impaired vasodilator function within the cerebral microcirculation.[360] Increased salt intake may also impair $VRCO_2$, as evidenced by the reduced MCA relaxation during hypoxia in rodents fed a high salt diet.[253] In a murine model, a high salt intake lead to a systemic inflammatory state and impaired NVC.[361] The mechanism by which excessive salt exposure leads to chronic hemodynamic modifications and HT is not entirely understood. Recent animal research has demonstrated a slow, sustained, widespread vasoconstriction response in the hypothalamic supraoptic nucleus after an acute systemic salt load. This inverse NVC response led to local hypoxia, which increased the firing of vasopressin-producing neurons, with increased vasopressin release from dendrites.[188] This could mean chronic high salt intake increases vasopressin circulating levels, causing systemic vasoconstriction and increasing vascular resistance and BP.

Although data indicate the harmful impact of salt on blood vessels and cerebral hemodynamics, there remains a noteworthy dearth of information regarding the influence of dietary salt on regulating CBF in humans.

Recently, Dumančić *et al*. studied healthy young adults after 7 days of a low-sal (LS) diet followed by 7 days of a high-salt (HS) diet to evaluate the impact of excessive salt on cerebral vasoreactivity to orthostatis and to study the potential role of the ANS. They found increased cerebrovascular resistance in the MCA after rising up, as well as blunted baroreflex sensitivity to orthostasis and attenuated sympathetic activity following the HS diet. These results occurred despite participants remaining normotensive and with preserved CBF in both diet conditions.[362] However, this hemodynamic response may be short-lived, and long-term excessive Na^+ consumption may have different effects on the microvasculature, since, as discussed previously, repeated activation appears to cause sensitisation and amplification of the sympathetic responses, and may have a role in the brain adaptations leading to HT.[199]

Migdal *et al*. found no impairment of $VRCO_2$ after either a single meal (considered an acute challenge) or ten days (considered a chronic challenge) of HS intake in healthy young adults.[363,364] The same authors found that a HS meal acutely caused a modest reduction in dCA during supine spontaneous fluctuations in BP in healthy young adults, but there was no effect of ten days of HS meals in dCA.[364,365] A ten-day salt challenge is an acute change when considering a lifetime of exposure to a HS diet, which could explain these negative results. In addition, these were healthy, young, non-hypertensive adults, who could be salt-resistant. Our results align with Migdal´s work, as we found no association between daily salt intake and dCA or $VRCO_2$ in our cohort of HT patients. Studies are needed to clarify whether this reflects a chronic adaptation of the cerebral vasculature to high levels of Na^+, similar to the preserved dCA observed in HT patients. However, despite these results, the effects of chronic excessive salt intake on dCA and $VRCO_2$ remain to be elucidated, preferably in at-risk populations and with a prospective design.

To our knowledge, there are no human studies on the impact of dietary salt on NVC besides ours, and no other studies have looked at its impact on dCA and $VRCO_2$ in healthy or diseased populations. Further investigation is warranted to evaluate the impact of varying salt intake on individual cerebral hemodynamic responses and to understand the cerebrovascular implications of excessive salt consumption. Additionally, identifying patients who would experience a clinically significant reduction of cerebral microvascular damage through Na^+ restriction, even in the absence of HT, would be invaluable. Our results, however preliminary, suggest that the NVC, particularly the natural frequency, is affected by excessive Na^+ and point to the possible role of NVC as a surrogate biomarker for studying the effects of salt restriction on the reduction of CSVD.

Microvascular dysfunction in the brain and the kidney

We found no association between renal function, measured by the eGFR and albumin-to-creatinine ratio (ACR), and the TCD hemodynamic tests in the MCA and PCA arterial territories. In addition, our results show no association between eGFR and ACR and MRI markers of CSVD or cognitive function.

