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ADVANCED TRANSCRANIAL DOPPLER TECHNIQUES FOR PREDICTING AND UNDERSTANDING COGNITIVE DECLINE IN CEREBRAL VASCULAR DISEASE

Juliana Patrícia Figueiras Ferreira

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PUBLICAÇÕES

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1 - Ferreira J, Ferreira P, Azevedo E, Castro P. Assessment of Neurovascular Coupling by Spectral Analysis of Cerebral Blood Flow Velocity with Transcranial Doppler. Ultrasound Med Biol. 2024 May;50(5):751-759. doi: 10.1016/j.ultrasmedbio.2024.02.003. Epub 2024 Feb 28. PMID: 38418342. **IF:3.12, Q1**

2 - Ferreira J, Alves F, Pedro T, Fonseca L, Gama G, Moreira G, Azevedo E, Castro P. Neurovascular Coupling assessment by Transcranial Doppler in Acute Stroke could be Informative of Long-term Cognitive Status *(under Review - Journal of Cerebral blood Flow and metabolism)*

3 - Castro P*, Ferreira J*, Malojcic B, Bazadona D, Baracchini C, Pieroni A, Skoloudik D, Azevedo E, Kaps M (2023). Detection of microemboli in patients with acute ischaemic stroke and atrial fibrillation suggests poor functional outcome. Eur Stroke J. 27:23969873231220508. doi: 10.1177/23969873231220508. **IF: 5.89, Q1.**

4 - Ferreira J, Pereira G, Alves F, Pedro T, Fonseca L, Gama G, Moreira G, Azevedo E, Castro P. Microemboli Detection in Acute Ischemic Stroke could be an Early Marker of Poor Cognitive Outcome *(under Review - Stroke Journal)*

Em cumprimento com o disposto no Art.º 8º do Decreto-Lei n.º 388/70, declaro que participei no planeamento metodológico de todos os trabalhos de investigação que constam desta tese, fui responsável pelo recrutamento dos participantes, pela recolha, armazenamento e tratamento de dados, bem como, pela redação de todas as publicações. Participei ativamente no processo de submissão dos manuscritos para publicação e no seu processo de revisão.

Outras publicações da autora que contribuíram para os trabalhos desta tese:

1 - Pedro, T., Costa, A., Ferreira, J., Rocha, A. L., Salgueiro, E., Pereira, G., Azevedo, E., & Castro, P. (2022). Changes in cerebral autoregulation and vasoreactivity after surgical aortic valve replacement: A prospective study. Experimental Physiology, 1–8. https://doi.org/10.1113/EP090502. **IF: 2.858, Q1.**

2 - Dias, L., Gonçalves, J., Ferreira, J., Fonseca, L., Moreira, G., Castro, P. (2024) Red Cell Distribution Width and Outcome in Acute Ischemic Stroke Patients. Journal of Stroke Cerebrovascular Diseases. https://doi.org/10.1016/j.jstrokecerebrovasdis.2024.108069 **IF: 2.136, Q1.**

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Pensei várias vezes sobre este momento, como seria escrever esta página e descrever um processo tão solitário e ao mesmo tempo tão cheio de pessoas. Este é um projeto com pessoas e sobre pessoas.

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ABBREVIATIONS

CVD – Cerebrovascular Diseases

TOAST - Trial of Org 10172 in Acute Stroke Treatment

Apo – Apolipoprotein

HDL - High Density Lipoproteins

VLDL - Very Low-Density Lipoproteins

LDL - Low Density Lipoproteins

IDL - Intermediate Density Lipoproteins

ICA - Internal Carotid Arteries

ACA - Anterior Cerebral Artery

AChA - Anterior Choroidal Artery

MCA - Middle Cerebral Artery

PCoA - Posterior Communicating Artery

ACoA - Anterior Communicating Artery

AChA - Anterior Choroidal Artery

VA - Vertebral Arteries

BA - Basilar Artery

PICA - Posterior Inferior Cerebellar Artery

AICA - Antero-inferior Cerebellar Artery

SCA - Superior Cerebellar Artery

PCA - Posterior Cerebral Artery

BBB - Blood-brain Barrier

CNS - Central Nervous System

K^+ - Potassium

Ca^{2+}- Calcium

O_2 - Oxygen

CO_2 - Carbon Dioxide

Na^+ - Sodium

Cl^- - Chloride

CBF - Cerebral Blood Flow

BP - Arterial Pressure

NO - Nitric Oxide

NVC - Neurovascular Coupling

CBv - Cerebral Blood Flow Velocity

fTCD - Functional Transcranial Doppler

CADASIL - Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy

T2DM - Type 2 Diabetes Mellitus

MES - Microembolic Signals

CAA - Cerebral Amyloid Angiopathy

ROS - Reactive Oxygen Species

TCD - Transcranial Doppler

CA - Cerebral Autoregulation

dCAR - Dynamic Cerebral Autoregulation

IQCODE - Informant Questionnaire on Cognitive Decline in the Elderly

mRS - Modified Rankin Scale for Neurologic Disability

MoCA - Montreal Cognitive Assessment

IADL - Instrumental Activities of Daily Living Scale

AF - Atrial Fibrillation

FFT - Fast Fourier transformation

SNR - Signal-to-Noise Ratio

SINAD - Signal-to-Noise and Distortion Ratio

PSCI – Post-stroke Cognitive Impairment

ABSTRACT

Introduction

Stroke is the most recognised type of cerebrovascular disease, yet the mechanisms underlying long-term cognitive decline after stroke remain poorly understood. Monitoring with Transcranial Doppler (TCD) allows real-time assessment of cerebral haemodynamic parameters compromised during stroke and complements information obtained from neuroimaging. Understanding the factors contributing to cognitive decline and poorer functional recovery, and their interaction with haemodynamic data, may help stratify risk and guide future interventions for these patients.

Objectives

This study aimed to contribute to the identification of factors that may predict cognitive function and functional recovery after stroke and to provide data on cerebral haemodynamics in patients with ischaemic stroke. The study prospectively investigated neurovascular coupling (NVC) and microembolic signals (MES) in patients who had experienced an ischaemic stroke, assessing their relationship with cognitive function and functional recovery.

Methods and Results

This prospective study included a cohort of 512 patients who had suffered an ischaemic stroke and were admitted to the stroke unit at the Centro Hospitalar Universitário de São João (Porto, Portugal) between November 2020 and July 2022. Follow-up assessments were conducted between February 2021 and May 2023. We also recruited an age-, sex- and vascular risk factor-matched control group of 40 participants, as well as a healthy control group of 20 participants. Both control groups were assessed at a single time point.

This work is divided into four studies addressing our research objectives.

The first study aimed to describe a novel approach for the non-invasive measurement of NVC using spectral analysis of cerebral blood flow velocity via TCD. In a sample of 20 healthy participants, we monitored systolic cerebral blood flow velocity in the left posterior cerebral artery (PCA) during resting (eyes closed) and visual stimulation (intermittent checkerboard) periods. The contralateral middle cerebral artery was simultaneously monitored as a control. Our findings suggest that this method represents an alternative to the classical overshoot approach, without the need for stimulus marking or synchronisation.

The second study assessed whether NVC status during acute ischaemic stroke could predict long-term cognitive impairment. In this prospective study, 144 stroke survivors and 40 matched controls were included. TCD monitoring was conducted within the first 72 hours after stroke to assess NVC. The primary outcome was cognitive status at 12 months, measured using a 7-level cognitive scale. Results showed that NVC assessment via TCD in the acute phase of ischaemic stroke may serve as a predictor of long-term cognitive impairment, suggesting that both diminished and exaggerated NVC responses are associated with poorer cognitive outcomes.

The third study was a multicentre, prospective investigation into the burden of MES in patients with acute ischaemic stroke and atrial fibrillation, examining the impact of these signals on functional outcomes. Sixty-one patients were included and monitored with TCD for one hour within 24 hours of symptom onset. The results indicated that the detection of MES within 24 hours of stroke onset may serve as a marker of poor functional outcomes.

In the fourth study, we explored the burden of MES in acute ischaemic stroke patients and its impact on long-term cognitive function. This prospective study included 316 patients who underwent TCD monitoring within the first 72 hours of symptom onset, with recordings lasting 30 minutes. Cognitive function at 12 months was assessed using a 7-level cognitive scale. The results showed that patients who were MES-positive during the acute phase experienced worse cognitive outcomes 12 months after the stroke.

Conclusion

Our study provided data on cerebral haemodynamics during the acute phase of stroke, MES and NVC parameters, their evolution over the first-year post-stroke, and their impact on patients' functional and cognitive recovery.

The detection of MES during the acute stroke phase using TCD may be considered an early marker of poorer cognitive performance, allowing for risk stratification in high-risk patients. Acute-phase NVC appears to offer valuable insights into long-term cognition and may serve as a target for therapeutic adjustments aimed at improving cognitive outcomes.

Keywords: Transcranial Doppler Techniques; Cerebrovascular Diseases; Cognitive Decline; Functional Recovery.

RESUMO

Introdução

O AVC é o tipo mais reconhecido de doença cerebrovascular e os mecanismos de declínio cognitivo a longo prazo após o AVC não são bem compreendidos. A monitorização com Doppler Transcraniano (DTC) permite avaliar parâmetros da hemodinâmica cerebral comprometidos no AVC, em tempo real, e complementa as informações recolhidas na neuroimagem. Compreender os fatores que contribuem para o declínio cognitivo, para uma pior recuperação funcional e a sua interação com os dados hemodinâmicos pode ser útil para estratificar o risco e planear futuras intervenções para estes pacientes.

Objetivos

Este estudo teve como objetivo contribuir para a identificação de fatores que possam ser preditores da função cognitiva e da recuperação funcional após o AVC, e fornecer dados sobre a hemodinâmica cerebral em pacientes com AVC isquémico. O estudo investigou prospectivamente o acoplamento neurovascular (ANV) e os sinais microembólicos (SME) em pacientes que sofreram AVC isquémico, avaliando a sua relação com a função cognitiva e a recuperação funcional.

Métodos e resultados

Este estudo prospetivo, incluiu uma coorte de 512 doentes que sofreram um AVC isquémico e foram admitidos na unidade de AVC (Centro Hospitalar Universitário de São João, Porto, Portugal) entre novembro de 2020 e julho de 2022. O seguimento foi realizado entre fevereiro de 2021 e maio de 2023. Recrutámos um grupo de controlo pareado por idade, género e fatores de risco vascular com 40 participantes e um grupo de controlo saudável com 20 participantes. Ambos foram avaliados num único momento.

Este trabalho está divido em quatro estudos para responder ao nosso objetivo de investigação. O primeiro estudo, teve como objetivo descrever uma nova abordagem para a medição não invasiva do ANV por meio da análise espectral da velocidade do fluxo sanguíneo cerebral utilizando DTC. Numa amostra de 20 participantes saudáveis, monitorizámos a velocidade do fluxo sanguíneo cerebral sistólica na artéria cerebral posterior esquerda (PCA) durante períodos de repouso (olhos fechados) e estímulo (tabuleiro de xadrez intermitente). Simultaneamente, a artéria cerebral média contralateral foi monitorizada como controlo. Os nossos resultados mostram que este método representa uma alternativa ao *overshoot* clássico, sem a necessidade de marcação ou sincronização do estímulo.

O segundo estudo, avaliou se o estado do ANV no AVC isquémico agudo pode prever comprometimento cognitivo a longo prazo. Neste estudo prospetivo foram incluídos 144 sobreviventes de AVC e 40 controlos pareados por idade, género e fatores de risco vasculares. A monitorização por DTC foi realizada nas primeiras 72 horas após o AVC para medir o ANV. O desfecho primário foi o estado cognitivo aos 12 meses, avaliado por uma escala cognitiva de 7 níveis. Os resultados mostraram que a avaliação do ANV por DTC na fase aguda do AVC isquémico pode atuar como um preditor de comprometimento cognitivo a longo prazo, sugerindo que tanto respostas reduzidas quanto exageradas de ANV estão associadas a piores desfechos cognitivos.

O terceiro estudo foi um estudo multicêntrico e prospetivo, onde investigámos a carga de SME em pacientes com AVC isquémico agudo e fibrilhação auricular, avaliando o impacto desses sinais nos desfechos funcionais. Foram incluídos 61 pacientes, monitorizados com DTC por uma hora até 24 horas após o início dos sintomas. Os resultados mostraram que a deteção de SEM dentro de 24 horas após o AVC pode significar um marcador de desfechos funcionais desfavoráveis.

No quarto estudo, investigamos a carga de SEM em pacientes com AVC isquémico agudo e seu impacto na função cognitiva a longo prazo. Este estudo prospetivo inclui 316 pacientes, com monitorizações por DTC nas primeiras 72 horas após início dos sintomas, durante 30 minutos. A função cognitiva aos 12 meses foi avaliada por uma escala cognitiva de 7 níveis. Os resultados mostraram que pacientes SEM-positivos na fase aguda se relacionam com piores desfechos cognitivos 12 meses após o AVC.

Conclusão

O nosso estudo forneceu dados sobre a hemodinâmica cerebral na fase aguda do AVC, sobre os parâmetros de SEM e ANV, a sua evolução ao longo do primeiro ano após o evento e seu impacto na recuperação funcional e cognitiva dos pacientes.

A deteção de SEM na fase aguda do AVC por DTC pode ser considerada um marcador precoce de pior desempenho cognitivo, permitindo a estratificação de risco em pacientes de alto risco. O ANV na fase aguda parece oferecer informações valiosas sobre a cognição a longo prazo e pode ser um foco para ajustes terapêuticos destinados a melhorar os desfechos cognitivos.

Palavras-chave: Técnicas de Doppler Transcraniano; Doenças Cerebrovasculares; Declínio Cognitivo; Recuperação Funcional.

CHAPTER I: GENERAL INTRODUCTION

1. CEREBROVASCULAR DISEASES

Cerebrovascular diseases (CVD) encompass a large group of conditions that can affect the cerebral blood flow or the cerebral vessels, excluding the traumatic causes. The most representative disease is stroke, which results from acute cerebral vessel occlusion and subsequent ischemia or from bleeding due to sudden vessel rupture. These events can lead to a broad spectrum of effects, ranging from silence lesions found in neuroimage studies, to dramatic acute clinical events. The episodes can also be transient or more prolonged, such as ischemic and haemorrhagic strokes, subarachnoid haemorrhages, and thromboses of the dural venous sinuses and cerebral veins. CVD also includes chronic cognitive impairment caused by the cumulative brain damage or lesions affecting strategically important brain locations (Caprio & Sorond, 2019; Ferrer & Vidal, 2017; Hu et al., 2017).

The consequences of cerebrovascular diseases are a major concern for global health (Tsao et al., 2023). The incidence of cerebrovascular diseases increases with age, varies by gender and racial differences, and is strongly influenced by personal risk factors. Atherosclerosis, small vessel disease, cardiovascular disease, and haemorrhagic stroke have also been associated with genetic alterations (Ferrer & Vidal, 2017; Iadecola et al., 2020). Among cerebrovascular diseases, stroke ranks as the leading cause of death in developed countries (Caprio & Sorond, 2019). Vascular dementia is considered the second leading cause of dementia in the elderly, even though it is often found alongside other neurodegenerative diseases. Additionally, vascular lesions exacerbate degenerative cognitive decline (Caprio & Sorond, 2019; Hu et al., 2022; Tsao et al., 2023).

Despite efforts for early diagnosis and treatment of modifiable risk factors, stroke remains one of the leading causes of mortality and morbidity worldwide (Caprio & Sorond, 2019). Both large vessel and small vessel diseases, once initiated, progress slowly over the years. Aging and vascular risk factors accelerate the progression rate of these diseases, while diet and genetics act as modifiers of this progression, potentially increasing or decreasing its rate. In 2023, despite a decrease in the impact of cerebrovascular diseases in developed countries, there was an observed increase in this impact in developing countries. (Tsao et al., 2023).

1.1. STROKE

Stroke is the most recognised type of cerebrovascular disease and is characterised as a rapidly occurring neurological syndrome presenting with focal signs and symptoms. Two types of stroke can occur— ischemic and haemorrhagic —, with different

aetiopathogenesis, although they may share common risk factors (Figure 1) (Beishon & Minhas, 2021; Caplan, 2006)

Ischemic stroke results from an occlusion in an artery, leading to a significant reduction in blood flow to a specific region of the brain. Approximately 70% of stroke cases are ischemic and can be further subdivided based on their cause. According to the Trial of Org 10172 in Acute Stroke Treatment (TOAST), a system developed to categorise ischemic strokes regarding to their main cause, the following groups were considered: Large Artery Atherosclerosis, Cardioembolic, Small Artery Occlusion (Lacunar Infarction), Stroke of Other Determined Cause, and Stroke of Undetermined Cause (Bamford et al., 1991; Beishon & Minhas, 2021; Goldstein et al., 2001; Knight-Greenfield et al., 2019)

Strokes caused by large vessel diseases resulting from atherosclerosis of cervical or proximal intracranial vessels are one of the main causes of acute stroke. The pathogenic mechanisms in these cases of stroke include artery-to-artery emboli, where parts of an atherosclerotic plaque move downstream; *in situ* thrombosis, where clots occlude the artery in the area affected by atherosclerosis; and haemodynamic compromise, where distal decreased blood flow is due to critical narrowing or blockage of the arteries. Cardioembolism, most notably atrial fibrillation, accounts for approximately 20% to 30% of cases and are characterised by multifocal ischemic lesions (Knight-Greenfield et al., 2019). More recently, stroke associated with *Foramen Ovale Patent* as gained its own position as a cause outside cardioembolism (Caso et al., 2024). Lacunar infarcts are attributed to small perforating artery occlusions and account for approximately 10% to 23% of acute ischemic stroke cases. Uncommon causes can be found in 2% to 11% of cases. However, despite throughout examination, as much as 25% of the patients have an undetermined source, because there are concurring causes, or their cause is cryptogenic (Knight-Greenfield et al., 2019; Regenhardt et al., 2018).

Haemorrhagic stroke has a prevalence around 20% of acute stroke cases. with hypertension and amyloid angiopathy being the most common causes of deep and lobar location, respectively. (Knight-Greenfield et al., 2019; Montano et al., 2021).

