Fiber correlational tractography with neurovascular coupling and cognition in hypertension

Manuel Fortunato[1,2*†], Ricardo Morais[2,3,4,6†], Isabel Santana[4,6], Pedro Castro[7,8], Jorge Polónia[9], João Paulo Cunha[1,2], Ana Monteiro[7,10]

[1*]Faculty of Engineering of University of Porto, R Roberto Frias, Porto, 4200-465, Portugal.
[2]Center of Biomedial Reseach (C-BER), Instituto de Engenharia de Sistemas e Computadores,Tecnologia e Ciência (INESC-TEC), R Roberto Frias, Porto, 4200-465, Portugal.
[3]Neuroradiology Department, Unidade Local de Saúde São João, Porto, Portugal.
[4]Faculty of Medicine, University of Coimbra, Coimbra, Portugal.
[5]Neuroradiology Department, Unidade Local de Saúde de Coimbra, Coimbra, Portugal.
[6]Centre for Innovative Biomedicine and Biotechnology (CIBB), Universidade de Coimbra, Coimbra, Portugal.
[7]Department of Clinical Neurosciences and Mental Health and RISE-Health Unit, Faculty of Medicine of the University of Porto, Porto,Portugal.
[8]Department of Neurology, Unidade Local de Saúde São João, Porto, Portugal.
[9]Cintesis/RISE Faculdade Medicina do Porto, Hospital Pedro Hispano, Matosinhos, Portugal.
[10]Neurology Department, Unidade Local de Saúde do Alto Ave - Hospital Senhora de Oliveira, Guimarães,Portugal.

*Corresponding author(s). E-mail(s): manuel.leao.fortunato@gmail.com;
Contributing authors: ricardofelixmorais@gmail.com;
isabeljsantana@gmail.com; pedro.castro@chsj.min-saude.pt;

jjpolonia@gmail.com; joao.p.cunha@inesctec.pt;
ana.mg.monteiro@gmail.com;
†These authors contributed equally to this work.

Abstract

Background: Hypertension is the primary risk factor for cerebral small vessel disease (CSVD). However, its mechanistic links are yet to be completely understood. White matter (WM) lesions visible on conventional neuroimaging reflect late and irreversible CSVD damage. Advancements in diffusion-weighted magnetic resonance imaging (dMRI) techniques have shown increased sensitivity in detecting subtle changes in WM structural integrity.
Methods: 44 hypertension patients without symptomatic CSVD underwent multi-modal evaluation of cerebral structure and function, including dMRI, neuropsychological tests and transcranial Doppler (TCD) monitoring of the right middle and left posterior cerebral arteries (MCA and PCA, respectively) to assess neurovascular coupling (NVC). In the PCA, the modeled NVC curve was studied. We examined the cross-sectional relationship of WM integrity with NVC and cognitive performance, using correlational tractography. Diffusion measures from two dMRI models were used: fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) from diffusion tensor imaging (DTI), and quantitative anisotropy (QA) and isotropy (ISO) from q-space diffeomorphic reconstruction (QSDR).
Results: Regarding the NVC in the PCA, vascular elastic properties and initial response speed markers indicated better functional hyperemia with better WM integrity. However, the amplitude suggested increased NVC with worse WM integrity. In the MCA, increased NVC was associated with lower WM integrity. Better cognitive performance associated with preserved WM integrity.
Conclusion: Increased functional hyperemia despite worse WM integrity may reflect less efficient NVC in hypertensive patients, potentially arising from (mal)adaptive mechanisms and brain network reorganization in response to CSVD. This work highlights the potential of TCD markers and the QA measure as susceptibility markers of pre-symptomatic CSVD.

Keywords: Cerebral small vessel disease, hypertension, correlational tractography, neurovascular coupling, cognitive performance

Introduction

Cerebral small vessel disease (CSVD) is a complex and heterogeneous disorder that represents the progressive impairment of the microvasculature that irrigates the white matter (WM), and deep gray matter of the brain [1]. It is a risk factor for cognitive impairment, Alzheimer's disease, ischemic strokes, and spontaneous intracerebral hemorrhages [2][3]. CSVD manifestations are frequently clinically silent and are not fully depicted by current conventional neuroimaging sequences [4][5][6]. Thus, novel

risk biomarkers for pre-symptomatic CSVD are needed for better clinical management and prevention. Hypertension is the strongest vascular risk factor for CSVD. However, our understanding of the mechanistic link between hypertension and CSVD, as well as the pathophysiology and causality of CSVD, is limited. Evidence suggests that neurovascular dysfunction in CSVD may precede conventional symptomatic manifestations [7][8][9]. Neurovascular coupling (NVC) is the process where changes in neural activity lead to rapid adjustments in local blood flow, a phenomenon known as functional hyperemia [10]. Very few studies have investigated NVC in hypertension, mainly using imaging modalities. The transcranial Doppler (TCD) is a non-invasive technique that allows the study of NVC by measuring cerebral blood velocity (CBv) as a perfusion correlate [11][12].

White matter hyperintensities (WMH) are lesions of presumed vascular origin independently associated with hypertension and CSVD seen on T2-weighted and FLAIR images, probably reflecting late and irreversible steps in the pathological process [14][13]. Advancements in diffusion-weighted magnetic resonance imaging (dMRI) fiber tracking techniques have shown increased sensitivity in quantifying subtle changes in the 'normal appearing' WM (NAWM) that progresses over time into WMH [14][15]. Correlational tractography uses the q-space diffeomorphic reconstruction (QSDR) model to track local connectivity patterns along the fiber pathways and identify the exact segments that express significant associations with a study variable [14][16]. Quantitative anisotropy (QA) is the diffusion measure of QSDR that characterizes the anisotropic diffusion along a given fiber orientation, and isotropy (ISO) is the background isotropic free water component. QSDR has been shown to outperform diffusion tensor imaging (DTI) tractography [15], a model that is unable to disentangle the individual microscopic contributions, such as different WM fiber populations and cerebrospinal fluid contamination [19]. Whereas DTI-based measures, such as fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AD), reflect the rate of water diffusion along vectors principle eigenvectors of the tensor model, the QA reflects the volume of water diffusing in a q-space distribution [20].