Despite evidence of increased incidence of stroke and markers of CSVD with renal dysfunction,[366] data on cerebral hemodynamics in CKD are scarce. CKD patients exhibit cerebral hyperperfusion due to secondary anaemia.[367] In a study with young CKD patients, increased CBF was observed compared to controls, and the reduced hematocrit explained most of the differences. In addition, there was an association between the eGFR and CBF in control subjects, also explained by differences in the hematocrit, whereas the association between eGFR and CBF was lost in CKD patients.[368] Other MRI studies have shown conflicting results regarding the association between eGFR and CBF.[236,243,369]

Sprick *et al.* showed preserved dCA in CKD patients in stages III-IV compared to controls, suggesting that other mechanisms may be responsible for the increased CSVD observed in these patients.[370] However, in patients with acute ischemic stroke, decreasing eGFR was associated with progressive impairment in dCA. Nevertheless, it is unclear whether the autoregulation dysfunction occurred before stroke onset or if stroke-induced dCA impairment is amplified in CKD.[242] There is evidence of impaired CA in acute kidney injury, with increased microvasculature permeability and damage to the BBB,[371] but data on dCA in CKD is mostly limited to hemodialysis (HD) populations, and the lower limits of autoregulation appear highly variable.[367]

$VRCO_2$ research has yielded conflicting results, with some studies reporting impaired cerebral vasoreactivity in patients with CKD, while most studies have found no significant difference compared to healthy controls.[367,372,373] Impaired $VRCO_2$ has been found to relate to the degree of anaemia and may be a response to the hypoxia, with maximal dilation at rest, rather than a failure to respond to the vasoactive stimuli.[374] Despite lower NO bioavailability in CKD,[375] other compensatory vasodilators may be responsible for the preserved $VRCO_2$ observed in CKD patients, as $VRCO_2$ is only partially NO-dependent.[376]

Regarding NVC, the literature focuses on HD patients, preventing associations to the eGFR, and shows impaired NVC in end-stage CKD compared to healthy controls.[377,378] A recent study suggested that the NVC uncoupling observed after HD could be reversed by correcting the anaemia and hyperhomocysteinemia, maintaining stable systolic BP, and fluid restriction.[379] To our knowledge, no other studies exist on the association of eGFR and NVC in patients with renal impairment.

Albuminuria represents a marker of microvascular endothelial damage and precedes the loss of kidney function and the development of markers of CSVD.[239,380] As discussed, damage to the endothelium is one of the key mechanisms for BBB breakdown, and hence the ACR could be a valuable early biomarker for the emergence of features of CSVD. However, despite the numerous reported associations of albuminuria, cerebral microvascular disease, and cognitive decline,[236,237,203238,380] there is little research on the link between this renal disease marker and cerebral microvascular hemodynamics. In addition, these associations may result from the shared VRF. The Rotterdam Study found no association between ACR and CBF after adjusting for the VRF.[243] Likewise, we did not find an association between the microvascular function tests and ACR, nor was there a correlation between the ACR and neuroimaging markers of CSVD or cognitive performance.

Taken together, our findings and the reviewed literature do not support the role of eGFR or ACR as surrogates for monitoring early cerebral microvascular dysfunction in CKD. Instead, the changes in eGFR and ACR and the CSVD markers appear to progress in parallel, and this cerebro-renal relationship may be driven by shared genetic factors and pathways.[381] The early effects of CKD on microvascular hemodynamics could be mediated through other pathological mechanisms, including (and probably not limited to) an exacerbated inflammatory response and increased oxidative stress. Rodent models of CKD show increased BBB permeability, which increases susceptibility to uremic toxins and increases cerebral microhemorrhages.[382] Several systemic inflammatory biomarkers have been associated with CKD and CSVD, including IL-1, IL-6 and tumour necrosis factor-α.[380,383] There is evidence of an altered gut microbiome in CKD, leading to the overgrowth of uremic toxins-producing bacteria, and these toxins correlate with systemic inflammatory markers, vascular stiffness, and increased mortality risk.[383] New evidence is now emerging on the gut-CSVD associations.[383] As CKD is a Na^+-avid state, the direct toxic effect of excess dietary Na^+, also supported by our research, is another possible pathological mechanism by which CKD contributes to the development of cerebral microvascular dysfunction. Further studies on the interplay between renal and cerebral microvascular dysfunction are needed.

The link between microvascular hemodynamics and cognition in HT

An unexpected finding in our study was that better dCA in the MCA among patients with HT was associated with worse memory performance. In addition, reduced NVC in the PCA predicted better memory performance and processing speed. Our cohort of HT patients showed overall preserved cognition, with only 7 patients scoring below the validated cutoff points for MCI in the MoCA test, considering patient age and education. There were no

associations between TCD markers of CBF regulation and the MoCA test or the total cognitive score.