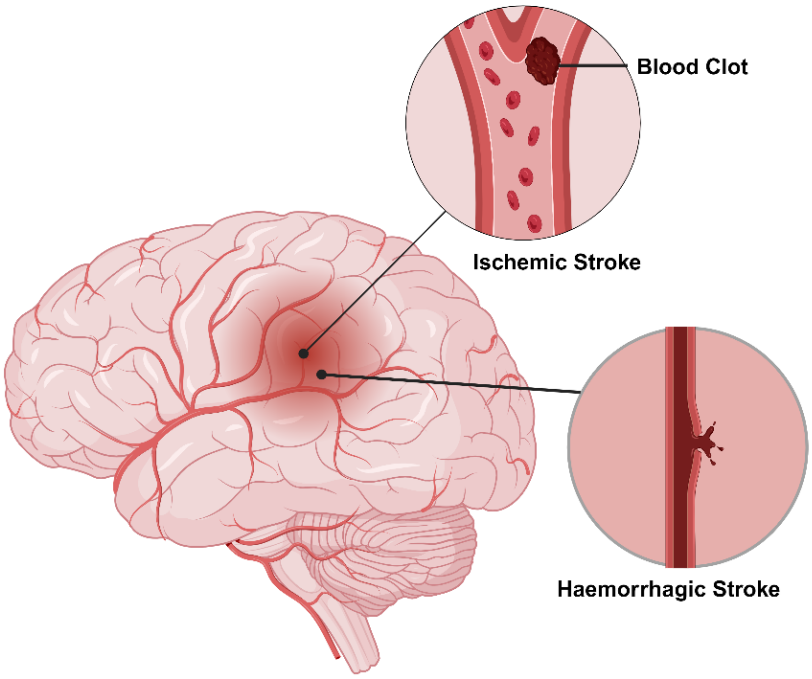

Figure 1. Types of Stroke (Created with BioRender.com)

Multicentric studies identify the following vascular risk factors: active smoking, diabetes mellitus, hypertension (blood pressure ≥ 140/90 mm Hg), abdominal obesity, depression and stress, lack of daily fruit and vegetable consumption, physical inactivity (absence of regular moderate or high-intensity physical exercise for at least 4 hours per week), regular alcohol consumption (3 or more times per week), and the Apo (apolipoprotein) B/ApoA1 ratio (Hankey, 2020; Kleindorfer et al., 2021; Smyth et al., 2021; Smyth et al., 2023; Teo & Rafiq, 2021; Wang et al., 2024).

1.1.1. Short Synopsis of Pathophysiology of Acute Stroke

Excitotoxicity, peri-infarct depolarizations, inflammation, and programmed cell death are the main mechanisms that occur because of the complex pathophysiological events triggered after ischemic brain injury. This cascade of events evolves over time following the injury and throughout the brain tissue (Dirnagl et al., 1999).

In the acute phase of ischemic injury, neurons that do not receive sufficient blood flow to meet their normal metabolic needs undergo depolarization, causing the release of excitatory glutamate. Because of glutamate release, there is an increase in intracellular levels of calcium (Ca^{2+}), sodium (Na^{+}), and chloride (Cl^{-}), along with the efflux of potassium (K^{+}) into the extracellular space. In peri-infarct regions, glutamate and Ca^{2+} induce waves of depolarization that can spread to other regions of the brain that were

not directly affected by the ischemic injury but are put at risk due to this disturbance in homeostasis (Dirnagl et al., 1999).

Local inflammation occurs due to the chemotaxis of extracerebral leukocytes and the activation of resident microglia, triggered by the increase in Ca^{2+} that activates a cascade of inflammatory mediators and cell apoptosis (Dirnagl et al., 1999). The osmotic imbalance caused by this cascade of events leads to edema, which exacerbates the injury. Besides edema, microembolization and micro-obstruction of the capillary network may also occur, which can prevent the recovery of the affected territory despite the recanalization of the main vessel (Balami et al., 2011; Simard et al., 2007).

Adding up to the dramatic ischemic and microvascular disruption, a neuroinflammatory process immediately begins. This process is characterised by the activation of resident immune cells, such as microglia and astrocytes, and the infiltration of peripheral immune cells that lead to the release of pro-inflammatory cytokines, chemokines, and reactive oxygen species (Figure 2) (Ferro et al., 2021; Pisco et al., 2023). The release of these inflammatory mediators results in the disruption of the blood-brain barrier (BBB), causes neuronal damage promotes neuronal apoptosis, impairs neuroplasticity, and exacerbates the neurological deficits associated with the lesion, thereby interfering with cognitive and functional recovery (see section 2. Mechanisms of Cognitive Decline in Cerebrovascular Diseases). However, this process also aids in the clearance of cellular debris and plays an important role in promoting tissue repair (Alsbrook et al., 2023; Candelario-Jalil et al., 2022; Shi et al., 2019).

In ischaemic stroke, the neuroinflammatory process begins minutes after the onset of ischaemia and continues for several days following the acute event. This not only affects the infarct area but derails into other non-affected regions. This makes of stroke not a local brain problem but a whole brain dysfunction (Alsbrook et al., 2023; Shi et al., 2019).

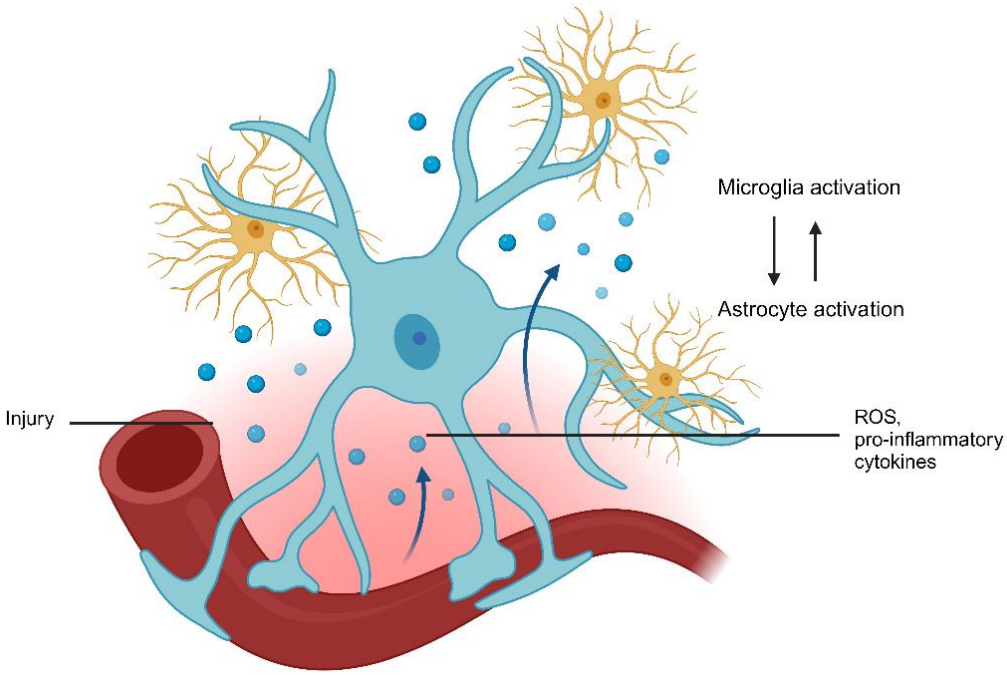

Figure 2. Neuroinflammation. Created with BioRender.com

1.2. CEREBRAL VASCULARIZATION

The blood supply to the brain has functional particularities that ensure it is adequate for physiological conditions, thereby maintaining the integrity of areas that control vital functions. Cerebral circulation does not exhibit relevant variation in blood pressure during the cardiac cycle; that is, during diastole, there is no significant decrease in blood supply to the brain, maintaining a minimum and adequate perfusion pressure for normal cerebral function (Ferrer & Vidal, 2017).

Cerebral blood circulation consists of an arterial blood supply system and a venous drainage circulation. The sources of arterial blood supply to the brain are the anterior circulation or carotid system, originating from the internal carotid arteries (ICA), and the posterior circulation or vertebrobasilar system, originating from the vertebral arteries. The branching of these arteries after entering the skull allows blood supply to both superficial and deep regions of the brain (Chandra et al., 2017; Cipolla, 2010).

1.2.1. CEREBRAL LARGE VESSELS

Anterior Cerebral Circulation

The carotid system, or anterior circulation, which supplies two-thirds of the brain, is composed of the internal carotid artery (ICA) with its extra- and intracranial segments

and its intracranial branches. Notable branches include the anterior cerebral artery (ACA), the middle cerebral artery (MCA) and its branches, and the posterior communicating artery (PCoA) (Figure 1). The anterior circulation is responsible for supplying a large part of the anterior portion of the brain, including the frontal and parietal lobes, as well as part of the temporal lobes, diencephalon and the internal capsule (Chandra et al., 2017; Cure, 2011).

The MCA is the largest cerebral artery, with the most complex distribution and the least inter-individual variability. It originates at the bifurcation of the ICA and runs along the ventral surface of the frontal lobe (M1), entering the Sylvian fissure between the temporal lobe and the insular cortex. In this area, the artery bifurcates into main trunks (M2), extending to the superior and inferior regions of the insular surface and supplying most of the 2/3 of lateral surface of the cerebral hemisphere: the insular cortex; opercular surface; superior and middle temporal gyri; inferior parietal lobe and part of the postcentral gyrus; inferior and middle frontal gyri; part of the precentral gyrus; and the lateral portion of the orbital surface (Chandra et al., 2017; Giotta Lucifero et al., 2021).

Posterior Cerebral Circulation

The vertebrobasilar system consists of the two vertebral arteries (VA) that merge to form the basilar artery (BA), which gives rise to the two posterior cerebral arteries and their perforating branches (Figure 3). The posterior cerebral circulation supplies the entire brainstem, as well as the cerebellum and the posterior third of the cerebral hemispheres, accounting for one-third of the total blood flow to the brain (Chandra et al., 2017; Ferrer & Vidal, 2017). This circulation has several characteristics of pathological interest, although it is less frequently associated with cerebrovascular pathology compared to the anterior circulation (Chandra et al., 2017).

The vertebral arteries (VA) are paired arteries that originate bilaterally from the subclavian vessels, and travel through the transverse foramina up to the posterior arch of the atlas. In this region, it penetrates the dura mater into the subarachnoid space to become intracranial and ending up both in a single basilar artery.

Important branches arising from the intracranial segment of the vertebral artery (VA) and the basilar artery include the cerebellar arteries, which supply the brainstem and cerebellum: the posterior inferior cerebellar artery (PICA), the anterior inferior cerebellar artery (AICA), and the superior cerebellar artery (SCA). Finally, BA bifurcates into the two posterior cerebral arteries (PCA) (Cure, 2011; Ferrer & Vidal, 2017).

The posterior cerebral arteries encircle the midbrain in the region immediately anterior to the root of the oculomotor nerve, running through the cerebral peduncles and reaching

the ventromedial region of the cortex. These arteries supply the occipital lobe, the inferior and middle portions of the temporal lobe, and a more posterior part of the inferior parietal lobe. Consequently, lesions in these arteries can result in visual field deficits as well as alexia and agnosia. The PCoA connects the ICA with the PCA and divides it into two segments: the pre-anastomosis segment (P1) and the post-anastomosis segment (P2) (Chandra et al., 2017; Ferrer & Vidal, 2017).

Collateralization System

The territories of the ICA and the VB connect at the base of the brain through the anterior and posterior communicating arteries in the circle of Willis. This anastomosis allows for a connection between the main vascular territories, helping to maintain cerebral perfusion and protect the brain against ischemia in cases of arterial disease (E. Lin et al., 2022). The anterior portion of the circle of Willis is composed by the bilateral terminations of the ICA, the A1 segments of the ACA, and the ACoA, which connects the right and left anterior circulations. The posterior portion consists of the P1 segments of the PCA, which form the most posterior boundaries of the circle, and the PCoA, which provides communication between the anterior and posterior circulations (Chandra et al., 2017).

There is intra-species variability in the circle of Willis, meaning that among the healthy population numerous anatomical variations of these collateralizations are found. Studies indicate that individuals with a complete circle of Willis have a better prognosis in the event of a stroke. These anastomoses provide an alternative pathway for cerebral blood circulation, helping to prevent the necrosis of penumbral tissue in the case of an ischaemic cerebral event. The arterial circuit network also facilitates the drainage of interstitial fluid and proteins from the brain (Jones et al., 2021; Rozeman et al., 2022).

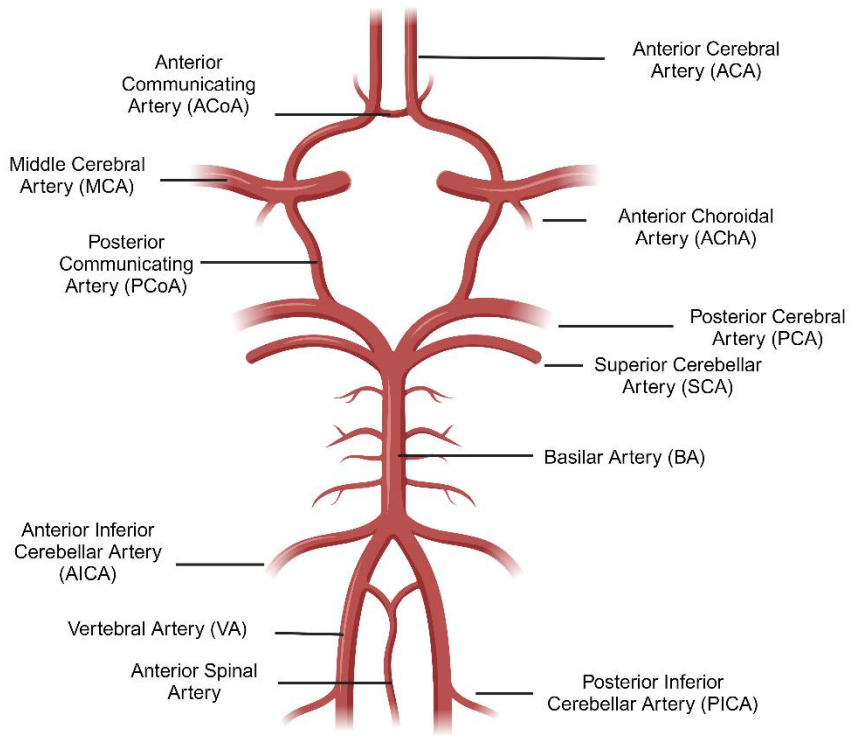

Figure 3. Circle of Willis (Created with BioRender.com).

1.2.2. Cerebral Small Vessels and Blood-brain Barrier

At the microcirculation, exchanges of oxygen and nutrients occur at the blood-brain barrier (BBB).This gas and nutrient exchange is crucial for maintaining optimal brain function, with a close relationship observed between neuronal activity, cellular metabolism, and cerebral blood flow (Chandra et al., 2017; Hoque et al., 2021).

In the highly dense network of tiny vessels located between the arterioles and venules, lies the main site for gas and nutrient exchange. The arterioles supply blood to the capillaries where gas and nutrient exchanges with the brain occur, and the venules collect blood from the capillaries and direct it back to larger veins. The contraction and dilation of pre-capillary arterioles and post-capillary venules regulate blood flow in this region, making blood flow in the capillaries highly dynamic. Local regulation of blood flow plays an important role, leading to the flow variations in the capillaries (Daneman & Prat, 2015; Hoque et al., 2021).

The BBB is a concept that describes the unique properties of the microcirculation in the central nervous system (CNS), formed by endothelial cells interconnected by tight junctions, pericytes, astrocytes, and a basal lamina. In the CNS, the blood vessels are continuous and non-fenestrated, their walls are composed of a continuous layer of endothelial cells that lack pores or fenestrations. These vessel properties allow for the regulation of the movement of molecules, ions, and cells between the blood and the CNS, functioning as a selective barrier and facilitating transport primarily through the endothelial cells. This restrictive capability ensures the proper functioning of neurons and protects the CNS against toxins, pathogens, inflammation, injuries, and diseases (Daneman & Prat, 2015).

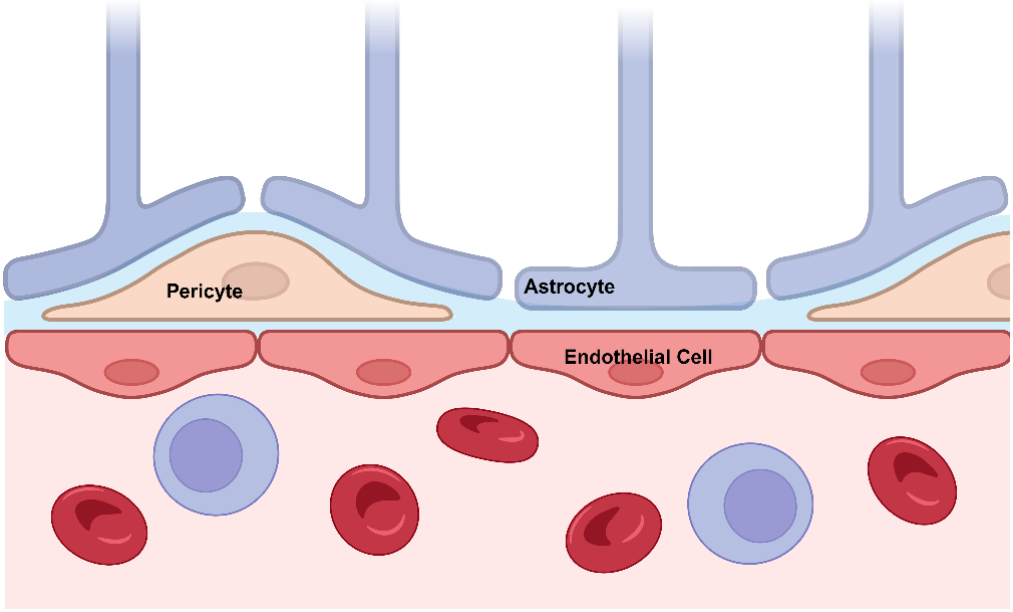

Figure 4. Blood Brain Barrier (Created with BioRender.com).

Endothelial Cells

Endothelial cells line the lumen of all vessels and are the basic and essential building blocks of blood vessels. These microvascular endothelial cells have unique properties that facilitate exchanges between the blood and the brain. These cells are much thinner compared to the endothelial cells in muscles and are held together by tight junctions. These tight junctions limit the flow of larger molecules or particles through their cell membrane via vesicles, a process known as transcytosis. Compared to peripheral endothelial cells, CNS endothelial cells have lower rates of transcytosis, meaning there is a restriction in the movement of vesicles through the cell (Daneman & Prat, 2015; Hoque et al., 2021).

In the CNS, endothelial cells possess two main categories of transporters: efflux transporters, which are polarised to the luminal surface and transport various lipophilic molecules; and nutrient transporters, which facilitate the transport of nutrients across the BBB into the CNS and the removal of byproducts resulting from CNS metabolism into the blood (Bernardo-Castro S, 2023; Daneman & Prat, 2015; Hoque et al., 2021; Sargento-Freitas et al., 2018).

Pericytes

Pericytes are mural cells associated with the microvasculature, extending along endothelial cells to promote vessel integrity. Pericytes can contract and control the diameter of the capillary, playing an important role in angiogenesis, wound healing, and the regulation of immune cell infiltration. These cells exhibit unique properties in the CNS compared to other tissues, with greater pericyte coverage in the CNS microvasculature than in other tissues (Daneman & Prat, 2015; Eltanahy et al., 2021). Pericytes play an important role in the regulation and formation of the BBB during development, as well as in maintaining its function throughout adulthood and aging (Eltanahy et al., 2021; Jokumsen-Cabral et al., 2019)

Astrocytes

Astrocytes are a very important type of glial cell and are the most abundant cells in the CNS. These cells perform functions such as structural support, formation of the BBB, neuronal metabolism, maintenance of the extracellular environment, regulation of cerebral blood flow through the contraction and dilation of arterioles and capillaries, support in cell communication, synthesis of neurotransmitters, and protection against oxidative stress. The branches of astrocytes envelop all cellular components of the CNS, contacting with all parts of neurons. These cells are an integral functional and structural part of the neurovascular unit and tripartite synapses, the physical barrier that limits the diffusion of neurotransmitters away from the synapse formed by the pre- and post-synaptic terminals enveloped by astrocytes (Daneman & Prat, 2015; Liu & Chopp, 2016).