We aimed to study associations between WM structural integrity, as assessed by DTI and QSDR, NVC measured by TCD, and cognitive performance in hypertensive patients without major CSVD-related impairment. We sought to provide a deeper understanding of hypertension-related CSVD and explore the potential surrogate biomarkers of pre-symptomatic disease.

Methods

Clinical evaluation

This was a prospective cohort study. Patients with a confirmed diagnosis of hypertension for a minimum duration of one year without neurological clinical manifestations of CSVD were included. Exclusion criteria included past stroke and clinically defined dementia, and other major brain conditions such as brain neoplasia, brain trauma, prior brain infection, or degenerative neurological disease. Additionally, patients with serious or unstable illnesses, an unsuitable acoustic temporal bone window, stenosis

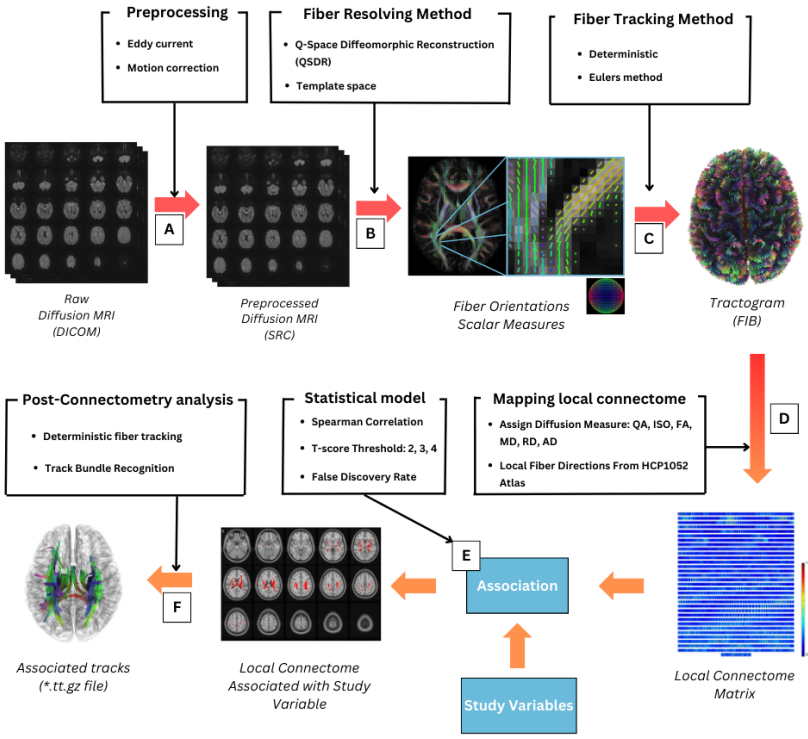


Fig. 1: Diffusion MRI connectometry pipeline for the correlational tractography using DSI Studio. (A) Diffusion-weighted images were corrected for motion and eddy current artifacts. (B) The Q-space diffeomorphic reconstruction (QSDR) resolved the fiber orientations normalized in the MNI template space. (C) A deterministic fiber tracking algorithm was used with the default settings. (D) The whole brain tractograms of each patient were grouped in 'databases' to form the local connectome matrices, one for each diffusion metric. A tractography atlas provided the fiber orientations for each voxel. (E) At each voxel, the associations were calculated by the Spearman correlation test. Variables were randomly permuted 4000 times to calculate the false discovery rate (FDR). (F) The local connectomes significantly associated (FDR<0.2) with the study variable were reconstructed and the resulting track bundles were anatomically identified.)

in extra- or intracranial arteries exceeding 50%, and inability to cooperate or provide informed consent were also excluded. The research followed the principles of the Declaration of Helsinki, and the local Ethics Committee approved the protocol (approval number 082/CE/JAS). Participants provided written consent and underwent the multimodal evaluations during a 3-month time frame to keep them as temporally close as possible.

Demographic and clinical information, including age, gender, health history, body mass index (BMI), and current medications, were systematically recorded. Routine

blood and urine tests were administered. Vascular comorbidities were quantified using a vascular comorbidity score (VCS), which included hypertension, diabetes mellitus, dyslipidemia, tobacco use, chronic heart failure, coronary artery disease, arrhythmias, valvular heart disorders, nephropathy, and peripheral vascular disease. Each condition was assigned a score of 1 if present or 0 if absent, resulting in a score ranging from 0 to 9 [21][22]. Methodology details regarding cognitive evaluation are available elsewhere [21]. The comprehensive neuropsychological evaluation included the Mini-Mental State Examination (MMSE), the Montreal Cognitive Assessment (MoCA) and tests for attention and executive function, processing speed, and memory domains. The total cognitive score was also calculated.