Processing speed mostly reflects structures supplied by the anterior circulation, particularly dorsolateral prefrontal and cingulate neuronal circuits, and impairments in this function are among the earliest and most noticeable cognitive signs of CSVD.[384,385] Conversely, memory was tested using the AVLT total score, which has been shown to correlate to several temporal structures, including the hippocampus, the rostral medial temporal cortex, and temporal pole, and the caudal middle frontal gyrus.[386] These structures receive blood from both the carotid and the vertebrobasilar systems.[387,388]

These results could reflect a reorganization of the brain vasculature to counteract the HT-induced subcortical small vessel damage, which is usually more severe in the frontal WM.[389] This reorganization could result from the chronic hemodynamic changes HT induces. As discussed above, we found improved dCA in our HT cohort, which has also been reported in several other studies and suggests permanent remodeling of the cerebral vasculature to accommodate the higher BP.

It is well-established that cerebral vasomotion has a significant impact on dCA. Hypocapnia-induced cerebral vasoconstriction has been found to improve dCA, while the opposite is seen during hypercapnia-induced cerebral vasodilation.[175] In healthy subjects, studies have shown attenuated dCA responses during visual stimulation.[390,391] Ogoh *et al*. observed impaired dCA during a cognitive challenge in healthy volunteers, irrespective of oxygenation.[392] Hence, the vasodilation induced by cognitive tasks may be the main mechanism behind the reduction in dCA, although there may be interference from other unknown factors. While the physiological role of modified dCA in response to cognitive challenges is not yet understood, it is plausible that it could reflect an evolutionary adaptation in which NVC mechanisms would increase CBF to the active brain regions independently of the hemodynamic status, allowing decision-making and prompt action in potentially harmful situations. The chronically enhanced dCA efficiency in HT, while improving the dampening capacity of the increased BP, may disrupt these adaptations during a cognitive task and account, at least in part, for the early cognitive impairment observed in the disease. As such, NVC could be a better and more precocious marker of CBF dysfunction. Further investigations are needed to understand the mechanisms governing the attenuation of dCA when the brain is challenged and to determine its biological significance and potential modifications in disease states.

In addition to the dCA modifications, the unanticipated association of reduced NVC in the PCA with better memory performance and processing speed could also reflect the remodeling

of the functional hyperemia responses, either compensatory or maladaptive, especially affecting the posterior circulation. As discussed above, NVC in the PCA was impaired in these patients compared to controls, despite preserved dCA, also indicating particular involvement of the hyperemia responses in the posterior circulation.

Jennings *et al.*, observed that parietal rCBF was positively associated with verbal working memory in HT patients, but the inverse was true in normotensive patients. HT patients who performed poorly also showed less prefrontal cortex rCBF activation. HT subjects also coactivated the amygdala/hippocampus during memory task performance, while control subjects did not.[284] Another group observed major functional reorganization in cognitive processing in uncomplicated HT irrespective of macrostructural damage, with significant hyperactivation of several brain areas during an executive function task, mostly in the medial parietooccipital cortex.[393] Hence, HT causes neuronal network modifications that include increased co-activation of temporal and parietooccipital areas to perform tasks related to the frontal cortex. This could suggest the development of compensatory changes, with the involvement of additional areas in processing, even before recognizable tissue damage, further adding to the hypothesis of the brain as an early target and a driver of HT.[199] Another group has shown unaltered NVC in the MCA of middle-aged well-controlled HT patients compared to healthy controls,[394] which aligns with our hypothesis of increased susceptibility of the PCA territory to these changes. We found better cognitive function in tasks dependent on the frontal lobe despite reduced NVC in the PCA area, which could suggest additional engagement of processing areas in posterior regions for these tasks with compromised cognition, leading to overcompensation of the NVC response in the PCA. Conversely, patients exhibiting better cognitive performance may not yet display this overactivation, with more efficient NVC in the PCA. The overall preservation of cognitive function in most patients may also indicate compensatory mechanisms contributing to cognitive maintenance within this population, supporting our hypothesis that TCD parameters could be reliable markers for early changes in the relationship between cerebral hemodynamics and cognitive performance.