At the synapses, astrocytes are responsible for maintaining the extracellular concentration of neurotransmitters, regulating ionic balance. Astrocytes remove potassium (K^+) resulting from neuronal activity, uptake glutamate, convert it into glutamine, and release it back to the presynaptic terminal. Astrocytes play a direct and dynamic role with neurons in synaptic transmission by the regulated release of synaptically active molecules such as glutamate, GABA, dopamine, serotonin, and acetylcholine, among others. These cells provide a pathway for glucose and other

metabolites to pass between the blood and neurons (Liu & Chopp, 2016; Zhang et al., 2021)

In addition to regulating the local extracellular environment, these cells also function as inflammatory cells, producing inflammatory factors and releasing various mediators, such as nitric oxide (NO), which influence the regulation of blood vessel diameter and blood flow (Liu & Chopp, 2016). Astrocytes are primary mediators in transmitting signals that regulate cerebral blood flow in response to neuronal activity. Thus, astrocytes play an essential role in maintaining normal brain function (Iadecola, 2017).

Basement Membrane

The basement membrane is a structure that surrounds the vascular tube and acts as an additional barrier between the vasculature and neurons for molecules and cells. The membrane performs many important functions, such as providing structural support, anchoring cells to the substrate or extracellular matrix, and converting extracellular signals into cellular responses. The disintegration of the basement membrane is a significant component in the dysfunction of the BBB, observed in many neurological pathologies (Daneman & Prat, 2015; Xu et al., 2019).

In the brain, there are two types of basement membrane: the endothelial basement membrane and the parenchymal basement membrane, which under normal conditions, appear to form a continuous layer. In areas lacking pericytes, these layers are indistinguishable. Biochemically, this membrane consists of proteins primarily synthesized by endothelial cells, pericytes, and astrocytes: collagen IV, laminin, nidogen, perlecan, and agrin. Functionally, these proteins seem to play an important role in maintaining BBB homeostasis and cerebrovascular function, although their exact role remains nuclear (Kang & Yao, 2020; Xu et al., 2019).

1.2.3. VENOUS CIRCULATION

After passing through the capillaries of the cerebral microcirculation, the blood receives metabolites and carbon dioxide (CO_2) resulting from neuronal activity and enters the venous circulation. The structures that make up the cerebral venous circulation show more anatomical variation than those of the arterial circulation. This variation is not only between individuals but also between hemispheres in the same individual, leading to high variability that complicates the classification of cerebral veins (Chandra et al., 2017).

The venous circulation is divided into the deep venous system, which includes the deeper cerebral veins, and the superficial venous system, which encompasses the cortical veins. Ultimately, both drain into the dural sinuses. The dural venous sinuses are

responsible for collecting venous blood from the veins and delivering it to the systemic venous circulation (Abdalkader et al., 2023; Chandra et al., 2017).

1.2.4. Cerebral Blood Perfusion

Brain function depends on the close relationship and coordination of responding to metabolic needs, supplying oxygen and nutrients, and removing waste products resulting from neuronal activity. Proper brain function relies on continuous regulation of cerebral blood flow (CBF), vascular reactivity to vasoactive stimuli, and the response of CBF to local changes in neuronal activity (Claassen et al., 2016).

Chemoregulation

The blood perfusion of the human brain is controlled by various systems, including chemoregulation. Chemoregulation involves vascular responses to changes in CO_2, oxygen, and blood pH levels. Hypercapnia and hypocapnia (increased and decreased CO_2 levels in the blood, respectively) lead to significant changes in cerebral blood flow (CBF) through vasodilation in response to elevated CO_2 levels (increasing CBF) and vasoconstriction in response to decreased CO_2 levels (decreasing CBF) (Madureira et al., 2017), A decrease in blood pH causes vasodilation, while an increase in pH causes vasoconstriction, also affecting CBF. Although changes in oxygen levels have the least impact on CBF, low oxygen levels can trigger compensatory mechanisms to increase CBF (Claassen et al., 2021).

CO_2 reactivity is the most extensively studied cerebrovascular response in humans, having been investigated under various physiological and pathological conditions. In patients with neurodegenerative and vascular cognitive impairment, previous studies have shown alterations in CO_2 reactivity. In patients following a stroke, impaired CO_2 reactivity appears to be associated with worse functional outcomes. Previous research has identified gaps in knowledge and inconsistencies regarding the association of CO_2 reactivity with the acute phase of CVD (Sforza et al., 2022).

Cerebral Autoregulation

Cerebral perfusion pressure is determined by arterial pressure (BP), intracranial pressure, and venous pressure. In healthy individuals, cerebral perfusion pressure values fall within the range of 50-150 mmHg, resulting from the mean arterial pressure (60-160 mmHg) minus the intracranial pressure. Under physiological conditions, cerebral perfusion pressure is primarily determined by BP and body position, as intracranial and venous pressures exhibit almost negligible variations (Armstead, 2016; Claassen et al., 2021). Cerebral autoregulation is a homeostatic mechanism that regulates and maintains

CBF constant despite fluctuations in BP (Armstead, 2016; Azevedo & Castro, 2016; Castro et al., 2018; Nogueira et al., 2021; Schaeffer & Iadecola, 2021).

Cerebral autoregulation primarily occurs through myogenic mechanisms, but also involves neurogenic, metabolic, and endothelial mechanisms that help maintain a constant CBF (Schaeffer & Iadecola, 2021). Smooth muscle contracts or dilates in response to changes in transmural pressure. There is extensive nerve supply to cerebral vessels through receptor activation, and metabolic changes in the environment, such as increased H^+ levels, can contribute to vasodilation. Additionally, endothelial factors like nitric oxide (NO) also play a role in cerebral autoregulation (Armstead, 2016; Claassen et al., 2021; Xiong et al., 2017).

Previous studies show that this mechanism remains preserved in healthy elderly individuals and in patients with hypertension. Most studies on autoregulation in patients after a stroke indicate that autoregulatory mechanisms are impaired following both ischemic and haemorrhagic strokes; however, there are stroke survivors who maintain preserved autoregulation. Impaired autoregulation after a stroke has been significantly associated with poorer functional recovery, suggesting that autoregulation should be considered in the management of these patients. Although there are currently gaps in the understanding of the pathophysiology of autoregulation in humans, this homeostatic mechanism has been extensively studied in patients following a stroke (Castro, Azevedo, et al., 2017; Castro et al., 2018; Castro, Serrador, et al., 2017; Nogueira et al., 2021)

Neurovascular Coupling

Neurovascular coupling (NVC) allows an interplay between neuronal activity and cerebral blood flow and is one of the most distinctive properties of cerebral circulation. The modulation of neuronal activity causes changes in local blood flow to ensure appropriate blood distribution. Therefore, increased neuronal activity leads to an increase in cerebral blood flow only in the activated area (functional hyperaemia). Functional imaging allows this activity to be assessed in spatial and temporal terms, as the spatial and temporal association between neuronal activity and the hemodynamic response is sufficiently precise (Schaeffer & Iadecola, 2021).

Cerebral perfusion is closely linked to cerebral metabolism and neuronal activation, with the primary role of NVC being the delivery of oxygen and glucose to active cells. NVC also plays a role in immune trafficking, the removal of waste products resulting from neuronal activity (such as lactate, CO_2, beta-amyloid peptide, and tau), regulation of body temperature, and proteostasis (Iadecola, 2017).

Various cells participate in NVC and perform different functions, as do the different types of blood vessels. Capillaries and arterioles have the greatest impact on NVC. Capillaries are where the main substance exchanges occur, while arterioles play a key role in modulating blood flow. Venules are responsible for removing metabolic waste products but do not play a primary role in blood flow modulation (Iadecola, 2017; Kugler et al., 2021).

Neurons initiate the local vascular response and regulate cerebral blood flow through signals that act on local blood vessels. Astrocytes, glial cells involved in NVC, serve as mediators due to their close relationship with synapses and local microvessels. Endothelial cells, pericytes, and smooth muscle cells also participate in NVC. These cells are associated with different types of vessels and influence vascular tone (Iadecola, 2017; Kugler et al., 2021). Due to the importance of each cell type in the NVC process and consequently in proper brain function, the neuro-glial-vascular unit has increasingly been recognized as a significant biomarker in cerebrovascular diseases (Kugler et al., 2021).

NVC effectively describes the brain's vasoregulatory mechanism, and previous studies provide potential pathophysiological explanations for the mechanisms involved in neurovascular coupling (Shabir et al., 2018). The proposed "feedback" mechanism to demonstrate this relationship is based on the vasodilatory capacity of byproducts resulting from neuronal activity, such as adenosine, CO_2, H^+, and lactate, and their potential to initiate the local blood flow response. This model assumes continuous adjustment of the system following a change to ensure homeostasis, meaning a better adjustment of blood flow in response to metabolic needs (Iadecola, 2017).

Previous studies have shown that the increase in cerebral blood flow exceeds the metabolic needs of the tissue, and this increase also occurs in situations where the tissue has an excess of O_2 and glucose, suggesting that this coupling may occur through a "feedforward" mechanism. The release of vasoactive substances that signal blood vessels to adjust their diameter is a consequence of glutamate release during synaptic activity. This glutamate release activates postsynaptic glutamate receptors, which in turn leads to the activation of Ca^{2+}-dependent signalling pathways. The local vascular response in arterioles and capillaries, driven by the release of vasoactive substances such as K^+, NO, and prostanoids, occurs independently of metabolism (feedforward). This indicates a preparatory adjustment of the system to the change, increasing blood flow in response to neuronal activity (Gonçalves et al., 2024; Goncalves et al., 2022; Iadecola, 2017; Schaeffer & Iadecola, 2021).

These two models may not be mutually exclusive. Microvascular studies have shown that a reduction in O_2 and/or glucose can precede the increase in cerebral blood flow. Although baseline O_2 levels vary across different regions, regional hypoxia promotes local vasodilation, depending on the local vascular topology and the activating stimulus (Iadecola, 2017).

Figure 5 demonstrates the regional increase in cerebral blood flow velocity (CBv). Following the presentation of a visual stimulus, an increase in CBv was observed in the PCA artery, with no increase in the MCA. In the observed functional hyperemia, neurons initiate this increase in CBF. Sustained increases in neuronal activity that lead to increases in CBF are highly restricted to the activated network.

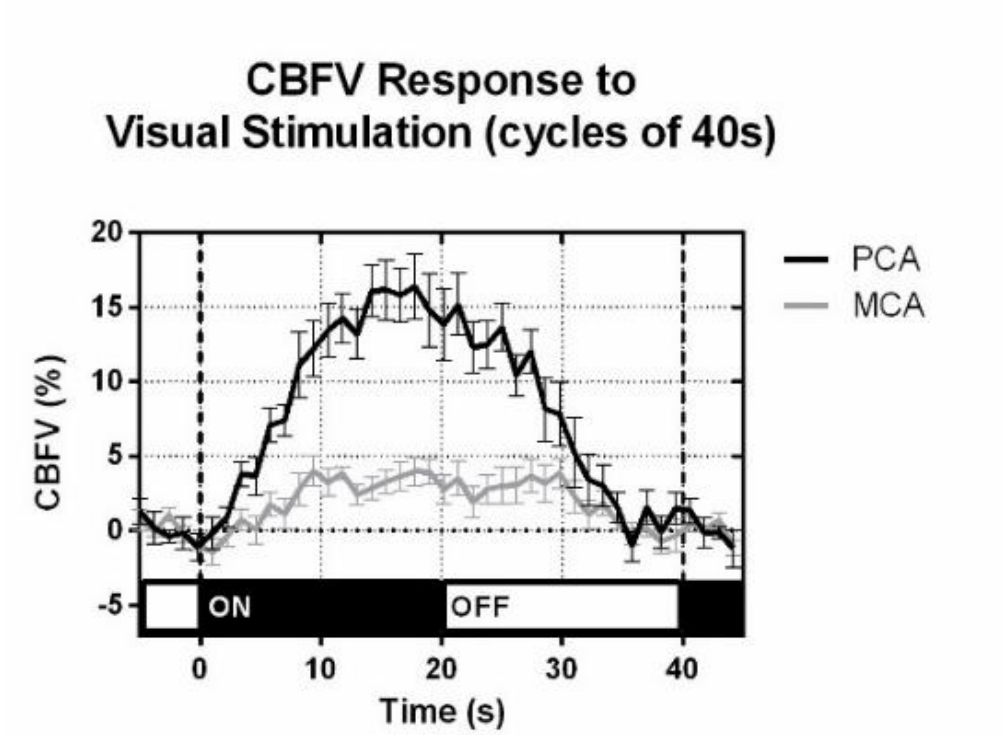

Figure 5. Variation of the percentage of cerebral blood flow velocity in the PCA in response to a visual stimulus 20s ON-20s OFF (Ferreira et al., 2024).

In response to neurovascular signalling, the feedforward mechanism may trigger an exaggerated blood flow response, but feedback mechanisms can help adjust blood flow to the actual metabolic needs. The vascular response tends to peak initially and then readjust to a lower level (Iadecola, 2017). Figure 6 shows an example of the hemodynamic response measured by Functional Transcranial Doppler (fTCD) in the P2 segment of the PCA in a healthy individual. In five cycles of visual stimulation, the peak

CBv was reached at 20 seconds into the first cycle, after which the increases were readjusted to a lower level.

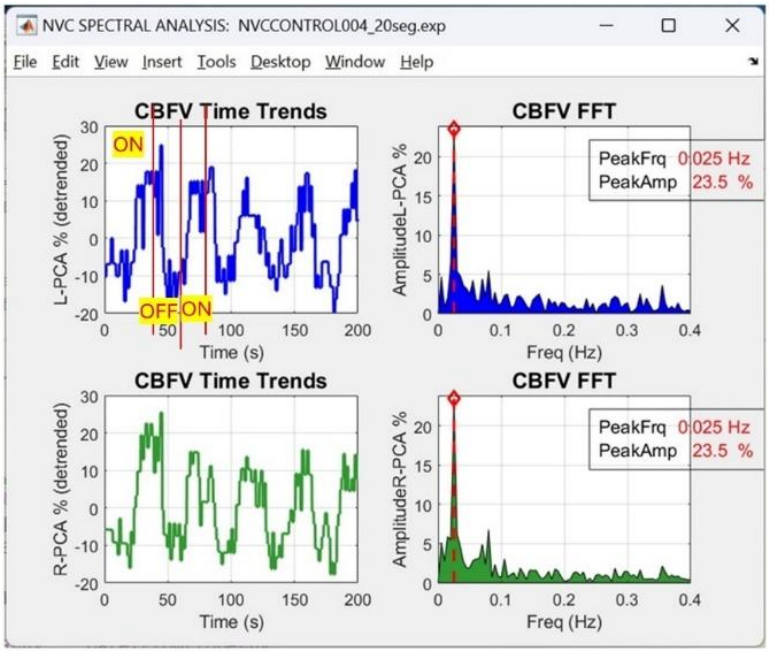

Figure 6. Example of a Healthy Control: Variation of the percentage of cerebral blood flow velocity in the PCA in response to a visual stimulus 5 cycles 20s ON-20s OFF. Image produced by the author.

Both feedback and feedforward mechanisms may be involved in the functional hyperemia observed. Functional hyperemia depends on the intensity and duration of the stimulus, as well as the brain region and developmental stage of the brain. It is important to note that this functional hyperemia differs in the developing brain (Schaeffer & Iadecola, 2021).

In recent decades, various studies have focused on the mediators of neurovascular coupling and their roles in the process: ions and neurotransmitters, metabolic mediators, gases, peptides and proteins, glial cells, vascular endothelium, and other mediators. Throughout the cerebrovascular tree, structural differences are observed, suggesting that each vascular segment plays a different role in regulating cerebral blood flow (CBF). The local response of the microvessels surrounding the activated area occurs in coordination with the upstream response in the larger arteries (Iadecola, 2017; Kugler et al., 2021; Schaeffer & Iadecola, 2021).

Capillaries are closer to neurons than arteries and influence cerebral blood flow (CBF) by changing their diameter. The release of extracellular K+ and increased O2 consumption occur because of synaptic activity. The release of K+ causes hyperpolarization of endothelial cells, which propagates retrogradely. Simultaneously,

the reduction in O2 leads to an increase in red blood cell deformability (see Figure 5). The increased deformability of red blood cells reduces blood viscosity, which in turn increases capillary flow velocity (Iadecola, 2017).

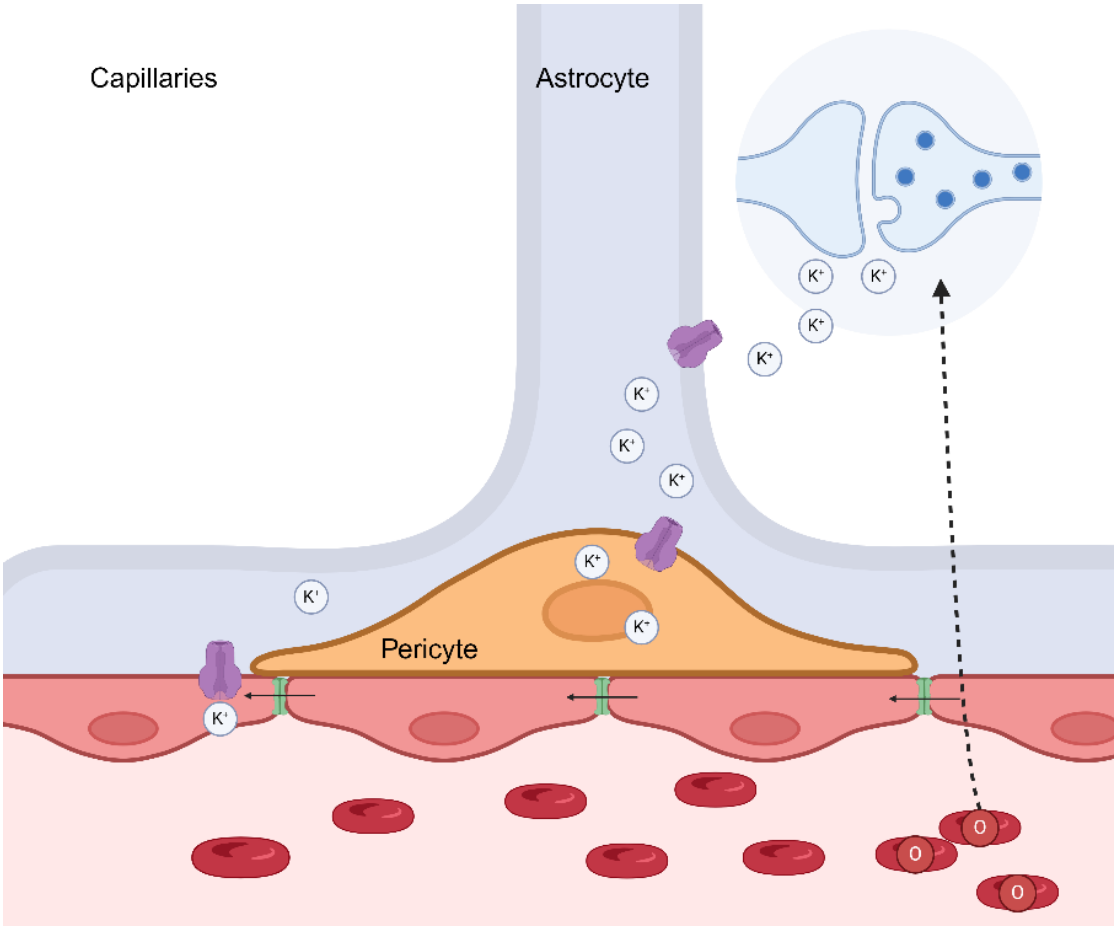

Figure 7. Neurovascular Coupling (Created with BioRender.com).