Transcranial Doppler monitoring

Patients were continuously monitored in the right MCA and left PCA by TCD, as described previously [21]. NVC in the right MCA was evaluated using the N-Back test, which includes 1-Back and 2-Back tasks, standardized to a reference task (Spot "X"). For this test, 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 (CBv_{NB}) relative to the reference task (CBv_{IDX}) using the following equation:

$$\frac{CBv_{NB} - CBv_{IDX}}{CBv_{IDX} \times 100}(\%) \tag{1}$$

NVC in the left PCA was assessed using the flickering checkerboard method, consisting of 10 cycles, each with a 20 s resting phase (eyes closed) and 40 s stimulating phase at 10 Hz. The maximum change in systolic CBv was derived to compute the overshoot metric, $\frac{CBv_{maximum} - CBv_{baseline}}{CBv_{baseline}} \times 100(\%)$. The systolic CBv curve was modeled into a second-order linear system to outline the NVC response dynamics over time, according to the formula: $G(s) = \frac{K(1+Tvs)}{\frac{s^2}{\omega^2} + 2\xi * \frac{s}{\omega} + 1}$, Where "K" symbolizes gain, "Tv" denotes rate time, "ω" indicates the natural frequency, and "ξ" represents attenuation. Gain represents the relative CBv shift between the resting and steady states during visual activation. Rate time indicates the initial steepness of the CBv increase. Natural frequency reflects the system's oscillatory properties, with higher values representing better NVC responses. Attenuation describes the dampening and tonus features, with lower values indicating better functional hyperemia [23].

Diffusion MRI correlational tractography

The diffusion images were obtained on a 1.5T Siemens Aera system using a 2D EPI diffusion sequence. A single-shell scheme was acquired with 48 diffusion sampling directions (b-value = 1000 s/mm^2) and 16 volumes as reference. The in-plane resolution was 1.79 mm, and the slice thickness was 6 mm. The echo time was 97 ms, and the repetition time was 4300 ms.

The diffusion MRI connectometry analysis was conducted in DSI Studio software (version May 17, 2024) (http://dsi-studio.labsolver.org/) to investigate how quantitative measures along WM pathways associated with the study variables [16]. As a primary outcome, we investigated the relation type (e.g., positive and negative) of the post-connectometry tractograms. As secondary outcomes, we studied the anatomical location of associated track bundles, particularly for the QA diffusion measure, across cognition and NVC domains, and compared how each diffusion measure associated with the same study variable.

First, the dMRI data were loaded and corrected for motion and eddy current (Figure 1). The WM masks were segmented with an anisotropy threshold randomly selected between 0.5 and 0.7 otsu thresholds. The diffusion data were reconstructed using QSDR [24] to obtain the spin distribution function (SDF) [20]. From the SDF, the QA and ISO maps were calculated in each participant's native space and then normalized to the Montreal Neurological Institute (MNI) space via non-linear registration. The FA, MD, RD, and AD maps were calculated at each voxel using DTI, which was also spatially normalized to the MNI space based on QSDR's warp fields.

With the default settings, a deterministic fiber tracking algorithm was used. The diffusion sampling length ratio was 1.25. Tracks shorter than 30.0 or longer than 200.0 mm were discarded. The output resolution was resampled to 2 mm isotropic to improve reconstruction quality.

Tractograms were aggregated into the local connectome matrix. The HCP1065 atlas provided the local fiber orientations [16]. A database was created for six diffusion measures. Thirteen study variables underwent connectometry analysis (six cognitive parameters and seven NVC markers). The effects of age, gender, VCS, and BMI were adjusted using a multiple linear regression model, for impacting WMH burden and tractography associations (see Supplemental material). A non-parametric Spearman correlation was used to derive the associations [18]. To ensure the stability of the findings, tracks were reported using t-score thresholds of 2, 3, and 4 [17]. The false discovery rate (FDR) was estimated after 4000 randomized permutations to obtain the null distribution. An FDR below 0.2 deemed the results significant. The bash script for correlational tractography is available in https://github.com/manelxpto/correlational-tractography-DSI-Studio. Tracks with less than 1% of the total streamline count were excluded. The cerebellum was excluded due the lack of irrigation from the MCA and PCA territories.

Results

Of the initial 60 patients enrolled in the study, 3 were excluded due to past lacunar stroke and 1 due to an unsuitable acoustic temporal bone window for TCD assessment. Only 52 of the enrolled patients attended the neuropsychological evaluation, and 48 consented to undergo cerebral MRI. However, 4 additional patients were excluded due to dMRI data corruption. Additionally, some patients had missing study variables (Table 1).

The post-connectometry tractograms and the recognized track bundles are documented in the supplemental material, for demographic variables and the t-score

Demographics and comorbidities, units (n)	*Mean (standard deviation)*
Age, y	65 (9)
Sex, M:F	24:20
Education, y	6.1 (4)
Diabetic, %	61
VCS	3.6 (1.2)
BMI, kg/m2	30.0 (5.2)
Neuropsychological tests	
MMSE (44)	27.5 (2.4)
MoCA (44)	20.6 (4.8)
Cognitive score - total (36)	-2.3 (4.8)
Cognitive score - executive Function (36)	-1.7 (2.3)
Cognitive score - processing speed (44)	-0.64 (3.0)
Cognitive score - memory (44)	-0.5 (0.8)
Neurovascular coupling	
MCA	
N-1 back, % (35)	-0.7 (5.8)
N-2 back, % (35)	2.9 (4.3)
PCA	
Overshoot systolic CBv, % (42)	21.7 (9.3)
Gain, % (39)	13.8 (7.4)
Natural frequency, Hz (39)	0.2 (0.1)
Attenuation, a.u. (39)	0.4 (0.3)
Rate time, s (38)	0.6 (1.8)
Global diffusion measures (44)	
QA, a.u.	0,105 (0,012)
ISO, a.u.	0,923 (0,075)
FA, a.u.	0.186 (0.011)
MD, $10^{-3}mm^2/s$	1,132 (0,080)
AD, $10^{-3}mm^2/s$	1.324 (0.081)
RD, $10^{-3}mm^2/s$	1,036 (0,081)

Table 1: Cohort demographics. Data are presented as *mean (standard deviation)*. Abbreviations: MCA, middle cerebral artery. PCA, posterior cerebral artery. y, years. M, male. F, female. VCS, vascular comorbidities score. BMI, body mass index. MMSE, mini-mental state examination. MoCA, Montreal cognitive assessment. EF, executive function. PS, processing speed. CBv, cerebral blood flow velocity. s, seconds. a.u., arbitrary units. FA, fractional anisotropy. MD, mean diffusivity. RD, radial diffusivity. AD, axial diffusivity. QA, quantitative anisotropy. ISO, isotropy.

thresholds of 2, 3, and 4. The following results focus on the t-score threshold of 3 (the intermediate significance level).