Several groups have observed a posterior-to-anterior shift with aging (PASA) in cortical activity, usually understood as compensatory and contributing to the maintenance of cognitive performance.[395-397] However, Morcom *et al*. demonstrated that this increased prefrontal activation reflects reduced efficiency or specificity rather than compensation.[398] The frontal cortex appears to be more sensitive to aging and becomes less specific, suggesting a possibility of less efficient neural function requiring greater hyperemia for the same level of processing.[397,399-401] The use of alternate networks has also been demonstrated in patients with AD.[402-404] Perhaps similar modifications occur in the HT brain, contributing to accelerated

brain aging and cognitive decline. In this regard, our results could indicate less efficient NVC in the PCA territory due to disrupted top-down signals from the prefrontal cortex (PFC). The PFC is crucial for selective attention to specific visual stimuli. It modulates the magnitude and selectivity of activation in the visual cortex according to the goal requirements.[405-407] Using fMFI, D'Esposito and co-workers showed that impairing the PFC function of healthy individuals resulted in decreased performance on a face/scene matching task.[408] Our findings of reduced NVC in the PCA among those with better processing speed and memory performance could reflect more efficient functional hyperemia with more selective visual stimulus processing in patients with less frontal lobe damage. Conversely, patients with worse PS and memory performance may have more extensive damage to networks regulating visual processing, resulting in an increased hyperemic response to visual stimuli.

In comparison to the control group, our analysis revealed reduced natural frequency in the patient cohort, indicating reduced vessel tonus and speed, indicative of vessel damage.[194] However, notably, we observed a concurrent reduction in rate time and overshoot systolic CBFV in the PCA in patients exhibiting superior cognitive performance. These metrics, reflective of the initial velocity surge and overall CBFV increase during visual stimulation,[194] may signify a modulation in the activation of the PCA´s NVC responses rather than structural vessel damage. These contrasting findings could further imply a more nuanced functional hyperemia activation in patients with preserved cognitive performance and that the natural frequency may serve as a sensitive marker for microvessel damage.

Further research is necessary to investigate the lasting impacts of HT on microvascular and brain network functionality and to elucidate whether our findings reflect adaptive changes or reduced efficiency or specificity of the neurovascular networks.

Regarding $VRCO_2$, we found no relation to cognitive performance in this cohort. We found no other studies on the relationship between $VRCO_2$ and cognition in HT patients. A study of older adults at increased risk of cognitive decline (including some HT and DM patients) using pseudo-continuous ASL showed that $VRCO_2$ in the whole brain was positively associated with cognitive performance. There was no subgroup analysis for the different VRF.[409] In DM patients, baseline $VRCO_2$ did not predict cognitive decline after four years.[255] In AD patients, $VRCO_2$ was reduced compared to controls and significantly predicted the MMSE in these patients.[410,411] However, this could reflect reduced metabolism from brain atrophy instead of loss of vasomotor reserve, as studies in MCI patients show $VRCO_2$ similar to controls.[411,412] Another study showed that baseline $VRCO_2$ was worse in MCI patients that progressed to dementia over one year compared to MCI patients that remained stable, and some patients in

the first group might have been early dementia patients considering the Clinical Dementia Rating Sum of Boxes scores.[413] These results may indicate that the relationship between $VRCO_2$ and cognition observed in dementia patients results from neurodegeneration. The role of $VRCO_2$ as a biomarker for cognitive decline in HT remains to be elucidated.

These observations contribute to our understanding of how HT-related neurological changes can manifest differently across various brain regions. However, more research is needed to fully unravel the complex interplay between dCA, $VRCO_2$, and NVC abnormalities and cognitive functions, specifically in HT individuals.

Limitations

This work is subject to several limitations that require acknowledgment to ensure a comprehensive understanding of the research findings.

Firstly, the cross-sectional design hampers our ability to establish causality or determine microvascular hemodynamic dysfunction as an early predictor of CSVD. Future longitudinal studies are needed to better understand the relationships reported in our work. However, it should be noted that all patients included in the study exhibited well-controlled HT based on average ABPM values and were devoid of any clinical manifestations suggestive of existing cerebrovascular disease.

Another limitation lies in the sample size employed for data collection, as already mentioned. Although it follows the average sample size for hemodynamic physiology studies using TCD dynamic tests, this may limit the ability to generalize the results and draw broad conclusions about a larger population. Furthermore, the historical control group used in our study consisted of a relatively small number of volunteers and were not precisely matched in age and gender. There were also variations in comorbidities between the two groups, which could introduce confounding factors that may obscure or influence the observed differences despite our efforts to account for this with the statistical methods employed. Additionally, the control group did not undergo the MCA NVC protocol, only the PCA NVC protocol, which hindered us from making group comparisons regarding this aspect and could have provided valuable insights.