Capillaries are uniquely positioned to capture signals from both neurons and astrocytes. Despite their limited ability to directly regulate vascular resistance, capillaries detect neuronal activity and transmit these signals to the mural cells (Iadecola, 2017).

NVC is a marker of healthy brain function, and its dysfunction is associated with various pathologies (Schaeffer & Iadecola, 2021). Previous studies have demonstrated NVC dysfunction in patients with more severe markers of small vessel disease, age-related NVC dysfunction, hypertension, persistent reduced ejection fraction and cardiac output, diabetes mellitus, Alzheimer's disease, cerebral amyloid angiopathy, autosomal dominant cerebral arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), and peripheral artery disease (Aires et al., 2020; Iadecola & Gottesman, 2019; Jokumsen-Cabral et al., 2019; Monteiro et al., 2021; Mukli et al., 2024; Owens et al., 2022; Ruan et al., 2023; Schaeffer & Iadecola, 2021; Yang & Webb, 2023). There is growing evidence of the association between NVC, and vascular cognitive impairment

observed in patients with small vessel disease (Monteiro et al., 2024; Yang et al., 2022; Yang & Webb, 2023).

In patients following a stroke, changes in hemodynamic responses are also observed compared to healthy individuals (Claassen et al., 2021; Schaeffer & Iadecola, 2021). The cause of these changes is still unclear, meaning it is uncertain whether these alterations reflect cerebral physiology or are solely due to the vascular injury occurring in this context. Recent studies suggest using hemodynamic measures after a stroke to aid in predicting functional and behavioural outcomes, based on observations of NVC in the subacute phase of stroke in animal models (Sunil et al., 2023).

In humans, there is also evidence of hemodynamic changes following a stroke. Previous studies have shown that increased cerebral blood flow in regions surrounding the brain lesion and in the contralateral hemisphere can lead to improvements in brain function. Differences in NVC values have been observed between healthy individuals and stroke patients. However, gaps remain in understanding the regulation of cerebral blood flow after stroke, the influence of pathological subtype on NVC values, the predictive capacity of NVC for long-term functional outcomes, and the implications for early intensive rehabilitation and pharmacological strategies. There are also many inconsistencies in cerebral blood flow behaviour over time and between hemispheres (Salinet, Haunton, et al., 2013; Salinet et al., 2019; Wang et al., 2021).

2. MECHANISMS OF COGNITIVE DECLINE IN CEREBROVASCULAR DISEASES

Cognitive impairment after a stroke (PSCI) is a common, highly prevalent, and disabling effect for stroke survivors. After a stroke, about 60% of survivors experience and report memory and reasoning problems. Of these patients, about 20% with mild PSCI fully recover their pre-stroke cognitive function; however, it is more common to maintain long-term cognitive impairment (El Husseini et al., 2023; Quinn et al., 2021).

Despite the relevance of PSCI, high rates of underdiagnosis are observed in clinical practice. Assessing cognitive function in post-stroke patients requires careful attention, as it is necessary not only to evaluate the patient's cognitive symptoms but also to understand the impact on daily activities and monitor its progression over time. This assessment should cover multiple cognitive domains, and thus, the MoCA test is widely recommended and used in clinical settings (Biesbroek & Biessels, 2023; Quinn et al., 2021).

CVD are highlighted as a cause of disability and cognitive impairment. Evidence gathered from clinical, pathological, and neuroimaging studies shows that cognitive impairment arises because of cerebrovascular lesions or reduced cerebral blood perfusion. Blood perfusion varies according to the type and size of the vessel where the brain lesion occurs, the origin and cause of the vascular occlusion, and the integrity of the vessel wall components, which consequently affect the severity of cognitive impairment. Vascular cognitive impairment thus encompasses a spectrum of disease severity ranging from mild cognitive impairment to dementia (Clancy et al., 2024; Kalaria, 2012; Stefano Govoni et al., 2020).

The relationship between CBF and cognitive impairment is not a recent discovery, but there is still a need to develop non-invasive and easy-to-apply methods to facilitate the study and understanding of the vascular contribution to brain health. Recent studies show that most dementias in the elderly are mixed dementias and that vascular dysfunction precedes cognitive decline in Alzheimer's disease (Clancy et al., 2024).

The multiple observations from neuroimaging and pathological studies have enabled a better understanding of the location of lesions and their contribution to cognitive decline. The classification into vascular dementia and vascular cognitive impairment requires cognitive, functional, and neuroimaging assessments. However, the timing of the neuroimaging evaluation presents an additional challenge, as small infarcts may disappear during the subacute phase and reappear later; they may not be visible or may appear only transiently (Clancy et al., 2024; Rundek et al., 2022).

Changes in the brain parenchyma can affect cognition and other brain functions. These changes vary due to the presence of haemorrhages that cause direct damage to the parenchyma, the arterial territory compromised by the lesion, the location of the lesion (cortical or subcortical), the size of the lesion, the number of lesions, and the hemisphere of the brain where the lesion occurs (Kalaria, 2012; Wong & Chui, 2022). The presence of vascular risk factors such as hypertension and diabetes mellitus can exacerbate the damage caused by brain injury. At the level of small cerebral vessels, arterioles may undergo changes that result in cognitive and functional impairment, such as thickening of the inner layer of the arterioles, which restricts blood flow, tissue cell death that leads to scarring and stiffening of the vessels, and the deposition of hyaline material (proteins) on the vessel walls (Rundek et al., 2022)

Age, genetic factors, environment, and lifestyle contribute to the development of vascular risk factors and CVD. Cardiovascular risk factors are fundamental in the pathogenesis of cognitive impairment and vascular dementia, through dysfunction of cerebral blood

flow and neuronal networks. These risk factors cause cerebrovascular diseases that lead to lesions resulting in cognitive impairment. Therefore, the best treatment for these conditions involves the early mitigation of cardiovascular risk factors. Smoking, hypertension, diabetes mellitus, and physical inactivity are the cardiovascular risk factors with the greatest impact on cognitive impairment. The process of neurodegeneration underlying the interaction of genetic and environmental risk factors is counterbalanced by individuals' cognitive and functional reserve and resilience (such as education, occupation, among others). As a result, the mechanisms contributing to cognitive decline are not well understood (Rundek et al., 2022; Wong & Chui, 2022).

Type 2 diabetes mellitus (T2DM) is associated with cognitive decline and dementia. Patients with prediabetes show no difference in global cognition, three to six months after the stroke, compared to patients with normal blood glucose levels (Lo et al., 2020). T2DM and impaired renal function are independently associated with poor performance in cognitive tests after stroke. Both conditions are associated with abnormal brain structure and when they coexist it quadruples the risk of cognitive decline (Ben Assayag et al., 2017). Blood pressure is associated with risk of vascular dementia (Emdin et al., 2016). Recent studies show that hypertension and prehypertension are related to a decrease in the global cognition score (Blevins et al., 2021; de Menezes et al., 2021). People assessed and diagnosed at the age of less than 55 years old showed lower scores on the memory parameter and people aged 55 years old or more showed worse results in verbal fluency (de Menezes et al., 2021).

Numerous cerebrovascular diseases cause vascular cognitive impairment and dementia, including small vessel disease, large artery atherosclerosis, brain haemorrhages, cardioembolism, and other types of stroke. The types of cerebrovascular pathologies and different vascular risk factors affect distinct regions of the cerebrovascular tree (Wong & Chui, 2022).

Cerebrovascular changes can occur in either the large or small blood vessels of the brain. Embolic events in large vessels include atherosclerosis, which is the formation of fibrofatty lesions on the walls of the arteries; plaque rupture, where the release of lipid material into the bloodstream can trigger thrombus formation; intraplaque haemorrhage, which is bleeding within atherosclerotic plaques that increases their size and makes them more prone to thrombus formation; thromboembolic occlusion, which is the blockage of a distant artery by a thrombus that has formed; embolism, which is the obstruction of a smaller vessel caused by a fragment that has broken loose into the bloodstream; and arterial dissection, where tearing of the artery can generate thrombi and subsequent

embolisation. These conditions can lead to macroinfarcts (Kalaria, 2012; Libby et al., 2019; Wong & Chui, 2022).

Stenosis, or arterial narrowing, due to atherothromboembolism occurs in cortical and subcortical regions and predominantly in vessels of the Circle of Willis. Arterial disease is considered more significant for cognitive impairment than venous disease (Kalaria, 2012). Heart failure, ischaemic heart disease, and atrial fibrillation have been associated with vascular cognitive impairment. Due to thromboembolic risk, association with small vessel disease, vascular inflammation, and genetic factors, atrial fibrillation is an important cause of cognitive impairment. The mechanism by which carotid stenosis causes cognitive impairment is not fully understood, but patients with carotid stenosis more frequently exhibit microembolic signals (Rundek et al., 2022).

The presence of cerebral microembolic signals (MES), which are platelet-fibrinogen embolic particles, is associated with clinical deterioration in patients and cognitive changes (Das et al., 2020). Studies in patients undergoing cardiovascular surgeries have shown a correlation between the number of MES detected and the results obtained in neuropsychological tests (C. J. Lin et al., 2022).

Previous studies have shown the association of cerebral microembolism as a cause of silent ischemic cerebral infarctions, contributing to cognitive decline (Goldberg et al., 2012). Patients with MES, mainly in greater numbers, are patients with a higher risk of stroke. The presence of MES in acute phase of stroke is little known, namely its role in relation to prognostic capacity (Bazan et al., 2020; Farina et al., 2018). Microembolic signs are indicated as predictive of new embolic events, but there is no relationship with worse clinical results at 90 days. There is no data to show whether the MES can predict long-term cognitive decline (Das et al., 2020; Sheriff et al., 2020). Therefore, prospective clinical studies are required to determine whether MES can lead to cognitive impairment.

Cognitive impairment commonly arises in association with microinfarcts or lacunar strokes, due to small vessel lesions and diffuse changes in the white matter. In response to variations in blood pressure and the loss of autoregulation, caused by disruption of the nerve plexuses, there is a decrease in the tone and elasticity of the walls of small vessels in the deep white matter and basal ganglia. This reduction in vessel elasticity leads to pronounced fluctuations in cerebral perfusion, affecting the deeper brain structures (Kalaria, 2012; Wong & Chui, 2022).

After a stroke, events such as cellular apoptosis, autophagy, and necrosis occur. The severity of the lesions varies depending on the occurrence of these processes, with autophagy and apoptosis potentially preventing inflammatory processes, whereas

necrosis can cause inflammation and damage to surround tissues. Other variables that influence the severity of the outcome include the volume of the lesion and the penumbra zone, the presence of oedema, neurons with condensed nuclei and cytoplasm indicating irreversible damage, dilated axons, and shrunken oligodendrocytes, which indicate cell death (Kalaria, 2012; Rost et al., 2021).

Lesions in strategic regions, such as the thalamus, angular gyrus, and the basal ganglia, can lead to cognitive impairment. In patients after a stroke, transient cognitive impairment may occur, which can be reversible to the pre-stroke state. This cognitive impairment does not include cases of post-stroke depression, which has a high prevalence (Rundek et al., 2022).

The endothelium of cerebral vessels and capillaries are vulnerable and can be chronically activated. In addition to haemodynamic events, the disintegration of the arterial wall can impair the BBB, and the uncontrolled passage of fluids and macromolecules over time will trigger an inflammatory response with an increase in perivascular neutrophils, lymphocytes, or macrophages (see section 2.1). Dysfunction of the neurovascular unit critically contributes to cognitive decline and neurodegenerative diseases (Kalaria, 2012; Rundek et al., 2022).

The process of cognitive impairment and dementia is also associated with cerebral haemorrhages, intracerebral haemorrhages, and microhaemorrhages. Studies have shown that microhaemorrhages are linked to cognitive impairment in patients even without other vascular risk factors. The mechanism behind this is not well understood, but it is suggested that it is due to disruption of neural network connectivity, making it less efficient (Rundek et al., 2022).

Cerebral Amyloid Angiopathy (CAA) is an independent factor in cognitive impairment, being a cause of both intracerebral and lobar haemorrhages. The accumulation of β-amyloid protein within or adjacent to the vasculature can lead to the degeneration of larger perforating arteries and cerebral capillaries. Cortical microinfarcts may occur as a consequence of decreased vascular integrity and hypoperfusion, which in turn leads to reduced perivascular clearance of β-amyloid protein. CAA has been associated with impairment in semantic memory and visuospatial skills (Rundek et al., 2022; Wong & Chui, 2022).

Subcortical leukoencephalopathy, the degeneration of the brain's white matter, is a common alteration in vascular dementia. Communication between cortical and subcortical regions of the brain is compromised by damage and loss of myelin and axons in the white matter. White matter degeneration arises from myelin loss and abnormalities

in axons, which result from vascular insufficiency and chronic hypoxia associated with conditions such as chronic hypertension and atherosclerosis (Kalaria, 2012).

2.1. Neuroinflammation and Oxidative Stress

Neuroinflammation results in the production of pro-inflammatory cytokines, chemokines, complex cytokines, and some molecular messengers, such as prostaglandins, NO, and reactive oxygen species (ROS), in response to pathological damage occurring in the central or peripheral nervous system. The inflammatory reaction acts as a defence against infections, injuries, or other stimuli affecting the nervous system. However, when this reaction becomes chronic, it leads to a pro-inflammatory response through the continuous release of inflammatory cytokines, which exacerbates inflammation and damages neurons (Wang et al., 2023).

The production of inflammatory cytokines is significantly higher in microglial cells compared to other glial cells. The role of microglia has been increasingly studied, particularly in Alzheimer's disease, as it is associated with memory difficulties, synapse loss, and tau protein phosphorylation. In stroke, neuroinflammation occurs during both the acute and chronic phases, being a critical component of its pathophysiology. The inflammatory response can exacerbate brain damage and hinder tissue recovery, which negatively impacts cognitive and functional recovery in patients following a stroke (Alsbrook et al., 2023; Wang et al., 2023).

Immediately following the injury, microglia activation occurs, leading to morphological changes and the secretion of cytokines. Astrocytes take on an active role in the immune response after brain injury by recruiting peripheral immune cells and secreting cytokines and chemokines. They also play a protective role for neurons during the acute phase of ischemia (Alsbrook et al., 2023; Wang et al., 2023).

The release of pro-inflammatory cytokines increases the permeability of the BBB due to the damage they cause to the tight junctions between endothelial cells. This increased permeability allows peripheral immune cells, such as leukocytes and neutrophils, as well as plasma proteins, to enter the brain, which in turn exacerbates the inflammatory process. The increase in ROS resulting from ischemia also damages the tight junctions of endothelial cells, further contributing to the heightened permeability of the BBB. Consequently, the increased permeability of the BBB leads to an influx of inflammatory mediators and ROS. This cyclical process acts as a catalyst for neuronal degeneration, as it continuously exposes the brain to toxic products (Figure 8) (Paul et al., 2021; Shi et al., 2019; Wang et al., 2023).

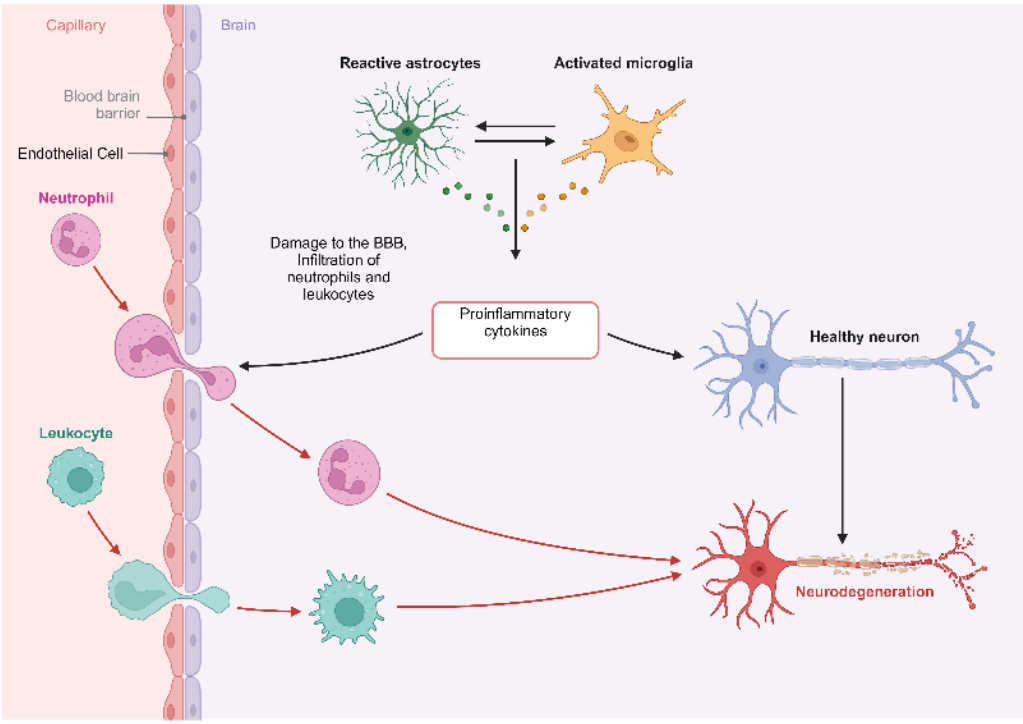

Figure 8. Neurodegeneration (Created with BioRender.com).

The disintegration of the BBB and the infiltration of leukocytes into ischaemic brain tissue initially occur as a consequence of the intravascular inflammatory process. BBB dysfunction is one of the main events in this pathophysiological response and can persist for days or weeks following a stroke. The permeability of the BBB increases within the first 6 hours after the stroke due to the sudden hypoxia that occurs and the early rupture of the BBB. Between 72 and 96 hours afterwards, the neuroinflammatory process further increases permeability. The peak of permeability observed during this phase is particularly due to reperfusion. This increased permeability can last for weeks after the stroke and may be associated with neurogenesis and neovascularization (Bernardo-Castro S, 2023; Bernardo-Castro et al., 2020).