A higher rise of CBv in the right MCA during the 1-Back task was associated with reduced QA, ISO, MD, RD, and increased FA (Figure 2). As an example, the reduced QA was seen along projection fibers such as the corticostriatal tract (CStr), left extreme capsule (EMC), and body of the corpus callosum (CC) (FDR<0.001) (Figure 3). A higher CBv performing the 2-Back task was associated with reduced QA and ISO and increased ISO, MD, and RD. The lower QA was observed in dorsomedial projection and association fibers connecting the frontal and parietal lobes, the corticospinal tract (CST), medial lemniscus (ML), parietopontine tract (PPT),

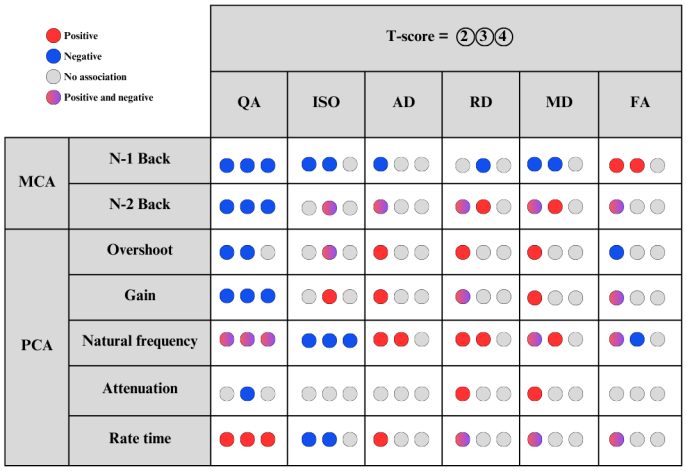


Fig. 2: Transcranial Doppler markers of neurovascular coupling in the MCA and PCA associated with DTI and QSDR diffusion measures. The connectometry tractograms were summarized into 3 circles, using a t-score threshold of 2, 3, and 4, respectively. The associations have four possible outcomes: positive (red), negative (blue), positive and negative (red-blue gradient), or no association (grey) (the false discovery rate was superior to 0.20). Abbreviations: MCA, middle cerebral artery. PCA, posterior cerebral artery. VLF, very low frequency. LF, low frequency. FA, fractional anisotropy. MD, mean diffusivity. RD, radial diffusivity. AD, axial diffusivity. QA, quantitative anisotropy. ISO, isotropy.

and inferior fronto-occipital fasciculus (IFOF) (FDR<0.001). Regarding the NVC in the PCA territory, an increased systolic CBv overshoot was associated with reduced QA in the FMA and FMI of the CC and projection fibers such as CST, corticopontine tract (CPT), ML, non-decussating dentatorubrothalamic tract (DRTT), and right CStr (FDR<0.001). ISO had both positive and negative associations, while DTI measures did not associate with the systolic CBv overshoot. A higher modeled gain was associated with increased ISO and reduced QA, the latter seen in anterior and deep projection fibers (e.g., CST, CPT, ML, DRTT) and the body and anterior portions of the CC (FDR<0.001). Increased natural frequency was associated with higher QA, AD, RD, and MD and lower QA, ISO, and FA. The QA was positively associated in several projection, commissural, and association tracts, while the inverse was observed in the FMI and FMA of the CC (FDR<0.001). Higher attenuation was associated with reduced QA in the CST, PPT, ML, and CStr (FDR<0.001). An increased rate time was associated with lower ISO and higher QA, the latter observed in the left EMC, body of CC, superior CStr, CST, CPT, ML, and ndDRTT (FDR<0.001).

Regarding the cognitive performance, higher MMSE test score was associated with lower ISO, AD, RD, and MD (Figure 4).

The MMSE had both positive and negative associations with QA, depending on the WM region (Figure 5). The positive QA associations were seen in the CST, ML, CPT,