Unlike the patient group, the control subjects, although without clinical evidence of cognitive impairment or vascular risk factors, were not subjected to neuropsychological examinations or MRI evaluations, and thus, we cannot definitively conclude that CSVD markers are absent in this control group, nor can we make comparisons between these two

populations regarding CSVD indicators. Hence, our conclusions are observational and exploratory in nature and must be regarded as preliminary. Moreover, we must recognize that the patients were directed to the Hypertension Unit due to severe or difficult-to-manage HT, but we lack information regarding the duration for which these individuals remained untreated prior. This knowledge gap could potentially impact our understanding of microvascular dysfunction and its degree within this cohort.

Furthermore, since the dietary salt intake was derived from an estimation based on 24h urine assessments, it may not reflect the lifelong effect of dietary salt on the cerebral vasculature.

The limited scope of the neuropsychological examination conducted on visuospatial and visuoconstruction abilities is an additional limitation that may have hindered the discovery of significant connections between hemodynamic changes in the PCA and cognitive performance related to parietoccipital functions. In addition, our cohort of patients displayed a low educational level, which is a prevalent sociocultural trait in the Portuguese population.[414-417] The low educational level influences the performance on the cognitive tasks and could have impacted our results. The presence of patients with scores below the cutoff for MCI are also noteworthy. In the future, larger longitudinal studies are required considering the influence of cognitive status and sociocultural variables. Moreover, a proportion of the patients exhibited symptoms of anxiety and depression, potentially influencing cognitive performance and which might represent initial symptoms of future cognitive decline.

Although TCD ultrasound is a reliable technique for estimating CBF under normal physiological conditions, it is important to acknowledge that the CBFV measurements obtained through TCD are approximations of CBF. This limitation arises because TCD cannot directly measure vessel diameter. Nonetheless, using CBFV as a surrogate for CBF is acceptable as long as the diameter of the insonated artery remains constant. Since data were acquired in a supine position and relied on spontaneous measurements, we do not expect any significant changes in artery diameter during the dCA and NVC protocols. However, despite previous studies reporting stable caliber diameter with CO_2 variations during vasoreactivity testing,[172] Coverdale *et al*. demonstrated that hypo and hypercapnia can lead to noticeable changes in MCA diameter. Thus, using CBFV would underestimate CBF during hypercapnia and overestimate CBF during hypocapnia.[418] However, the hypercapnic and hypocapnic stimuli were applied for 5 minutes in Coverdale´s work, while our stimuli were limited to 2 minutes, which may have limited this effect, although this would have to be confirmed by further research.

CHAPTER V – POTENTIAL APPLICATIONS AND FUTURE DIRECTIONS

Our study showed no correlations between TCD markers or cognitive performance and WMH burden. As discussed, prior research has highlighted significant heterogeneity in cognitive symptomatology among patients exhibiting similar burdens of CSVD markers on brain imaging. Presently, we are conducting a project within this cohort to investigate potential associations between normal-appearing white matter alterations, as measured by DTI, and TCD measures of CBF regulation, alongside cognitive performance.

The findings of this research project open several other promising avenues for understanding and managing CSVD in HT. The availability of TCD as a non-invasive, cost-effective, and versatile tool for studying cerebrovascular dynamics offers potential applications in clinical practice, some of which have already been discussed:

- **Understanding the long-term effects of HT on the brain:** as acknowledged, a cross-sectional design was adopted in this research. Future investigations should consider conducting longitudinal studies to investigate the changes in microvascular and brain networks in individuals with pre-hypertension and hypertension, starting from young adulthood. These studies should include multimodal biomarkers, but the affordability and safety of the TCD make it suitable for more frequent evaluations that could offer valuable information regarding the progression of CSVD.
- **Risk stratification:** our findings suggest that an altered NVC in the PCA may be a sign of asymptomatic microvascular dysfunction in HT, and this dysfunction is even worse with comorbid DM. This has the potential to assist clinicians in identifying hypertensive patients at an elevated risk of developing CSVD. Early detection would facilitate timely interventions, potentially preventing or at least delaying disease progression. Knowing the influence of comorbid VRF is instrumental in devising individualized therapeutic regimens. Future studies should further explore the role of TCD markers in risk stratification and the influence of other VRF in HT-induced CSVD.
- **Personalized therapeutic interventions:** Evaluating specific microvascular dysfunction signs may allow more accurate therapeutic intervention tailoring. Based on their unique physiological responses, as reflected by their dCA, $VRCO_2$, and NVC measurements, it might be feasible to design patient-specific treatment plans, including target BP values and specific medication regimens.
- **Outcome measures in clinical trials:** recent trials have now started to incorporate TCD-based outcome measures to evaluate the efficacy of therapeutic interventions for CSVD, such as the OxHARP trial (www.clinicaltrials.gov NCT03855332),[419] the