Acute deprivation of oxygen and glucose that occurs immediately after a stroke result in increased oxidative stress. The oxidative stress and its deleterious effects on the patient also arise from reperfusion in tissues that have previously experienced ischaemia; the increased oxygen supply also elevates the production of ROS. However, recanalization strategies remain the primary treatment following a stroke (Jurcau & Simion, 2021).

The focal inflammation that occurs during the acute phase is responsible for the exacerbation of brain injury. Excitotoxicity, which involves excessive calcium influx into

cells due to increased glutamate levels, causes mitochondrial damage and cell death; direct cytolysis, where activated immune cells release enzymes and free radicals that directly destroy brain cells; oxidative stress, marked by elevated ROS that damage lipids, proteins, and DNA in brain cells; and thrombo-inflammation, characterized by clot formation and immune system activation, all occur during the acute phase of stroke and lead to microvascular dysfunction, increased oedema, and consequently worsen the functional and cognitive recovery of patients (Jurcau & Simion, 2021).

Previous studies in animal models and humans suggest that immune responses occur in areas distant from the site of injury. Global brain inflammation following a stroke occurs in both the acute and chronic phases and presents components similar to focal brain inflammation. Glial cells, such as microglia and astrocytes, react to damage in the myelin, meaning these cells can be activated by damage to the white matter in remote locations. Extensive lesions in the white matter occur as a result of both retrograde and anterograde degeneration following a stroke. In the first 24 hours after the event, diaschisis may still occur, which is the functional loss of distant brain regions due to the interruption of neural pathways connecting these areas to the site of injury (Shi et al., 2019)

2.2. Endothelial disfunction

Endothelial cells line the cerebral microvessels and are involved in the regulation of the BBB (Abbott et al., 2006). The endothelium is a highly selective barrier and a metabolically active organ, responsible for regulating vascular tone, cell formation, and interactions with leukocytes, platelets, and the arterial wall (Park & Park, 2015; Ray et al., 2023).

Under normal conditions, nitric oxide released by the endothelium promotes vasodilation, inhibits platelet aggregation, inflammation, and oxidative stress. A reduction in nitric oxide production or an imbalanced distribution of vasodilation and vasoconstriction by endothelial cells are characteristics of endothelial dysfunction. The underlying pathophysiology of endothelial dysfunction and its relationship with various pathologies has been the subject of study, particularly its association with cerebrovascular diseases and cardiovascular risk factors such as diabetes and hypertension (Pacholko & Iadecola, 2024; Ray et al., 2023).

2.3. Neurodegeneration

Inflammation is a critical mechanism responsible for the progression of neurodegeneration, serving as a common factor in various neurodegenerative diseases.

The various metabolic alterations lead to a chronic inflammatory state, which consequently results in cell death (Figure 8) (Andjelkovic et al., 2023; Shi et al., 2019).

Microvascularisation and the complex pathogenesis of numerous CNS diseases play fundamental roles in the neurodegenerative process. In both stroke and Alzheimer's disease, the cascade of events related to BBB dysfunction, endothelial cell changes, and oxidative stress leads to the loss of cognitive function and other basic brain functions (Gallego et al., 2022).

3. TRANSCRANIAL DOPPLER AND FUNCTIONAL STUDIES

Transcranial Doppler (TCD) is a non-invasive method that is robust and has high temporal resolution. Compared to other neuroimaging techniques used to assess cerebral blood flow, this method is more accessible and easier to use; however, it does not allow for direct observation of neuroanatomy (Egger et al., 2021; Wan et al., 2022). TCD is commonly used in the study of cerebral haemodynamics, including the investigation of pathophysiological changes in various neurological and psychiatric conditions. The metrics that describe cerebral haemodynamics exhibit inconsistency in their terminology, which has been the focus of research in recent years (Egger et al., 2021; Skow et al., 2022).

The clinical utility of TCD has already been demonstrated, and it is widely used in intensive care units. TCD allows for the measurement of cerebral blood flow velocity beat by beat, enabling the assessment of cerebrovascular function. Cerebrovascular function can be studied through the collected values of systolic, diastolic, and mean blood flow velocity, as well as allowing for the calculation of other cerebral parameters (Skow et al., 2022; Titianova & Vastagh, 2016).

The measurement of cerebral blood flow by TCD is an indirect assessment using ultrasound waves that detect the linear velocity of red blood cells at a specific time and location within a cerebral blood vessel. This measurement is based on the assumption that changes in cerebral blood velocity are proportional to changes in cerebral blood flow, provided that the diameter of the insonated vessel remains constant (Skow et al., 2022).

Figure 9. Evaluation by Functional Transcranial Doppler (Photo taken by Audiovisual Resources Centre, FMUP - The author during the study data collection)

The ultrasound transducer is placed on the temporal window of the skull, where the bone is thinner and allows ultrasonic waves to penetrate. The high-frequency waves emitted by the transducer pass through the bone and return after reflecting off the red blood cells circulating in the vessels. TCD is operator-dependent, meaning that factors such as the insonation angle, penetration depth, sample volume, and response to specific stimuli influence the evaluator's selection of the blood vessel of interest (Skow et al., 2022; Titianova & Vastagh, 2016).

TCD offers numerous advantages, such as being portable, cost-effective, and easy to use. However, it requires specialized professionals, and approximately 10% of the healthy population has an insufficient temporal window, making them unsuitable for examination using this method. Nevertheless, TCD is a very useful tool for detecting microvascular dysfunction and is widely researched (Lohmann H, 2006; Titianova & Vastagh, 2016).

3.1. Study of Cerebrovascular Regulatory Mechanisms: Cerebral Autoregulation, Neurovascular Coupling, and Vasoreactivity

Cerebral autoregulation, neurovascular coupling, and cerebrovascular vasomotor reactivity are distinct regulatory mechanisms of cerebral blood flow (see 1.2.4. Cerebral Blood Perfusion).

The quantification of cerebral autoregulation (CA) in different physiological states may be essential and fundamental for clinical decision-making, especially if CA is

compromised. This quantification involves measurements of CBF and cerebral perfusion pressure (the difference between arterial pressure and intracranial pressure). Intracranial pressure is a stable value under normal conditions; therefore, only changes in arterial pressure are considered. CA plays a crucial role in brain function (Kostoglou et al., 2024).

The clinical and experimental protocols for assessing CA most commonly utilise TCD. This method allows for the measurement of changes in cerebral blood flow and, consequently, the velocity of blood flow in the main cerebral arteries. This measurement is performed simultaneously with the measurement of blood pressure (Azevedo & Castro, 2016; Castro et al., 2018; Kostoglou et al., 2024).

Classical studies of CA investigated static CA, which examines how cerebral blood flow remains constant despite gradual changes in blood pressure over hours or weeks. These studies demonstrated that for blood pressure values ranging from 60 to 150 mmHg, cerebral blood flow remains stable. However, rapid changes in blood pressure can affect this autoregulatory capacity (Kostoglou et al., 2024).

Dynamic cerebral autoregulation (dCAR) has greater clinical applicability and is therefore more commonly used, as it does not require continuous periods of blood pressure changes for assessment. dCAR can be evaluated by quantifying the relationship between blood pressure and CBv or cerebral perfusion pressure and CBv over shorter periods, through the induction of blood pressure changes (for example, rapid release of pressure cuffs on the thighs) or by using spontaneous fluctuations in blood pressure. TCD is one of the methods that allows for the evaluation of dCAR (Castro et al., 2018).

In the assessment of dCAR using TCD, Transfer Function Analysis (TFA) is widely employed to characterize dCAR. TFA considers dCAR as a linear, time-invariant system, with mean arterial pressure as the input and cerebral blood flow as the output. dCAR acts as a high-pass filter, not attenuating high-frequency oscillations (>0.2 Hz) but dampening slight oscillations in blood pressure. This calculation is performed using the Fast Fourier Transform to compute the power spectral density and cross-spectral density of the mean arterial pressure and cerebral blood flow signals. The Welch method is employed to enhance the estimation of the power spectral density and the transfer function, allowing for a detailed description of the relationship between the input and output of the linear system in terms of frequency. The transfer function aids in quantifying the system's behaviour, such as its ability to dampen or amplify pressure fluctuations and its impact on cerebral blood flow (Azevedo & Castro, 2016; Castro et al., 2018).

The interpretation of dCAR and its clinical applicability have been studied, with the most commonly used measures being phase, gain, and coherence. The phase parameter

indicates the temporal discrepancy between the oscillation of arterial pressure and the response of cerebral blood flow, ranging from 0 to 90 degrees—indicating a lack of autoregulation to preserved autoregulation. Regarding the coherence parameter, it shows the linear relationship between variations in arterial pressure and cerebral blood flow, varying between 0 and 1—indicating no relationship to a perfect correlation. A lower gain value reflects good autoregulation, as this parameter indicates the amount of variation in blood flow that results from a change in arterial pressure (Azevedo & Castro, 2016; Claassen et al., 2016)

Robust and reproducible measurements have been the subject of study, as despite the extensive literature on these topics, gaps still exist. The quality of the measurements varies according to the characteristics of the signal, the sampling rate, the length of the data, and the signal-to-noise ratio (Kostoglou et al., 2024).

To improve the collected signal, it is important to consider the following recommendations: the signal collected with spontaneous fluctuations of arterial pressure and cerebral blood flow for TFA analysis should last at least 5 uninterrupted minutes, discarding the first 10 to 30 seconds to achieve stable oscillations; the signal collection should take place in a noise-free environment with controlled temperature; participants should avoid caffeine, nicotine, alcohol, and chocolate for 12 hours prior to collection; monitoring both cerebral hemispheres is preferable; the signal should be visually inspected before analysis to ensure it is free from excessive noise and artifacts; the partial pressure of carbon dioxide ($PaCO_2$) should be evaluated; studies should include a properly matched control group to compare results and should provide detailed clinical and sociodemographic data of the sample (Panerai et al., 2023).

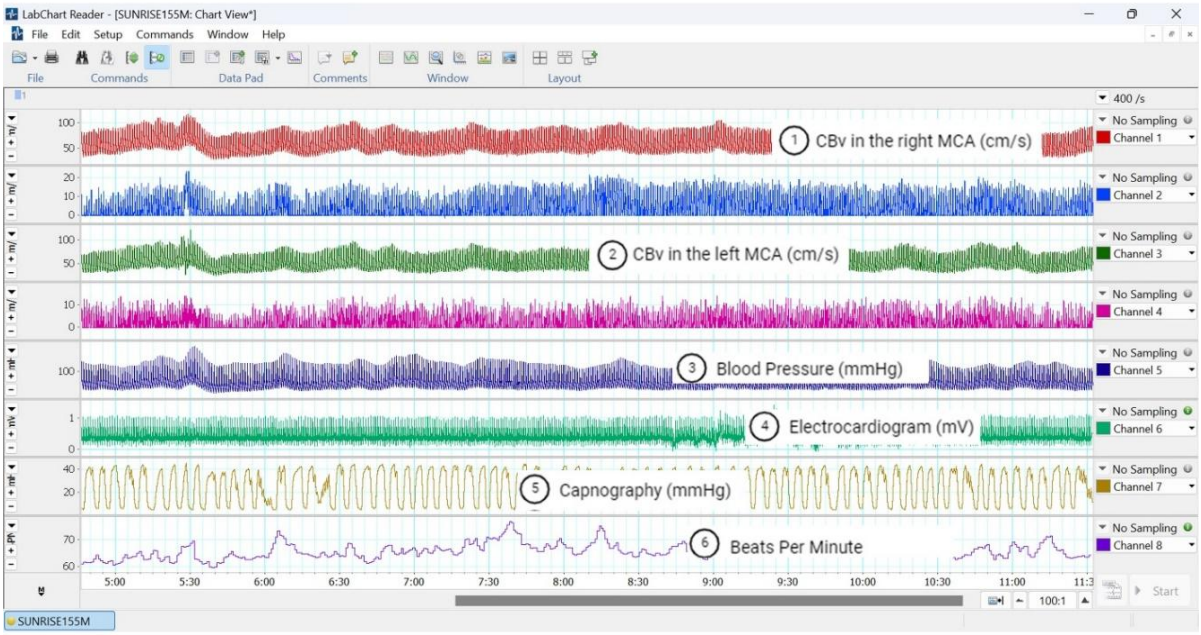

Figure 10. Analog signals from Powerlab® during supine rest. Data collected by the author. *Unpublished data.*

To assess dCAR with spontaneous fluctuations, a basic set of physiological measurements is necessary (see Figure 10). To obtain CBv in the arteries of both cerebral hemispheres, the most used method is TCD, as shown in Figure 10 (points 1 and 2). For measuring blood pressure (Figure 10 – point 3), a non-invasive and continuous arterial volume clamping method is typically employed (for example, Finometer®). Capnography (Figure 10 – point 5) allows for measuring the partial pressure of $PaCO_2$ based on the end-tidal carbon dioxide pressure during expiration.

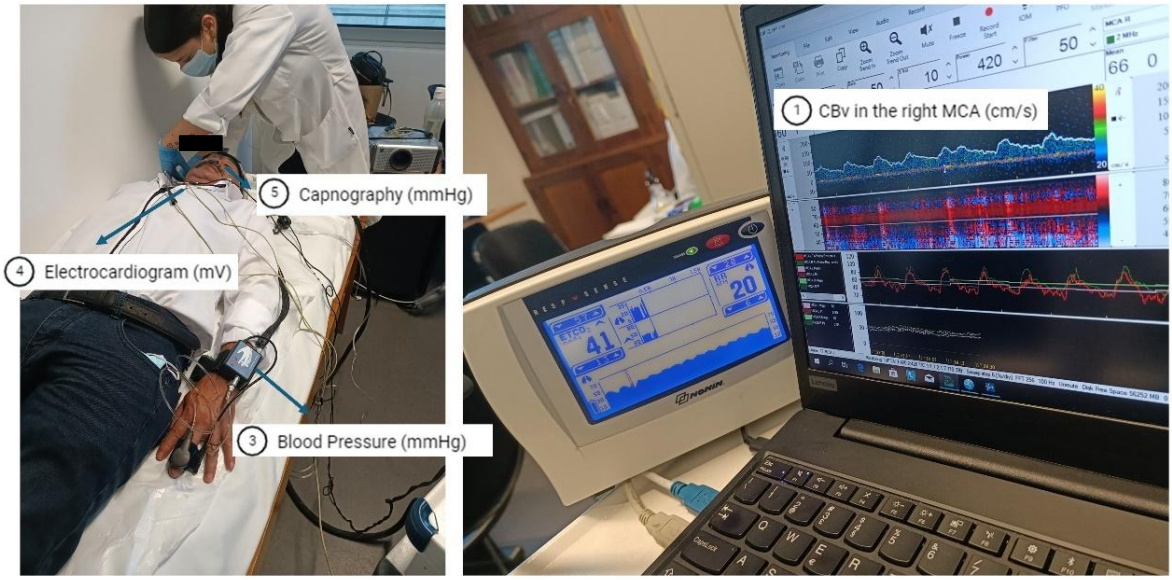

Figure 11. dARC measured by TCD. Data collected by the author. *Unpublished data.*

The effects of carbon dioxide on cerebral circulation play a significant role in autoregulation. The differences in cerebral blood flow between resting states and following hypercapnia reflect the state of cerebrovascular reactivity (VMR) (see Figure 12) (Gur et al., 2016).

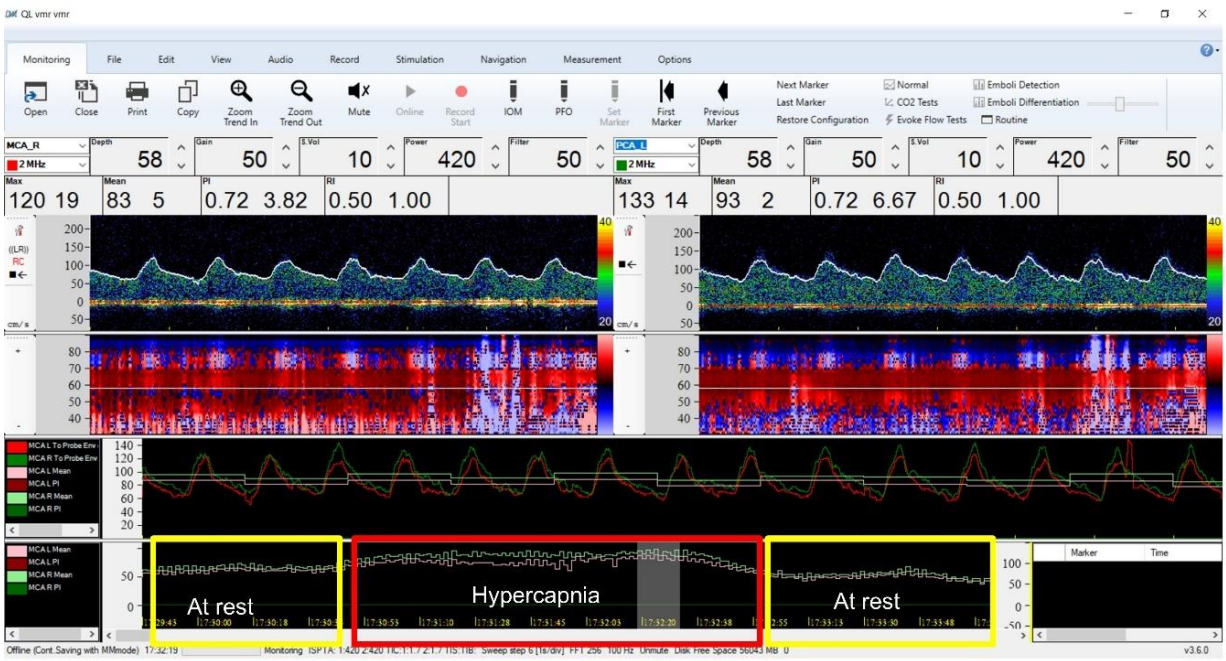

Figure 12. Cerebral Vasomotor Reactivity. Data collected by the author.

The VMR is the ability of the arteries to dilate in response to external stimuli, such as an increase in $PaCO_2$ and a decrease in extracellular pH. The VMR provides important data regarding the cerebral haemodynamic state, showing the difference in CBv before and after a vasodilatory stimulus (Gur et al., 2016). In Figure 11, an image of CBv at rest, during hypercapnia, and during hypocapnia, measured with TCD, is observable.

One of the most distinctive properties of cerebral circulation is the interaction between neuronal activity and cerebral blood flow, known as NVC. The modulation of neuronal activity induces changes in regional cerebral blood flow to meet the demands of the activated neuronal region; thus, this haemodynamic response is sufficiently precise to be assessed with TCD (Schaeffer & Iadecola, 2021). TCD allows for continuous measurement of CBv in the large cerebral arteries, including the PCA, MCA, and ACA. Its high temporal resolution enables the detection of differences in cerebral blood flow during functional changes in brain activity (Egger et al., 2021; Hage et al., 2021).