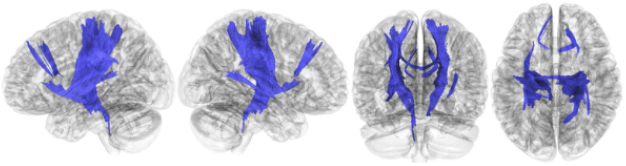
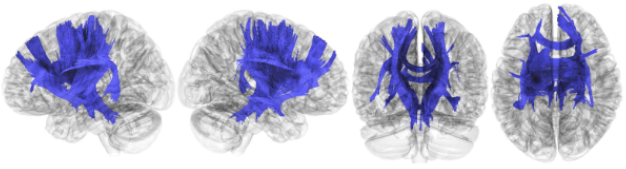
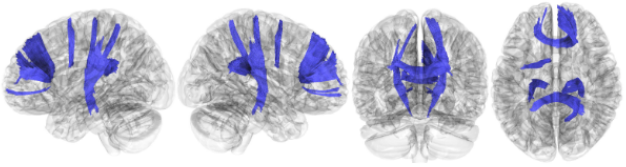
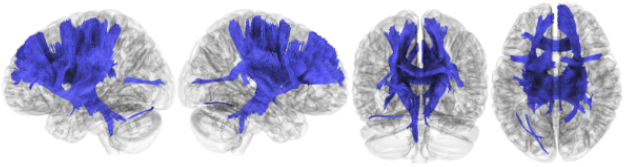
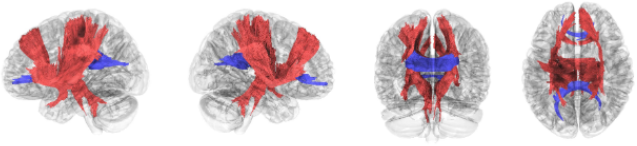
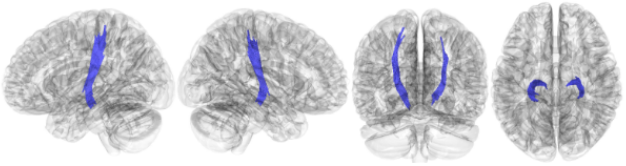
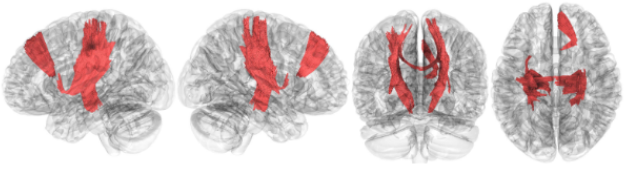
Study variable	Quantitative anisotropy (t-score = 3; minimum length = 40 mm)
1-Back	
2-Back	
CBv Overshoot	
Gain	
Natural frequency	
Attenuation	
Rate time	

Fig. 3: Post-connectometry tractograms in the saggital, coronal and axial planes showing the tracks where quantitative anisotropy (QA) was associated with the neurovascular coupling parameters in the MCA (1-Back and 2-Back tasks) and PCA (systolic cerebral blood velocity overshoot, gain, natural frequency, attenuation and rate time). Associations are positive (red) or negative (blue). The t-score threshold was 3 and the minimum track length was 40 mm (20 voxel distance).

Fig. 4: Neuropsychological tests associated with QSDR (QA and ISO) and DTI diffusion measures. The connectometry tractograms were summarized into 3 circles, using a t-score threshold of 2, 3, and 4, respectively. The associations have four possible outcomes: positive (red), negative (blue), positive and negative (red-blue gradient), or no association (grey) (the false discovery rate was superior to 0.20). Abbreviations: MMSE, Mini-Mental State Examination. MoCA, Montreal Cognitive Assessment. FA, fractional anisotropy. MD, mean diffusivity. RD, radial diffusivity. AD, axial diffusivity. QA, quantitative anisotropy. ISO, isotropy.

CStr, and body of CC (FDR=0.007), while the negative associations were observed in the left FMA of the CC (FDR<0.001) (Figure 5). Increased MoCA test scores were associated with decreased QA, and ISO and increased QA, RD, and MD. The QA negative association was observed in the ML, CST, and body of CC, while the positive was seen in the right IFOF (FDR<0.001 and FDR=0.0034). A higher total cognitive score was associated with increased QA, ISO, and FA and reduced AD, RD, and MD. The QA positive association was observed in portions of the CC, projection fibers, and the left AF and inferior longitudinal fasciculus (ILF) (FDR<0.001). Increased executive function was associated with higher QA and FA, and lower ISO and RD. The associations with higher QA were seen in the dorsomedial CST, ML, CPT, DRTT, body and tapetum of the CC, occipital anterior commissure (AC), AF, and ILF (FDR<0.001). Increased memory was associated with higher QA and lower ISO. The former was positively associated in the ML, CST, CPT, DRTT, CStr, and left EMC (FDR<0.001). Increased processing speed was associated with higher ISO and lower QA, AD, RD, and MD. The QA was negatively associated with CST, PPT, and left corticobulbar tract (FDR=0.0011).

Discussion

To our best knowledge, this correlational tractography study was the first to investigate WM structural integrity associations with cerebral blood flow regulation, assessed

by TCD, in the setting of hypertension without CSVD. Our results suggest that lower arterial rigidity in the PCA territory (as indicated by natural frequency and attenuation) and faster neurovascular coupling responses (rate time) were associated with higher WM integrity. Hypertension promotes abnormal material deposition in the vessel walls, which contributes to vessel stiffening and neuronal damage [26][27][28], and these results may indicate a mechanistic link between vessel wall changes and neurovascular unit dysfunction. However, increased functional hyperemia during visual stimuli was observed in association with reduced WM structural integrity, as indicated by the associations with the overshot systolic CBv and gain parameters. Additionally, increased functional hyperemia in the right MCA in response to the N-back tasks was also associated with reduced WM structural integrity. These unexpected associations may reflect less efficient NVC due to hypertension-induced damage to neural networks. More severe damage to WM tracks may require the recruitment of adjacent cortical areas, during cognitive and visual stimuli, leading to heightened functional hyperemic responses for the same level of processing. A prior study on this cohort showed that increased NVC in the PCA was associated with worse cognitive performance [22], which aligns with this hypothesis. The present work further suggests that the observed decline in cognitive function associated with increased NVC responses is driven by a loss of WM integrity. Another study on this cohort failed to detect an association between WMH and TCD markers of microvascular dysfunction [29], underscoring the importance of evaluating NAWM damage to better elucidate the pathophysiology of hypertension-induced neurovascular uncoupling and cognitive impairment. Morcom and colleagues found increased prefrontal activation during cognitive tasks in aging, suggesting reduced efficiency or specificity [30]. Our results could indicate less efficient NVC due to disrupted modulation from the prefrontal cortex (PFC). The PFC adjusts the magnitude and selectivity of cortical activation in response to different stimuli [31][32][33]. Other research has shown that impairing PFC function in healthy individuals led to decreased performance on a face/scene matching task [34]. Our findings of increased NVC with greater WM damage could reflect more extensive damage to long association fibers that regulate top-down signals from the PFC, resulting in impaired modulation of the hyperemic response.