PERFORM trial (www.clinicaltrials.gov NCT05583266),[420] the ADTSVD trial (www.clinicaltrials.gov NCT02730065),[421] the GAPP-SVD trial (www.clinicaltrials.gov NCT05356104),[422] the TMSCA-CSVD trial www.clinicaltrials.gov NCT05914623),[423] and a trial studying the use of DL-NBP in patients with mild CSVD-associated vascular dementia (www.clinicaltrials.gov NCT03906123).[424] These trials mostly use measures of cerebral autoregulation and vasoreactivity. This research further underscores the value of TCD-based biomarkers in clinical trials and suggests NVC measures as sensitive biomarkers for future trials.

- **Novel therapeutic targets:** targeting the NGVU and improving NVC responses could be explored as a therapeutic strategy to mitigate CSVD progression. Modulating factors like angiotensin II, inflammation, and pericyte function may hold promise in restoring cerebral microvascular health. Studies have shown that targeting the carbonic anhydrases may protect against NGVU dysfunction, and these treatments are already available for other conditions.[149] Both cocoa and deferoxamine have improved microvascular hemodynamics in elderly individuals.[232,425] It would be interesting to test these treatments in HT patients.
- **Dietary recommendations:** The finding that dietary salt consumption is an independent predictor of microvascular dysfunction in HT may provide further rationale for restricting salt intake. Future research should focus on providing additional data on the effects of chronic excessive salt intake that may help shape dietary recommendations to attenuate the risk trajectory of CSVD in hypertensive individuals. Studies with prospective designs should evaluate the efficacy and threshold of dietary salt restriction in improving TCD hemodynamic markers and, ultimately, cognitive health.
- **Preventive interventions in cognitive decline:** a comprehensive understanding of how microvascular (mal)adaptations affect cognitive function could offer valuable insights into potential preventive measures. As discussed, studies indicate that long-term changes in the brain may occur well before hypertension reaches clinically diagnosable levels. By prospectively assessing cognitive and microvascular function early in the disease course, we can gain a better understanding of and potentially prevent detrimental modifications to both the microvasculature and cognition.
- **Management of incidental findings:** CSVD is highly prevalent in the general population. Imaging markers of CSVD often appear as incidental findings, and there are no guidelines for managing these findings. Studying the value of TCD markers of microvessel dysfunction for identifying patients at risk of developing CSVD-associated

cognitive dysfunction and vascular events could help decision-making in these patients, offering an opportunity to prevent complications effectively.

- **Implications for Alzheimer's Disease:** the links between HT, AD and NGVU dysfunction raises intriguing questions about shared mechanisms between CSVD and AD. Future studies using TCD markers to explore these connections could have implications for AD research and interventions.

CHAPTER VI – CONCLUSION

This research seeked to contribute to the field of HT-related CSVD, a very prevalent yet understudied disease. The primary objective of this investigation was to advance our understanding of the intricate relationships between HT and cerebral microvascular dysfunction, with a particular emphasis on assessing dCA, $VRCO_2$, and NVC as prospective biomarkers for CSVD. The recognition that NVC i CHAPTER II – n the PCA, particularly the modeled parameter natural frequency, is impaired in HT patients, more so in those with comorbid DM and excessive daily salt intake, is a noteworthy discovery.

It should be acknowledged that the investigation of CSVD using TCD is still in its infancy, mirroring the broader landscape of CSVD research. Nevertheless, the observed impairment in NVC, particularly the natural frequency, underscores the potential value of TCD as a tool for identifying hypertensive individuals with incipient microvascular dysfunction who may be at an elevated risk of developing clinically significant CSVD. The recognition of NVC as a plausible, sensitive, and early biomarker for CSVD in the context of HT holds substantial promise.

Although in its preliminary stages, this research lays the groundwork for developing novel diagnostic and therapeutic strategies for early intervention and risk stratification. By identifying individuals with subclinical cerebrovascular dysfunction, particularly within the HT population, we may be better equipped to prevent or mitigate the devastating consequences of CSVD. Thus, this work represents a small step toward a more profound understanding of HT-related CSVD and underscores the need for continued exploration in this evolving field.

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