The initial studies of NVC using TCD focused on monitoring the PCA during visual stimulation, where significant changes in cerebral blood flow occur due to the activation of the visual cortex. More recently, there has been an increase in NVC studies utilizing TCD and the methods employed, particularly the monitoring of NVC in other arteries and brain regions, such as the MCA and ACA. This diversity of studied territories and corresponding stimuli has enhanced our understanding of the influence of various sensory modalities on cerebral hemodynamics (Hage et al., 2021; Klingelhöfer, 2016; Smirl et al., 2016).

Using TCD, various indices of blood flow can be obtained in the assessment of NVC. The peak systolic flow velocity describes the cerebral blood flow signal during systole, the end-diastolic flow velocity represents the lowest cerebral blood flow until the end of diastole, and the mean flow velocity reflects the average obtained throughout the cardiac cycle. In NVC studies, a very rapid increase in cerebral blood flow, known as overshoot, is typically observed, followed by a stabilization phase. The recording of cerebral blood flow with TCD should be continuous, and the envelope of the frequency spectra should be stored for subsequent analysis (Klingelhöfer, 2016; Rosengarten & Kaps, 2010).

The orthostatic position and a heart rate between 60-90 beats per minute do not affect functional haemodynamic responses. Overall, changes in blood pressure, heart rate, or ventilation do not influence haemodynamic responses in studies involving tactile or visual stimuli. In studies with more complex cognitive stimuli, alterations in blood pressure and respiration may occur, and an increase of 1 mmHg in $PaCO_2$ can result in a 5% increase

in CBv, influencing the collected data (Azevedo E, 2007; Klingelhöfer, 2016; Rosengarten & Kaps, 2010).

NVC studies using visual stimuli and measuring CBv in the PCA record greater variation in CBv, as the visual cortex is predominantly supplied by the PCA. Changes in CBv in the MCA and ACA associated with tasks show an average variation of up to 5%. Additionally, the functional heterogeneity of the cortex should be considered when tasks activate the territories supplied by the MCA and ACA (Klingelhöfer, 2016; Smirl et al., 2016).

A significant number of NVC studies use a visual stimulus to activate the territory supplied by the PCA. The visually evoked flow velocity responses typically follow a characteristic temporal course: after a slight initial delay, the CBv increases and exceeds the baseline level before stabilising at a constant level (Figure 13). In the OFF phase, following the end of stimulation, the CBv decreases with a delay of 2 to 3 seconds. During the ON phase, the overshoot (peak maximum) occurs around 8 to 12 seconds, and the CBv stabilises approximately between 10 and 20 seconds. Methodologically, it is advisable to monitor for about 5 seconds before initiating stimulation to ensure a stable initial CBv and to monitor a greater number of ON-OFF cycles to enhance the signal-to-noise ratio, thereby enabling the researcher to differentiate the increase in CBv resulting from the stimulus from spontaneous fluctuations (Klingelhöfer, 2016; Rosengarten et al., 2001).

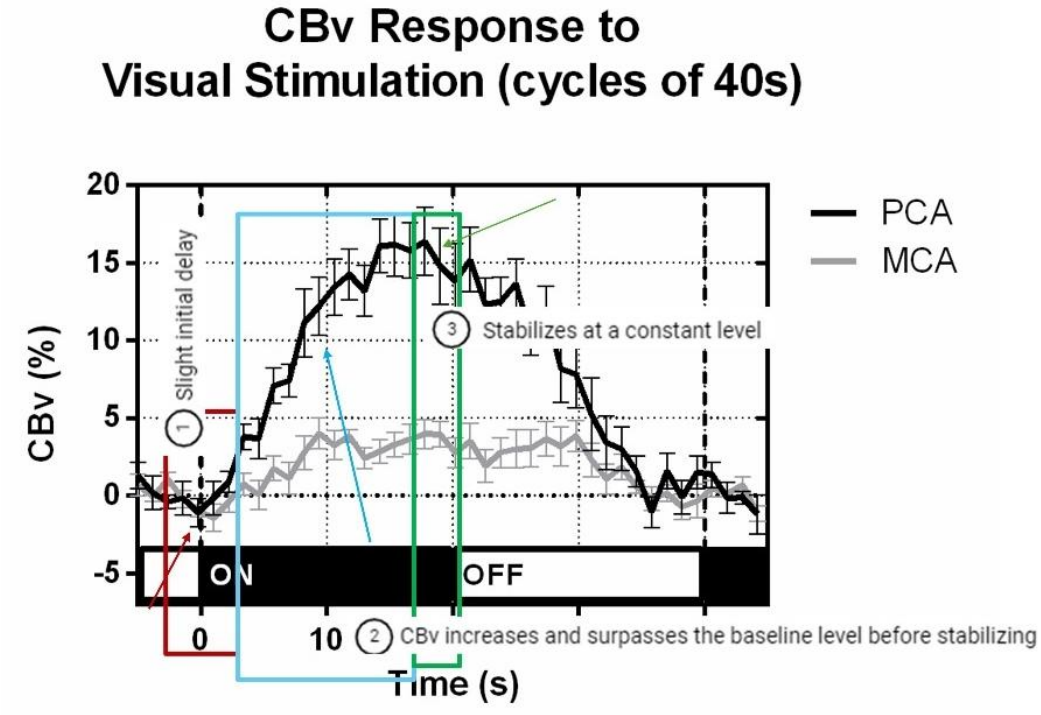

Figure 13. NVC measured by TCD. Data collected by the author.

TCD has been widely used to study NVC in various neurological conditions. Due to the characteristics of the technique, it has the potential to be employed in investigations involving large cohorts, encompassing individuals of all ages and those with reduced cooperative ability. TCD is regarded as the ideal instrument for functional testing in clinical settings to assess brain function; however, some gaps in its use still exist (Klingelhöfer, 2016). The intermittent checkerboard is the most common and widely used visual stimulus; however, it requires additional equipment to synchronize the event marker with CBv. (Rosengarten et al., 2001; Shabir et al., 2018) The need for these additional devices may discourage many researchers from adopting it, especially in clinical laboratories that only have TCD available and in more challenging settings for equipment installation, such as in intensive care units (Egger et al., 2021; Hage et al., 2021).

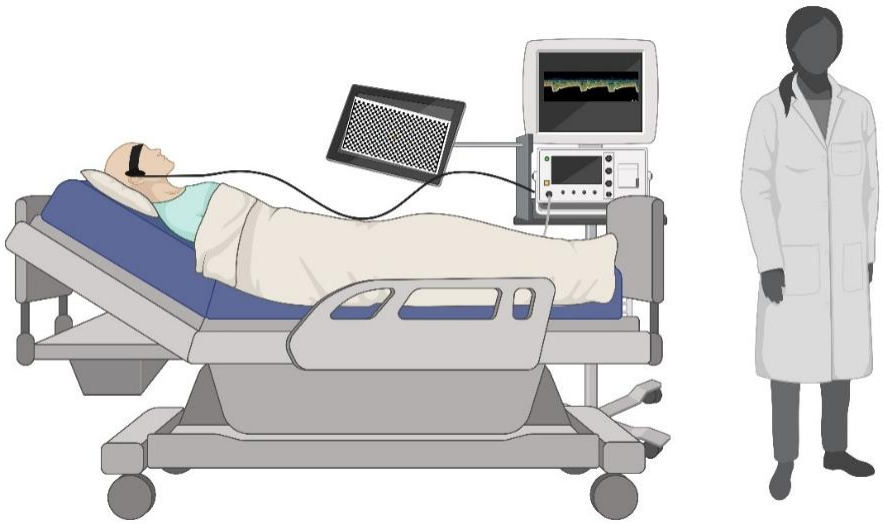

Figure 14. Assessment of NVC by TCD in the context of hospitalization (Created with BioRender.com).

3.2. MICROEMBOLIC SIGNALS

Microembolic signals (MES) correspond to the micro-embolic particles present within the cerebral arteries, which can be composed of gas or debris (Sudheer et al., 2021; Wan et al., 2022).

With TCD, it is possible to monitor MES in real time and continuously in cerebral circulation. This measurement is based on the detection of the backscattering of the emboli, which, being greater than the backscattering of the surrounding moving blood, appears as a short-duration, high-intensity transient signal in the TCD spectrum (Figure 15). In TCD evaluation, MES are identified as a short-duration, unidirectional signal with an intensity increase (>3 dB) that appears randomly within the cardiac cycle and

produces a 'whistling' sound as it passes through the sample volume (Németh et al., 2016; E. Ringelstein et al., 1998).

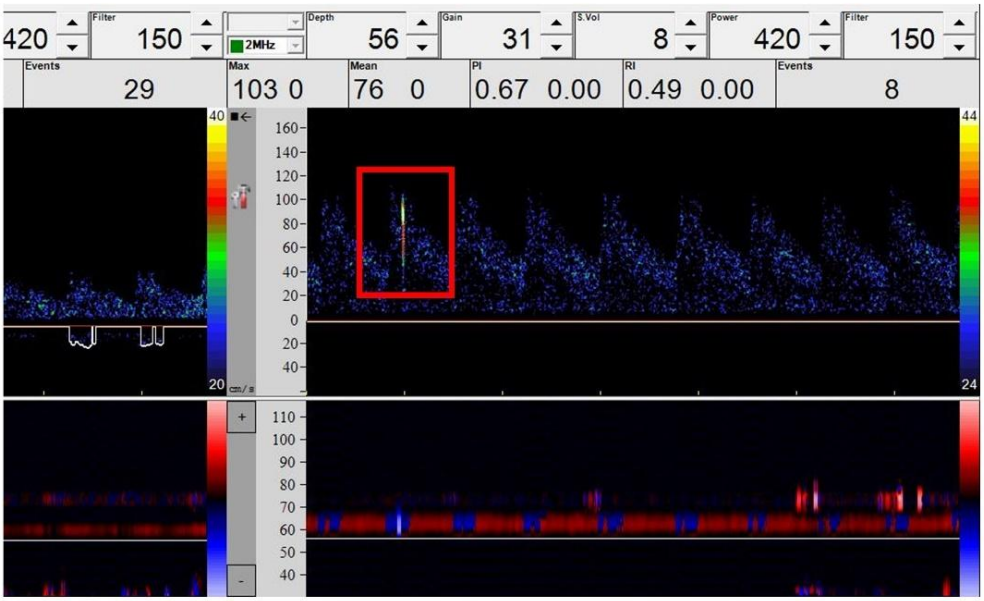

Figure 15. Microembolic signal. Data collected by the author.

The protocol for monitoring MES with TCD should ideally last between 30 and 60 minutes. It requires the patient to remain still during the monitoring, and the head support must be securely fixed to avoid signal artefacts caused by movement. The transducer should be attached to the temporal bone window to monitor the MCA, ideally with bilateral monitoring and a depth between 65 and 51 mm to assess the M1 segment of the MCA. TCD with M-mode aids in identifying the MCA, facilitating the determination of the optimal recording depth, angle, and sample volume. The gain parameter should be set as low as possible to eliminate noise and improve the quality of the collected signal (Németh et al., 2016).

Previous studies have demonstrated the clinical importance of investigating MES, showing their predictive value. Patients who are MES-positive after a stroke have a higher propensity for recurrence of ischaemic episodes and poorer functional recovery (Bazan et al., 2020; Das et al., 2020; Sheriff et al., 2020). However, a recent meta-analysis identified gaps and inconsistencies in the association between MES-positive status and unfavourable functional outcomes and mortality in patients after a stroke (Sudheer et al., 2021). There are still gaps in the understanding of the consequences of MES in clinical outcome besides stroke recurrence. Moreover, remains unclear how to implement it clinical applicability in risk stratification and among different patient groups. The applicability of TCD is evident, but it is still necessary to address the limitations of

its use in identifying MES, such as the user-dependent nature of its analysis protocol (Aarli et al., 2024; Sudheer et al., 2021).

CHAPTER II: THESIS AIMS

The mechanisms of long-term cognitive decline after stroke are not well understood, but 6 months after stroke the prevalence is approximately 52%. Hemodynamics parameters are described as altered in poststroke patients. **Rationale:** TCD ultrasonography allows to evaluate parameters of cerebral hemodynamic that are compromised with the stroke (See section 3. Transcranial Doppler and Functional Studies). The monitoring with TCD allows to complement the information collected in neuroimaging and to evaluate blood flow in real time. Numerous factors associated with vascular diseases contribute to cognitive decline (See section 2. Mechanisms of Cognitive Decline in Cerebrovascular Diseases). Understanding these factors and their interaction with the clinical and hemodynamic data collected in clinical practice can be useful to stratify the risk and management of patients.

Main Objective: This study aims to contribute to the identification of factors that may be predictive of cognitive function and to provide data on cerebral hemodynamic in ischemic stroke. This study will prospectively study neurovascular coupling (NVC) and microembolic signs (MES) in cerebrovascular patients with ischemic stroke and its relationship with cognitive function and functional recovery.

Specific objectives:

1. Describe a new approach to non-invasive measurement of NVC by spectral analysis of the cerebral blood flow velocity with transcranial Doppler.
2. Investigate the relationship between the value of NVC measured by transcranial Doppler in the acute phase after ischaemic stroke and long-term cognition.
3. Understand the difference in NVC between patients in the acute phase after ischaemic stroke and controls without brain injury.
4. Investigate the relationship between the value of NVC measured by transcranial Doppler in the acute phase after ischaemic stroke and medium- and long-term functional recovery.
5. Understand the difference in NVC between the hemisphere with and without brain injury in the acute phase after ischaemic stroke.
6. Investigate the relationship between MES-positive measured by transcranial Doppler in the acute phase after ischaemic stroke and long-term cognition.
7. Investigate the relationship between MES-positive measured by transcranial Doppler in the acute phase after ischaemic stroke and medium- and long-term functional recovery.

CHAPTER III: METHODS

1. Overall Design

Prospective and case-control study.

2. Participants

We included participants who suffered an ischemic stroke, admitted in the stroke unit (Centro Hospitalar Universitário de São João, Porto, Portugal) between November 2020 and July 2022. The follow-up was performed between February 2021 and May 2023.

We included patients that provided signed and dated informed consent; age> 18 years; with previous mRS<4 and patients with Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) final score≤3.0. We excluded patients with history of dementia or other central nervous system disease associated with cognitive impairment; uncollaborated; with absence of adequate temporal bone window for TCD monitoring; with serious kidney and systemic diseases; active cancer and patients with severe hearing or visual loss; and severe aphasia.

We recruited an Age-, gender-, and vascular risk factor-matched control group (Control Group I) and a healthy control group (Control Group II). They were assessed at a single time point, with NVC measured and sociodemographic and clinical information collected.

Control Group I was recruited from our institution's professionals and relatives to match age, sex and vascular risk factors.

Healthy subjects aged ≥ 18 years-old were recruited in Faculty Medicine of University of Porto (Porto, Portugal) for Control Group II. The volunteer participants included faculty staff and their relatives. We excluded volunteers with vascular risk factors, neurological and cardiovascular disease, or absence of temporal bone window for transcranial Doppler. All subjects were submitted to ultrasound examination to exclude cervical or intracranial arterial stenosis.

The procedures were explained and written informed consent was obtained from all participants. This study was approved by the local committee of ethics.

3. Assessments

This study required the assessment of demographics and biometrics, medical history, prior and concomitant medication, vital signs (Blood Pressure and Heart Rate), atrial cardiopathy markers, blood test parameters, stroke characteristics, cerebral hemodynamics, cognition and independence in daily living. Data collection followed the study periods described in the table 1.

Table 1. Schedule of Activities - Overview.

Study periods	**Screening**		**Intervention period**			
Number (t)	0		1		2	
Type	☎[a] (caregivers /family)	site	☎[b]	site	☎[b]	site
Study day and allowed deviations	Until 72 hours	Until 72 hours	3±1 months after onset	3±1 months after onset	12±1 months after onset	12±1 months after onset
Administrative Procedures						
Inclusion and exclusion criteria	x	x	x[c]		x[c]	
Signed informed consent		x		x		x
Demographics and biometrics		x[d]				
Medical history		x[d]				
Prior and concomitant medication		x[d]		x		x
Clinical procedures/ assessments						
mRS[1]		x		x		x
Vital signs (Blood Pressure and Heart Rate)		x		x		x
Abdominal circumference (cm)				x		x
Routine Blood Test Parameters / Hemostasis		x[d]				
Stroke Characteristics		x[d]				
IQCODE[2]	x					
MES[3] (PCA[4] and/or MCA[5])		x		x		x
NVC[6] (PCA)		x		x		x
MoCA test[7]				x		x
IADL[8]				x		x
New Vascular events			.- - - - - Continuously - - - - - -			

Abbreviations: [1]Modified Rankin Scale for Neurologic Disability (mRS); [2]Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE); [3]Neurovascular coupling (NVC); [4]posterior cerebral artery (PCA); [5]Middle cerebral artery (MCA); [6]Microembolic signals (MES); [7]Montreal Cognitive Assessment (MoCA); [8]Instrumental Activities of Daily Living Scale (IADL);

[a]Telephone contact is established with the patients's family/caregivers in t0.

[b]In the telephone contact established with the patient after the acute phase, his intention to continue in the study is again questioned. The study procedures and objectives are explained again.

[c]In the contact for scheduling the reassessment, the inclusion criteria are verified (before the site visit);

[d]Data obtained from clinical process.

Figure 16. Assessment of the participants: a) Collection of sociodemographic and clinical data - described in Table 1; b) Cognition and functional assessment; c) TCD assessment.

a) COLLECTION SOCIODEMOGRAPHIC AND CLINICAL DATA

b) COGNITION AND FUNCTIONAL ASSESSMENT

i. *Modified Rankin Score (mRS):* 0 - The patient has no residual symptoms; 1-The patient has no significant disability; able to carry out all pre-stroke activities; 2-The patient has slight disability; unable to carry out all pre-stroke activities but able to look after self without daily help; 3-The patient has moderate disability; requiring some external help but able to walk without the assistance of another individual; 4-The patient has moderately severe disability;

unable to walk or attend to bodily functions without assistance of another individual; 5-The patient has severe disability; bedridden, incontinent, requires continuous care; 6-The patient has expired.; (Saver et al., 2021)

ii. *Lawton Instrumental Activities of Daily Living Scale (IADL):* On the IADL scale, it is considered for the female sex: 0-1 total dependence, 2-3 severe dependence, 4-5 moderate dependence, 6-7 slight dependence and 8 independence. For males, the following values are considered: 0 total dependency, 1 severe dependency, 2-3 moderate dependency, 4 mild dependency and 5 independence; (Lawton & Brody, 1969)

iii. *Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE):* pre-existing level of cognition based on Portuguese Long Version - previous cognitive decline with score > 3.0; (Burton et al., 2021)

iv. *Montreal Cognitive Assessment (MoCA):* European Portuguese Version applied by certificated administrator Adm. ID PTFERJU235883-01. (Freitas et al., 2012)

C. TCD ASSESSMENT

i. *NVC measurement and analysis parameters:* For the assessment of the NVC, a previous study was carried out (Ferreira et al., 2024). The systolic flow velocities of the P2 segments of the posterior cerebral artery (PCA) were recorded continuously. Visual stimulus protocol consisted of 3 successive sessions, each having 5 cycles composed of periods of stimulation and resting with similar time duration: the first with 20-second ON-OFF cycles, the second 10-seconds and the last session with 5 seconds periods. The investigator will give the verbal / auditory command to close or open the eyes. TCD measured the changes in flow velocity in the PCA with visual stimulus, the activation of the visual cortex caused an increase in the flow velocity of this artery. We record with Doppler Box X, DWL (Sipplingen, Germany and San Juan Capistrano, CA) equipped with 2-MHz transducers secured by a probe-holder. Data was exported for offline analysis with costume software based on MATLAB® (Natick, US). NVC is classically determined by the peak relative increase in cerebral blood flow velocity during a visual stimulus. We developed a simpler analytic method to obtain the same amplitude of response by spectral analysis. By doing this we avoid additional instrumentation to synchronize stimulus with CBv trends. This

was particularly important in more challenging clinical scenarios like stroke (Ferreira et al., 2024)

ii. *MES measurement and analysis parameters:* The presence and rate of MES were assessed by 30 minutes of TCD monitoring. Bilateral M1 segments were insonated at a single depth. An MES was defined as a signal with duration < 300 ms, amplitude > 3dB, unidirectionality, and with a typical sound. The MES appears randomly on the signal and produce a “whistle”, “chirping” or “click” sound. The analysis is operator dependent (Németh et al., 2016; E. Ringelstein et al., 1998).