We observed better cognitive performance with higher WM structural integrity, namely there was a positive association between the MMSE, total cognitive score, executive function, and memory score and QA. The processing speed showed inconclusive associations, and the MoCA test was unexpectedly associated with lower anisotropy measures. However, an important caveat exists in using the MoCA test scores, as we used the total score for the associations without considering the influence of patient age and education [35]. Most DTI studies observe similar associations between microstructural damage in WM lesions and increased cognitive impairment [36][37]. Interestingly, a correlational tractography study also observed MoCA negatively associated with WM connectivity in hypertension patients [14].

In this study, QSDR was responsible for the reconstruction process. However, DTI and QSDR measures quantified the diffusion in the WM for posterior associations with the clinical variables. Regarding the 'behavior' of QA and FA, these had unexpected opposite associations with the same study variable (e.g. N-1 back task) despite both

representing anisotropy. Regions known for their complexity of crossing and bending can affect the dMRI accuracy, particularly of the DTI measures [38]. QA has the advantage of not quantifying the free-water diffusion background signal, being less affected by edema [19]. Moreover, the QA was more often associated with the study variables in connectometry analyses using higher t-score thresholds. Thus, QSDR-derived metrics show greater sensitivity than DTI in association with the study variables.

This study was limited by the dMRI acquisition with a non-isotropic slice thickness of 6 mm, while the in-plane resolution was 1.79 x 1.79 mm^2. The tractograms should be carefully interpreted, especially in regions with crossing fibers. The absence of a control group weakens our conclusions and a small sample size inherently introduces a reduced statistical power. The tractography atlas biased the anatomical identification of tracks.

The current findings extend beyond prevailing evidence of the underlying mechanism of hypertension-induced damage in WM and cerebral microvasculature, highlighting the potential of NVC assessed by TCD and QA as susceptibility markers of pre-symptomatic CSVD. More studies are needed combining structural diffusion data with cerebrovascular hemodynamics. Exploring other vascular comorbidities and performing differential tractography using normotensive controls would complement our findings [38]. The longitudinal evaluation of this cohort is warranted to validate if hypertension is the responsible factor for these associations and if NAWM regions will progress into WMHs.

Declaration of Competing Interests. We declare no competing interests.

Acknowledgements. We would like to acknowledge the invaluable contributions of Dr. Gilberto Pereira and Dr. Carmen Ferreira from Centro Hospitalar Universitário São João for their expertise in performing cervical and transcranial Doppler evaluations and monitoring. We received financial contributions from Associação para o Estudo das Doenças Cerebrovasculares for performing the MRI studies. This work was a collaboration between the Faculty of Medicine and Faculty of Engineering of the University of Porto, and the Center for Biomedical Research at INESC-TEC, Portugal. The patients were recruited from the Hypertension Unit of Unidade Local de Saúde de Matosinhos, and we extend our gratitude to them for making this study possible.

References

[1] Joanna Chojdak-Łukasiewicz, Ewa Dziadkowiak, Andrzej Zimny, and Bogusław Paradowski. Cerebral small vessel disease: A review. Advances in Clinical and Experimental Medicine, 30(3):349–356, 2021. doi: 10.17219/acem/131216.

[2] N. Prins, E. V. van Dijk, T. den Heijer, S. Vermeer, J. Jolles, P. Koudstaal, A. Hofman, and M. Breteler. Cerebral small-vessel disease and decline in information processing speed, executive function, and memory. Brain: a journal of neurology, 128 Pt 9:2034–41, 2005. doi: 10.1093/BRAIN/AWH553.

[3] Z. Arvanitakis, A. Capuano, S. Leurgans, D. Bennett, and J. Schneider. Relation of cerebral vessel disease to alzheimer's disease dementia and cognitive function

in older persons: A cross-sectional study. The Lancet. Neurology, 15:934 – 943, 2016. doi: 10.1016/S1474-4422(16)30029-1.

[4] Jokumsen-Cabral, A., Aires, A., Ferreira, S., Azevedo, E., and Castro, P. (2019). Primary involvement of neurovascular coupling in cerebral autosomaldominant arteriopathy with subcortical infarcts and leukoencephalopathy. J. Neurol. 266, 1782–1788. doi: 10.1007/s00415-019-09331-y

[5] Sorond, F. A., Kiely, D. K., Galica, A., Moscufo, N., Serrador, J. M., Iloputaife, I., et al. (2011). Neurovascular coupling is impaired in slow walkers: the MOBILIZE Boston study. Ann. Neurol. 70, 213–220. doi: 10.1002/ana.22433

[6] Pantoni, L. (2010). Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. Lancet Neurol. 9, 689–701. doi: 10.1016/S1474-4422(10)70104-6

[7] Wardlaw, J. M. (2010). Blood-brain barrier and cerebral small vessel disease. J. Neurol. Sci. 299, 66–71. doi: 10.1016/j.jns.2010.08.042

[8] Freeze, W. M., Jacobs, H. I. L., Schreuder, F., van Oostenbrugge, R. J., Backes, W. H., Verhey, F. R., et al. (2018). Blood-brain barrier dysfunction in small vessel disease related intracerebral hemorrhage. Front. Neurol. 9:926. doi: 10.3389/fneur.2018.00926

[9] Castro, P., Gutierres, M., Pereira, G., Ferreira, S., Oliveira, J. P., and Azevedo, E. (2020). Evaluation of cerebral microvascular regulatory mechanisms with transcranial doppler in fabry disease. Brain Sci 10:528. doi: 10.3390/brainsci10080528

[10] Girouard H, Iadecola C. Neurovascular coupling in the normal brain and in hypertension, stroke, and Alzheimer disease. J Appl Physiol (1985). 2006; 100(1): 328-35.

[11] Lohmann H, Ringelstein EB, Knecht S. Functional transcranial Doppler sonography. Front Neurol Neurosci 2006; 21: 251-60.

[12] Wolf ME. Functional TCD: regulation of cerebral hemodynamics–cerebral autoregulation, vasomotor reactivity, and neurovascular coupling. Front Neurol Neurosci 2015; 36: 40-56.

[13] Joanna M Wardlaw, Maria C Valdés Hernández, and Susana Muñoz-Maniega. What are white matter hyperintensities made of? relevance to vascular cognitive impairment. Journal of the American Heart Association, 4(6):e001140, 2015. doi: 10.1161/JAHA.114.001140.

[14] Kuang Q, Huang M, Lei Y, Wu L, Jin C, Dai J and Zhou F (2023) Clinical and cognitive correlates tractography analysis in patients with white matter hyperintensity of vascular origin. Front. Neurosci. 17:1187979. doi:

10.3389/fnins.2023.1187979

[15] Fang-Cheng Yeh, Timothy D Verstynen, Yu-Chun Wang, Juan C Fernández-Miranda, and Wen-Yih Isaac Tseng. Deterministic diffusion fiber tracking improved by quantitative anisotropy. PLoS ONE, 8(11):e80713, 2013. doi: 0.1371/journal.pone.0080713.

[16] F.Yeh, D. Badre, and T. Verstynen. Connectometry: A statistical approach harnessing the analytical potential of the local connectome. NeuroImage, 125:162–171, 2016. doi: 10.1016/j.neuroimage.2015.10.053.

[17] Kritikos, M., Huang, C., Clouston, S. A. P., Pellecchia, A. C., Santiago-Michels, S., Carr, M. A., Hagan, T., Kotov, R., Gandy, S., Sano, M., Horton, M., Bromet, E. J., Lucchini, R. G., Luft, B. J. DTI Connectometry Analysis Reveals White Matter Changes in Cognitively Impaired World Trade Center Responders at Midlife. Journal of Alzheimer's Disease, 2022, 89(3), 1075-1089. doi: 10.3233/JAD-220255.

[18] Thomas E. Nichols and Andrew P. Holmes. Nonparametric permutation tests for functional neuroimaging: A primer with examples. Human Brain Mapping, 15(1):1–25, Jan 2002. doi: 10.1002/hbm.1058.

[19] Maurizio Bergamino, Elizabeth Keeling, Virendra Mishra, Ashley Stokes, and Ryan Walsh. Assessing white matter pathology in early-stage parkinson disease using diffusion mri: A systematic review. Frontiers in Neurology, 11, May 2020. doi: 10.3389/fneur.2020.00314.

[20] Fang-Cheng Yeh and Wen-Yih Isaac Tseng. Ntu-90: a high angular resolution brain atlas constructed by q-space diffeomorphic reconstruction. NeuroImage, 58:91–99, 2011. doi: 10.1016/j.neuroimage.2011.06.021.

[21] A. Monteiro, P. Castro, G. Pereira, et al. Neurovascular coupling is impaired in hypertensive and diabetic subjects without symptomatic cerebrovascular disease. Frontiers in Aging Neuroscience, 13:728007, 2021.

[22] A Monteiro, P Castro, G Pereira, C Ferreira, J Polonia, M Lobo, and E Azevedo. Cerebral blood flow regulation and cognitive performance in hypertension. Journal of Cerebral Blood Flow & Metabolism, May 13 2024. doi: 10.1177/0271678X241254680.

[23] B. Rosengarten, C. Budden, S. Osthaus, and M. Kaps. Effect of heart rate on regulative features of the cortical activity-flow coupling. Cerebrovascular Diseases, 16(1):47–52, 2003.

[24] Fang-Cheng Yeh, Van J. Wedeen, and Wen-Yih Isaac Tseng. Estimation of fiber orientation and spin density distribution by diffusion deconvolution. NeuroImage, 55(3):1054–1062, 2011. doi: 10.1016/j.neuroimage.2010.11.087.

[25] Fang-Cheng Yeh et al. Automatic removal of false connections in diffusion mri tractography using topology-informed pruning (tip). Neurotherapeutics: The Journal of the American Society for Experimental NeuroTherapeutics, 16(1):52–58, 2019b. doi: 10.1007/s13311-018-0663-y.

[26] M. Baudrimont, F. Dubas, A. Joutel, E. Tournier-Lasserve, and M. G. Bousser. Autosomal dominant leukoencephalopathy and subcortical ischemic stroke. a clinicopathological study. Stroke, 24:122–125, 1993. doi: 10.1161/01.STR.24.1.122. Originally published 1 Jan 1993.

[27] Costantino Iadecola and Robin L. Davisson. Hypertension and cerebrovascular dysfunction. Cell Metabolism, 7(6):476–484, Jun 2008. doi: 10.1016/j.cmet.2008.03.010.

[28] Costantino Iadecola and Rebecca F. Gottesman. Neurovascular and cognitive dysfunction in hypertension: Epidemiology, pathobiology, and treatment. Circulation Research, 124:1025–1044, 2019. doi: 10.1161/CIRCRESAHA.118.313260. Originally published 28 Mar 2019.