4. BENEFIT/RISK ASSESSMENT

The results of the study may be useful in stratifying patients' risk and in their medium/long-term follow-up. There will be no risk to study participants. The expected inconvenience will be the time spent and the travel to the hospital, which will be scheduled on days of other appointments and at times chosen by the participants.

5. ETHICAL CONSIDERATIONS

Approved by the Ethics Committee of Centro Hospitalar de São João nº303/2020.

6. INFORMED CONSENT PROCESS

Written information is given to the patient in addition to the verbal information provided at the time of recruitment.

7. DATA PROTECTION

The evaluations of cerebral hemodynamics parameters, cognitive function and questionnaires are stored in computers and physical spaces of the educational institution. These documents and files are identified with a unique code, assigned on the date of inclusion in the study.

CHAPTER IV: ASSESSMENT OF NEUROVASCULAR COUPLING BY SPECTRAL ANALYSIS OF CEREBRAL BLOOD FLOW VELOCITY WITH TRANSCRANIAL DOPPLER

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Original Contribution

Assessment of Neurovascular Coupling by Spectral Analysis of Cerebral Blood Flow Velocity With Transcranial Doppler

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Vasoreactivity
Photic stimulation
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Objective: Neurovascular coupling (NVC) represents the increase in regional blood flow associated with neural activity. The aim here was to describe a new approach to non-invasive measurement of NVC by spectral analysis of the cerebral blood flow velocity (CBFV) with transcranial Doppler.
Methods: In a sample of 20 healthy participants, we monitored systolic CBFV in the left posterior cerebral artery (PCA) during off (eyes closed) and on (flickering checkerboard) periods. The contralateral middle cerebral artery was simultaneously monitored as a control. Each participant was submitted to three experiments, each having five cycles, with increasing duration of the cycles, from 10 s (0.1 Hz) to 20 s (0.05 Hz) and lastly 40 s (0.025 Hz), half the time for on and for off periods, constituting a total of 6 min. The successive cycles were expected to cause oscillation in CBFV in a sinusoidal pattern that could be characterized by spectral analysis. We also measured the classic CBFV overshoot as the relative increase in percentage of systolic CBFV from baseline. The relationship and agreement between the two methods were analyzed by linear regression and Bland–Altman plots. In every participant, a clear peak of amplitude in the PCA CBFV spectrum was discernible at 0.1, 0.05 and 0.025 Hz of visual stimulation.
Results: On average, this amplitude was 7.1 ± 2.3%, 10.9 ± 3.5% and 17.3 ± 6.5%, respectively. This response contrasted significantly with an absent peak in middle cerebral artery monitoring ($p < 0.0001$). The spectral amplitude and classic overshoot were highly correlated and linearly related ($p < 0.0001$).
Conclusion: NVC can be quantified by the spectral amplitude of PCA CBFV at slower and higher frequencies of visual stimulation. This method represents an alternative to classic overshoot without the need for stimulus marking or synchronization.

Introduction

Neurovascular coupling (NVC) represents the relationship between neural activity and cerebral blood flow [1–3]. Neuronal activity causes local hyperemia to meet the needs of these neurons. Cerebral perfusion is closely linked to cerebral metabolism and neuronal activation, allowing this activity to be evaluated in spatiotemporal terms [3,4].

The neurovascular unit, comprising brain cells and the cerebral vasculature, plays a vital role in supplying energy to the brain, removing harmful proteins, facilitating immune cell traffic and maintaining overall brain homeostasis [5–8]. Disruptions in the neurovascular unit can contribute to various brain disorders, such as cardiovascular disease and Alzheimer dementia [2,7].

NVC has been studied in patients with hypertension, diabetes and other cardiovascular risk factors [9], and in genetic conditions associated with cerebral small vessel disease [10,11]. Therefore, ensuring the proper functioning of this unit, which is responsible for neurovascular coupling, is crucial for maintaining a healthy brain [5,12,13].

Changes in cerebral blood flow velocity (CBFV) or subsequent changes in energy substrates can be measured using brain imaging techniques such as functional magnetic resonance imaging dependent on blood oxygen level, positron emission tomography and functional transcranial Doppler (f-TCD) and are used as an indicator of neuronal activity [1,4].

Functional transcranial Doppler is a non-invasive method and allows continuous measurement of the velocity of cerebral blood flow with high temporal resolution. It has been used to measure NVC in various paradigms, with the visual stimulation been the most common [4,9,14,15]. The flickering checkerboard is the standard visual stimulus that requires additional apparatus to correct synchronous event markers with CBFV. Additional instruments can deter many researchers from using it, particularly clinical labs equipped with only f-TCD. Some f-TCD devices do not allow including the stimulus signal for further analysis, and in devices that do include it, the stimulus marking can be susceptible to synchronization errors with the collected signal [4,8,16]. A simpler, non-invasive method may enable its replication in more centers

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and various clinical contexts, making it a valuable tool for studying NVC in different pathologies and contributing to the advancement of research in this field. Therefore, the aim of this study was to describe a new approach to non-invasive measurement of NVC by spectral analysis of CBFV with f-TCD. We hypothesized that NVC can be quantified by the spectral amplitude of CBFV.

Methods

Population

Healthy participants aged ≥18 y were recruited in the Faculty of Medicine, University of Porto (Porto, Portugal). The volunteer participants included faculty staff and their relatives. We excluded volunteers with vascular risk factors and neurological and cardiovascular disease and those lacking a temporal bone window for transcranial Doppler. All participants were submitted to ultrasound examination to exclude cervical or intracranial arterial stenosis.

The procedures were explained, and written informed consent was obtained from all participants. This study was approved by the Ethics Committee of Faculty of Medicine, University of Porto/Centro Hospitalar Universitário São João, Porto, Portugal (No. 303/2020) and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Monitoring protocol

Evaluations were carried out in a dim lighted room, at ambient temperature and with the participant in the sitting position. Participants were to refrain from caffeine, alcohol, exercise and vasoactive drugs for at least 12 h before evaluation. CBFV was recorded at around 50 mm of depth in the M1 segment of the right middle cerebral artery (MCA) and at around 60 mm of depth in the P2 segment of the left posterior cerebral artery (PCA), with 2-MHz TCD probes secured with a headframe (Doppler BoxX, DWL, Singen, Germany). Data were synchronized and digitized at 400 Hz with Powerlab (AD Instruments, Oxford, UK) and stored for offline analysis in a customized software.

Visual stimulation protocol

Each participant was submitted to three experiments, each having 5 cycles of equal length of ON and OFF periods, as illustrated in Figure 1. The first experiment includes cycles of 10 s or 0.1 Hz (5 s ON and 5 s OFF). The next experiment included cycles of 20 s or 0.05 Hz (10 s ON and 10 s OFF), and lastly, the final experiment included cycles of 40 s or 0.025 Hz (20 s ON and 20 s OFF). The total visual paradigm required 6 min to be completed. Each experiment started with the participants' eyes closed (OFF period). During the visual stimulation ON period, participants were instructed to open their eyes and look at a flickering checkerboard at a frequency of 2 Hz, displayed in a monitor with a resolution of 1920 × 1080 pixels (LG) at a distance of 2 m. Visual stimulation typically evokes a rapid increase in CBFV, which overshoots ~10 to 15 s and then stabilizes [16].

Data analysis

Peak systolic beat-to-beat data were calculated in the machine software (DWL, Singen, Germany) and exported. Data points that exceeded 300% of the precedent beat were excluded as outliers. Neurovascular coupling indices were calculated for each of the three experiments in both time domain and frequency domain, using a professional software tool (MATLAB, The MathWorks, Natick, MA, USA).

First, we obtained the most commonly used maximum systolic CBFV change to calculate the overshoot parameter as [9]

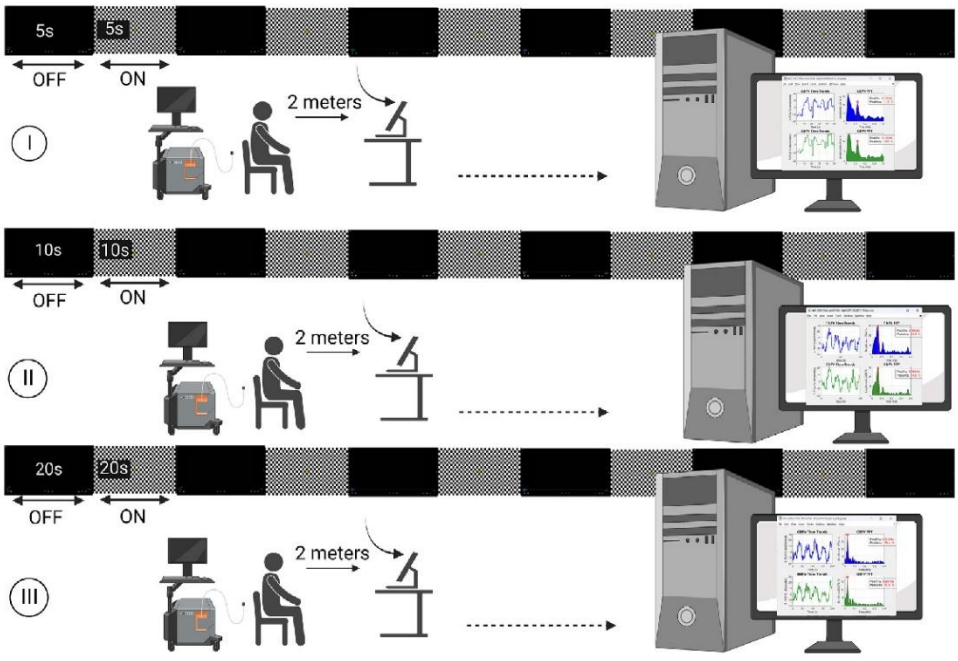

Figure 1. Monitoring protocol. Experiment I: visual stimulation protocol with 5 s ON−5 s OFF; Experiment II: Visual stimulation protocol with 10 s ON−10 s OFF. Experiment III: Visual stimulation protocol with 20 s ON−20 s OFF. Created with BioRender.com.

$$\frac{\text{Maximum CBFV} - \text{Baseline CBFV}}{\text{Baseline CBFV}} \times 100\%$$

We also determined the time to peak by averaging all 5 cycles of each experiment by the exact time mark of ON period. The baseline was the 5 s OFF period just before visual activation.

In a second approach, the CBFV signal was analyzed in the frequency domain. As the ON and OFF periods have even time spans, we hypothesized that the systolic CBFV response of activation and decoupling would generate a sinusoidal trend over the length of the 5 cycles of each experiment. As depicted in Figure 2, a pure sinusoidal signal can be depicted in the frequency domain, and the amplitude at the given frequency is maximal and equivalent to the amplitude of variation of the sinusoid in the time domain, which is the previously mentioned overshoot. To determine the amplitude, we applied fast Fourier transform (FFT). Data were first detrended and expressed as percentage of the mean. We further resampled the signal to have a uniform time basis to obtain an equal number of data points for FFT. For each frequency, the depicted amplitude represents the oscillations and would peak at the specific frequency of the experiment (0.1, 0.05 or 0.025 Hz). We calculated additional indices that express the distortion and noise from the sinusoidal oscillation based in reference to the fundamental frequency of oscillation of each experiment. These were the total harmonic distortion (THD), signal-to-noise ratio (SNR) and signal-to-noise-and-distortion ratio (SINAD). For this purpose, we first calculated the total power of the spectrum, the power of the spectrum at the fundamental frequency of each experiment (0.1, 0.05 or 0.025 Hz), the sum of the power of the first 10 harmonics frequencies (5 even and 5 odd) of the fundamental frequency and noise, as the sum of spectrum power minus the power at fundamental frequency and harmonics. THD was derived with the formula [17]

$$\text{THD} = \frac{\sqrt{\text{Total harmonic power}}}{\sqrt{\text{Total spectrum power}}} \times 100\%$$

The signal-to-noise ratio was derived with the formula [17]

$$\text{SNR} = 10 \times \log \frac{\sqrt{\text{Power fundamental frequency}}}{\sqrt{\text{Total noise power}}}$$

and SINAD was derived with the formula [17]

$$\text{SINAD} = 10 \times \log \frac{\sqrt{\text{Total spectrum power}}}{\sqrt{\text{Total harmonic power} + \text{Total noise power}}}$$

Artificial sinusoids

As a proof of concept, we generated three artificial pure sinusoidal signals with fixed distinct frequencies of 0.1, 0.05 and 0.025 Hz,

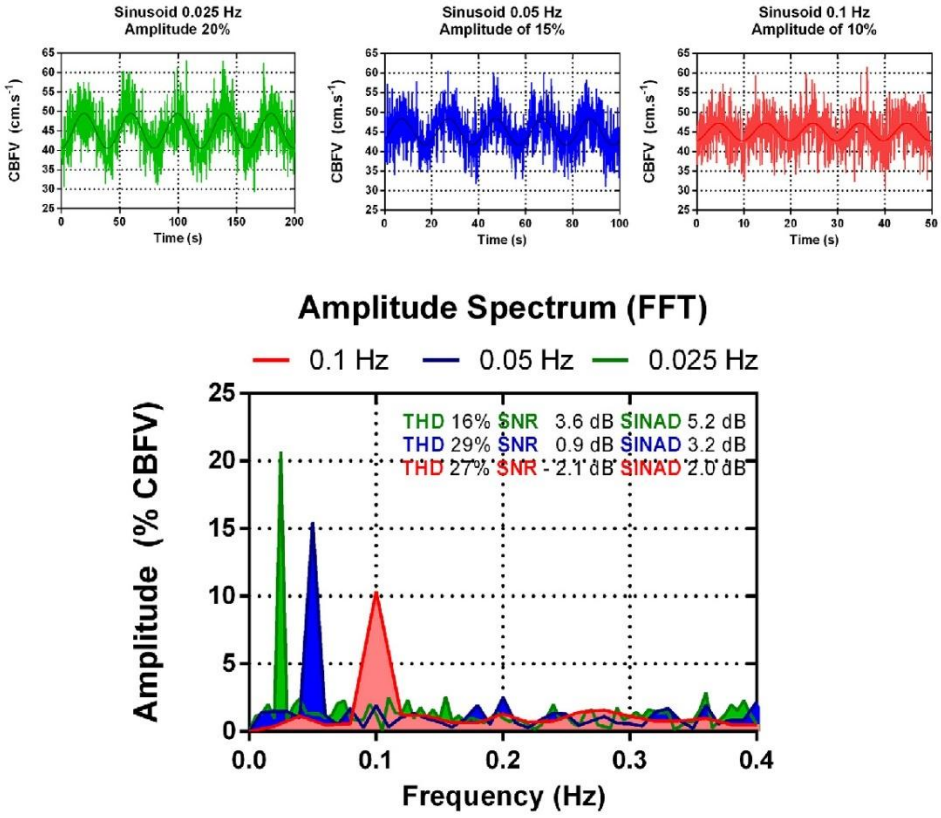

Figure 2. Representative artificial noisy pure sinusoidal signal at frequencies of 0.025, 0.05 and 0.1 Hz and its spectral representation. CBFV, cerebral blood flow velocity; FFT, fast Fourier transform; SNR, signal-to-noise ratio.

representative of expected CBFV changes during each experiment. We added 10% of random noise, the normal variability in CBFV measured by f-TCD [18]. We tested different levels of Gaussian noise (from 5% to 25%), and the amplitude in the spectral analysis was similar (Fig. S1 and Table S1, online only). FFT was then applied to calculate the amplitude as previously explained.

Statistics

Normality of the variables was determined with the Shapiro–Wilk test. Repeated-measures analyses of variance (ANOVAs) were used to determine the differences in each parameter accordingly to experiment (frequency of visual stimulation) and arterial territory (PCA vs. MCA).

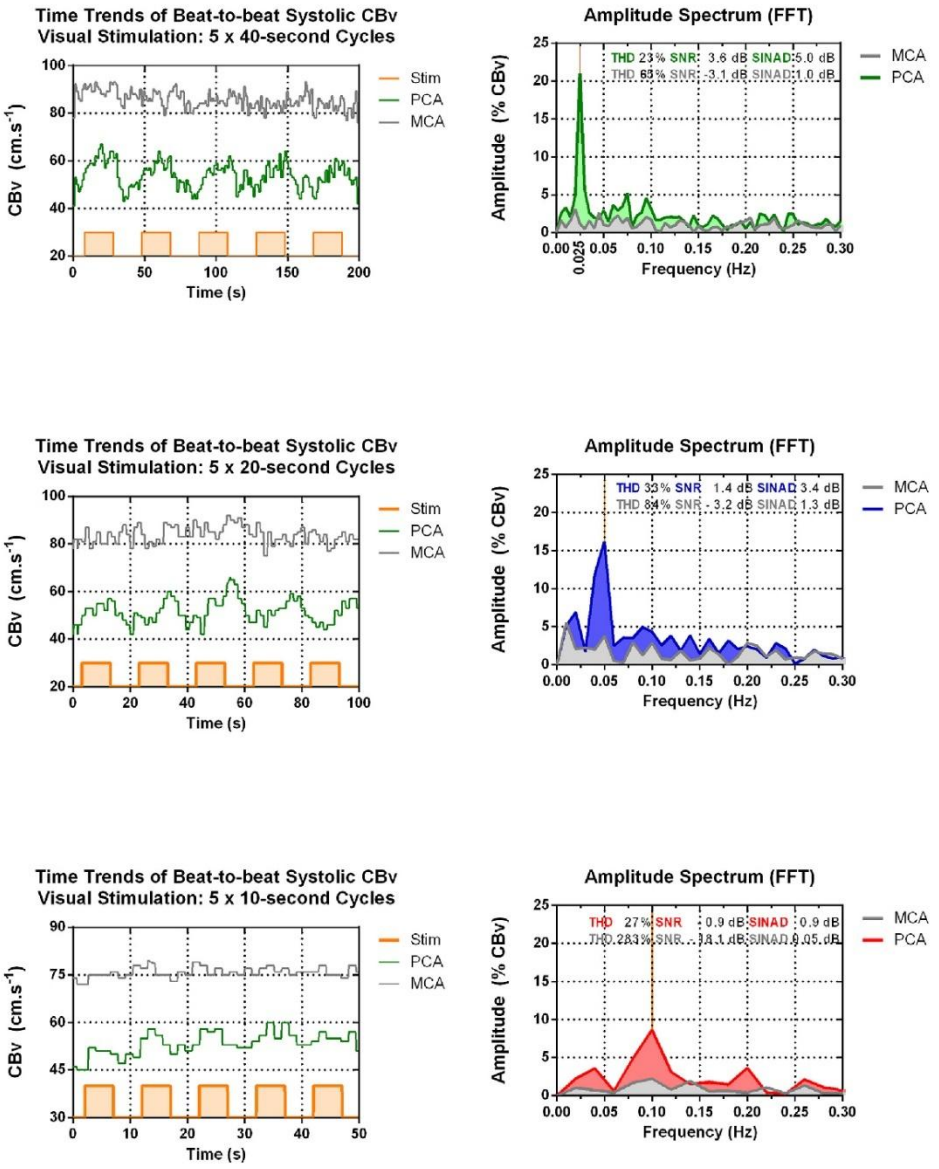

Figure 3. Spectral amplitude and classic overshoot were highly correlated and linearly related ($p < 0.0001$). The standard deviation was lower with a spectral method. CBv, cerebral blood flow velocity; FFT, fast Fourier transform; MCA, middle cerebral artery; PCA, posterior cerebral artery; SINAD, signal-to-noise-and-distortion ratio; SNR, signal-to-noise ratio; THD, total harmonic distortion.