[29] Ana Monteiro, BioRiMiCH: novel pre-symptomatic Biomarkers of Risk in Microvascular Cerebral disease in arterial Hypertension, May 2024.

[30] Morcom AM, Henson RNA. Increased Prefrontal Activity with Aging Reflects Nonspecific Neural Responses Rather than Compensation. J Neurosci 2018; 38(33): 7303-13.

[31] Gazzaley A, Cooney JW, McEvoy K, Knight RT, D'Esposito M. Top-down enhancement and suppression of the magnitude and speed of neural activity. J Cogn Neurosci 2005; 17(3): 507-17.

[32] Murray SO, Wojciulik E. Attention increases neural selectivity in the human lateral occipital complex. Nat Neurosci 2004; 7(1): 70-4.

[33] Chen AJ, Britton M, Turner GR, Vytlacil J, Thompson TW, D'Esposito M. Goal-directed attention alters the tuning of object-based representations in extrastriate cortex. Front Hum Neurosci 2012; 6:187.

[34] Lorenc ES, Lee TG, Chen AJ, D'Esposito M. The Effect of Disruption of Prefrontal Cortical Function with Transcranial Magnetic Stimulation on Visual Working Memory. Front Syst Neurosci 2015; 9: 169.

[35] Goncalves J, Gerardo B, Nogueira J, et al. Montreal cognitive assessment (MoCA): an update normative study for the portuguese population. Appl Neuropsychol Adult 2023; 14: 1–7

[36] An-Ming Hu, Yan-Ling Ma, Yue-Xiu Li, Zai-Zhu Han, Nan Yan, and Yu-Mei Zhang. Association between changes in white matter microstructure and cognitive impairment in white matter lesions. Brain Sciences, 12(4):482, 2022. doi: 10.3390/brainsci12040482.

[37] Shuning Wang, Fan Zhang, Ping Huang, Han Hong, Yefan Jiaerken, Xinfeng Yu, Ruiting Zhang, Qi Zeng, Yongchao Zhang, Ron Kikinis, Yogesh Rathi, Nikos Makris, Min Lou, Ofer Pasternak, and Mingjun Zhang. Superficial white matter microstructure affects processing speed in cerebral small vessel disease. Human Brain Mapping, 43(17):5310–5325, 2022. doi: 10.1002/hbm.26004.

[38] Fang-Cheng Yeh, Ibrahim M Zaydan, Valerie R Suski, David Lacomis, R Mark Richardson, Joseph C Maroon, and Jessica Barrios-Martinez. Differential tractography as a track-based biomarker for neuronal injury. Neuroimage, 202:116131, 2019.

Supplementary material. Available supplementary results contain tractograms and anatomical identification of the tracks for each t-score and each variable, including demographics.

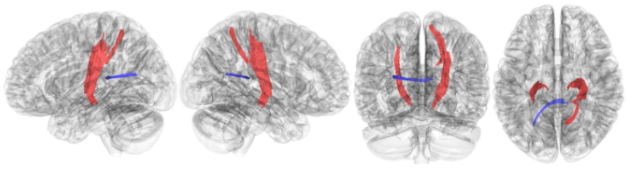
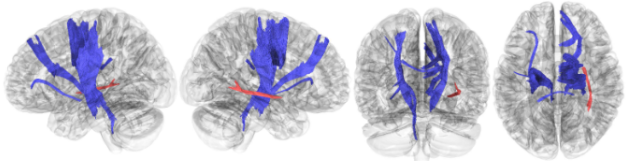
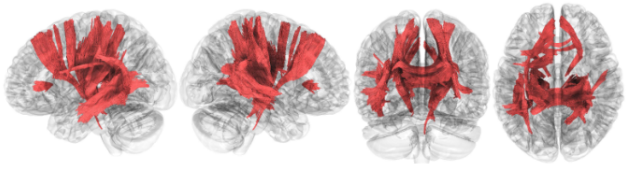
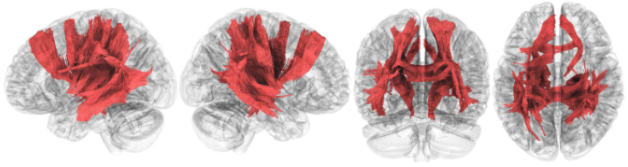
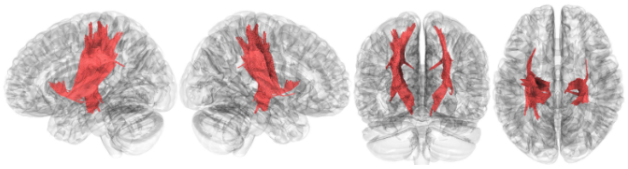
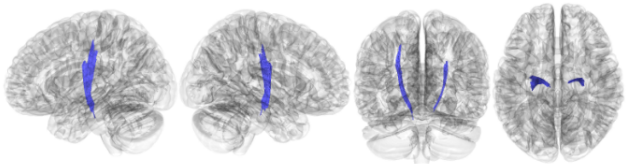
Study variable	Quantitative anisotropy (t-score = 3; minimum length = 40 mm)
MMSE	
MoCA	
Total score	
Executive function	
Memory	
Processing speed	

Fig. 5: Post-connectometry tractograms in the saggital, coronal and axial planes showing the tracks where quantitative anisotropy (QA) was associated with the neuropsychological tests. Associations are positive (red) or negative (blue). The t-score threshold was 3 and the minimum track length was 40 mm (20 voxel distance). Abbreviations: MMSE, Mini-Mental State Examination. MoCA, Montreal Cognitive Assessment.