Table 1
Neurovascular coupling calculated parameters in healthy volunteers

Parameter	0.1 Hz		0.05 Hz		0.025 Hz	
	MCA	PCA	MCA	PCA	MCA	PCA
Mean CBFV at rest (%)	82.8 (21.3)	46.3 (10.8)[a]	84.4 (20.2)	45.3 (10.2)[a]	86.1 (21.4)	44.3 (10.1)[a]
CBFV covariance (%)	3.8 (1)	5.6 (0.9)[a]	4.2 (1.5)	7 (1.6)[a]	5 (1.2)	8.7 (2.2)[a,b]
Time domain						
Overshoot (%)	1.4 (3.3)	7.5 (3.1)[a]	3.4 (4.3)	12.5 (3.5)[a,b]	4.8 (4.1)	18.7 (6.4)[a,b,c]
Time to peak (s)		5 (1.6)		9 (2.2)[b]		16.9 (3.9)[b,c]
Frequency domain						
Spectral amplitude at frequency of stimulation (%)	4.1 (1.5)	7.1 (2.3)[a]	3.7 (1.2)	10.9 (3.5)[a,b]	5.3 (1.9)	17.3 (6.5)[a,b,c]
Total spectral power (%^{2})	36.1 (16.9)	73.8 (19.9)[a]	56.6 (57.3)[b]	140.5 (64.6)[a,b]	76.7 (42.6)[b]	283 (140)[a,b,c]
Noise power (%^{2})	27.3 (14.1)	51.8(18.2)[a]	43.6 (46.8)[b]	65.7 (30.5)[a,b]	53.1 (34.8)[b]	97 (48.9)[a,b,c]
Total harmonic distortion (%)	129 (62.7)	125.3 (68.9)	111.2 (58.5)	55.2 (25.4)[a,b]	101.3 (82.4)	34.4 (16.7)[a,b,c]
Signal-to-noise ratio (dB)	−8.5 (3.4)	−8.3 (4)	−11.1 (6.6)	−1.3 (3.7)[a,b]	−6.3 (5.1)	1.8 (3.2)[a,b,c]
Signal-to-noise-and-distortion ratio (dB)	0.7 (0.4)	0.9 (0.7)	0.5 (0.4)	2.4 (1.3)[a,b]	1.1 (0.9)[b,c]	3.9 (1.7)[a,b,c]

All values are expressed as the mean (standard deviation).
CBFV, cerebral blood flow velocity; MCA, middle cerebral artery; PCA, posterior cerebral artery.
[a] $p < 0.01$ for differences between arteries (MCA vs. PCA).
[b] $p < 0.01$ for differences compared with 0.1 Hz experiment results.
[c] $p < 0.01$ for differences compared with 0.05 Hz experiment results.

The relationship and agreement between the overshoot and spectral methods of NVC estimation were analyzed by linear regression, and Bland–Altman plots were computed to detect bias.

Results

Twenty volunteers (15 women, 5 men) aged between 22 and 39 y (mean age: 30 ± 6 y) participated in the study. No data were discarded because of artifacts.

In every participant, a clear peak in amplitude in the CBFV spectrum was discernible at 0.1, 0.05 and 0.025 Hz of visual stimulation (Fig. 3). On average, this amplitude was 7.1 ± 2.3%, 10.9 ± 3.5% and 17.3 ± 6.5%, respectively (Table 1). As expected, the spectral amplitude of simulation increases with increasing time for visual stimulation of ON time. This response contrasted significantly with an absent peak at the MCA side (Fig. 4, $p < 0.01$) interpreted as no CBFV response to visual stimulation.

The experiments at 0.05 and 0.25 Hz of visual stimulation, slower and longer in total time length, revealed increased total spectrum and noise powers. PCA increased significantly more than MCA (Table 1, $p < 0.01$). However, THD decreased significantly in PCA progressively with the lowest values in the 0.025 Hz experiment.

Conversely, SINAD and SNR increased significantly in the 0.05 and 0.025 Hz experiments (Table 1, $p < 0.01$). These were similar between the arterial territories in the 0.1 Hz experiment.

The distortion of the measured signal in each experiment as compared with a pure sinusoidal wave was inferred from the analysis of Table 1 and the pure sinusoidal signal of Figure 2. Although THD was always higher and SNR and SINAD values were lower in observed spectral data as compared with the pure sinusoid created in Figure 2, these difference parameters were closer in the 0.05 and 0.025 Hz experiments.

Concordance and reliability

The spectral amplitude and classic overshoot were highly correlated and linearly related, with β ranging from 1.00 to 1.04 in all experiments (Fig. 5, $p < 0.0001$). This is also depicted in the mean and standard deviation (SD) values in Table 1. In the Bland–Altman graphical analysis (Fig. 5), concordance between the two methods is observed, as most of the points are close to the mean difference line and within the limits of agreement in the different experiments. Regarding the bias analysis between the two methods, the points are evenly distributed around the mean difference line. This indicates that the methods have a centered tendency.

Discussion

Our study has indicated that NVC can be quantified by calculating the spectral amplitude of PCA CBFV during a visual task. Visual stimulation of the occipital cortex resulted in a statistically significant increase in cerebral blood flow velocity in the PCA compared with the MCA, according to what should be the concept of NVC of regional hyperemia [16,19].

Previous methodological studies have demonstrated the usefulness of visual stimulation paradigms for studying neurovascular coupling mechanisms or alterations in the regulation of cerebral blood flow caused by vascular pathologies [16,19,20]. However, methodological differences continue to be identified as a barrier in the study of NVC [1,13,21].

To be consistent with previous studies and the f-TCD, our spectral analysis used a flickering checkerboard visual stimulus, which has excellent temporal resolution and can measure the blood flow velocity oscillations in real time [22]. Biologically, this model translates the rapid increase in cerebral blood flow velocity and the maximum amplitude reached in percentage at the frequencies of 0.025, 0.05 and 0.1 Hz in the PCA. Our method used FFT to determine the maximum flow oscillation amplitude using signal spectral analysis. The FFT is a more efficient technique for calculating the Fourier transform, especially for longer signals, and it allows for a significant reduction in computation time [23]. Beyond time, the FFT is more effective for large data sets and widely used across various domains, including signal processing, because of its efficiency. The FFT is an optimized and swift version of the discrete Fourier transform [23].

In our analysis, the FFT was applied directly to the collected signal, and the Welch method was not used. Overlap between the windows is important because it helps to smooth the transitions between adjacent windows and obtain a more accurate estimate of spectral density [24]. However, in our exploratory analysis, it was found that its application reduced the accuracy of amplitude peak calculation.

In our experiments, our goal was not to calculate spectral power in frequency bands, but rather at precise frequencies—0.025, 0.05 and 0.1 Hz. Therefore, we did not segment into windows. The signals collected in the three experiments (Fig. 1) had a sampling rate of 100 Hz and durations of 50 s (experiment I), 100 s (experiment II) and 200 s (experiment III). Therefore, the windows had a number of samples per window of 5000, 10,000 and 20,000, respectively.

The maximum peak amplitude of CBFV observed in the spectral analysis at 0.025 Hz, corresponding to the 20 s ON−20 s OFF experiment (experiment III) of ~17 %, is close to the maximum overshoot values presented in previous studies of 16% at around 15 s of stimulation (ON) [16]. On observation of the relative CBFV time trend to visual stimulation in Rosengarten et al.'s study [16], the approximate values of the CBFV relative changes at the first 5 and 10 s are similar to the values obtained in our 5 s ON−5 s OFF and 10 s ON −10 s OFF experiments (7.5 ± 3.1 and 12.5 ± 3.5, respectively). Once more, these are similar to the values obtained at equivalent

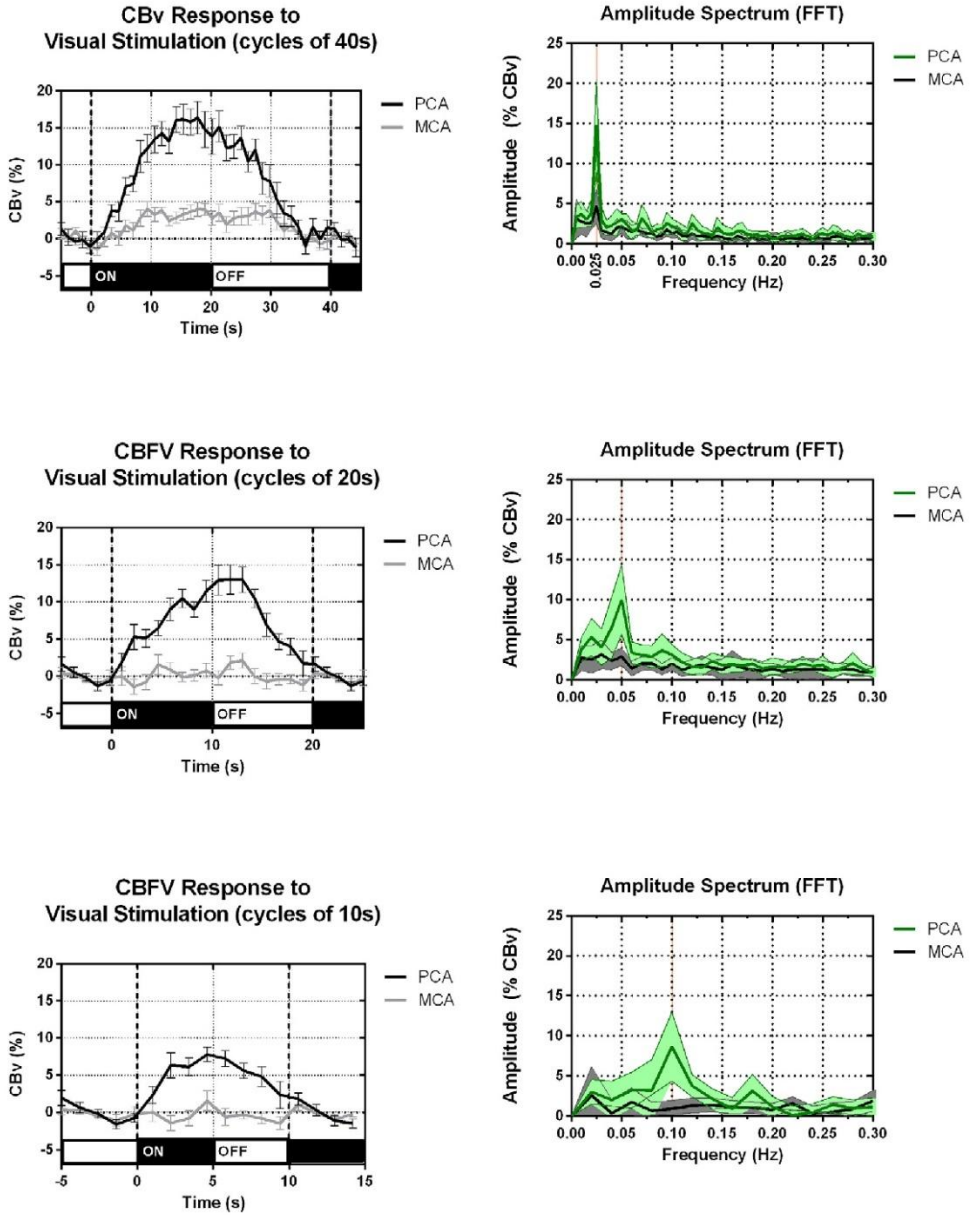

Figure 4. In every participant, a clear peak in amplitude in the CBFV spectrum was discernible at 0.1, 0.05 and 0.025 Hz of visual stimulation of PCA. On average, this amplitude was 17 ± 2%, 11 ± 1% and 7 ± 3%. This response contrasted significantly with an absent peak at the MCA side ($p < 0.0001$). CBv/CBFV, cerebral blood flow velocity; MCA, middle cerebral artery; PCA, posterior cerebral artery.

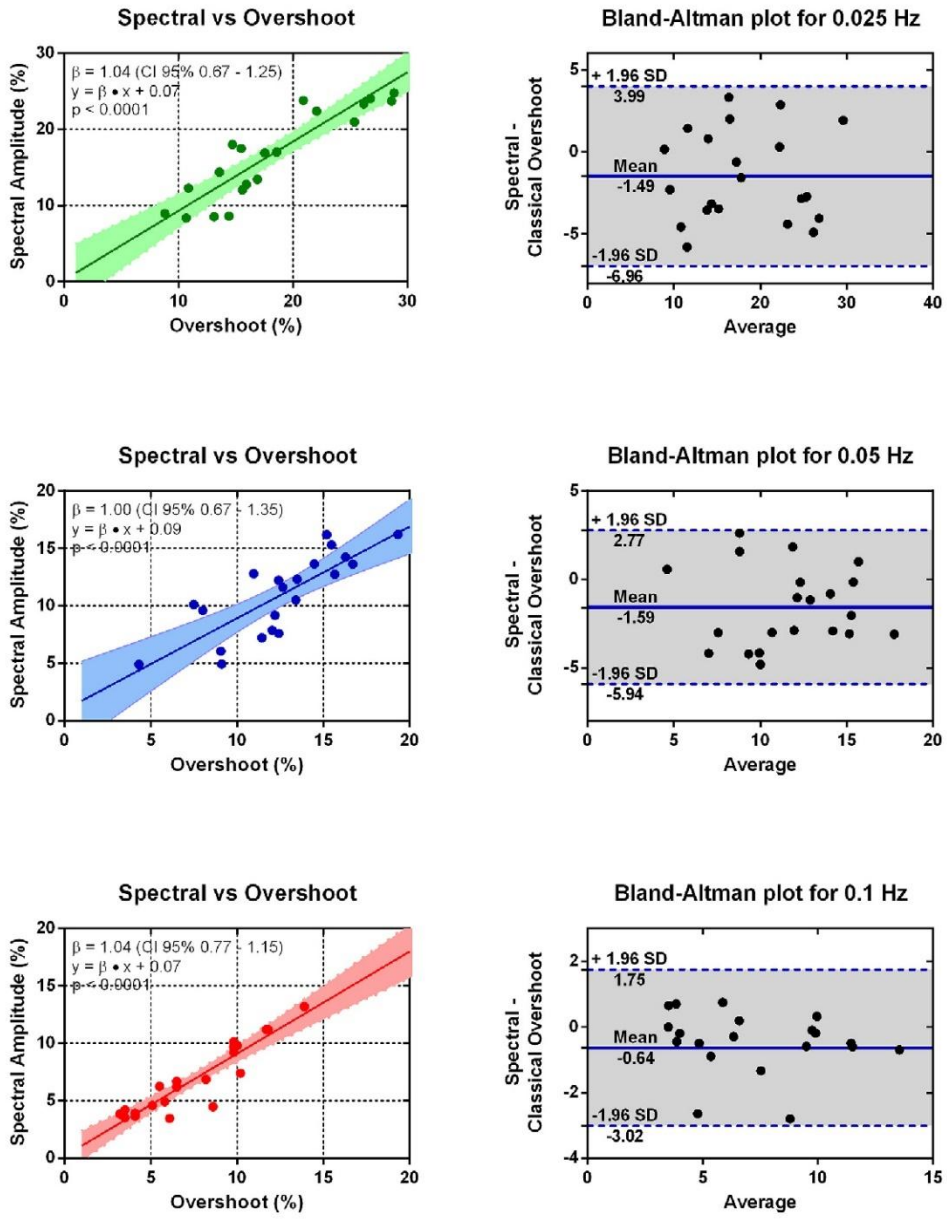

Figure 5. Group averaged cerebral blood flow responses to visual stimulation. CI, confidence interval; SD, standard deviation.

time points of visual stimulation, the 0.1 and 0.05 Hz experiments (7% and 10%, respectively) [16].

In the 0.025 and 0.05 Hz experiments, the signal recording in the PCA exhibits higher SNR and SINAD values and, therefore, a cleaner and more reliable signal at the frequency of visual stimulation.

Comparing the correlation of values found in the spectral analysis with the classic overshoot model, we can affirm that in the 0.025 and 0.05 Hz experiments, a central tendency is observed, with no bias between the methods. In the 0.1 Hz experiment, however, more points were observed above the mean difference line, which indicates a positive bias. In other words, the classic overshoot method tends to produce larger measurements than the spectral analysis, but within acceptable and clinically relevant limits.

The 0.1 Hz experiment is less reliable because the SNR and SINAD values in the PCA measurement are similar to the values in the MCA, despite being able to visually detect the peak maximum amplitude in the PCA. Among the three experiments, the 0.1 Hz experiment has lower SINAD and SNR values in the PCA signal analysis (Fig. 3), indicating that this signal has lower quality compared with the others. This signal quality analysis supports our graphical analysis, illustrating that, although within clinically relevant and acceptable limits, the 5 s ON–5 s OFF experiment is the least reliable for evaluating NVC values.

In assessments of NVC where the task used in the experiment is repeated over time, gradual adaptation may occur. This gradual adaptation can lead to a different vascular response compared with the initial response to the task, and it is important to consider the nature of the task, experimental conditions and complexity of the vascular and neuronal systems involved [25]. The visual stimulus presented in our study was arranged in increasing order of ON–OFF time intervals, specifically, Experiment I consisted of 5 cycles of 5 s ON–5 s OFF (total of 50 s), followed by Experiment II with 5 cycles of 10 s ON–10 s OFF (total of 100 s) and, finally, Experiment III with 5 cycles of 20 s ON–20 s OFF (total of 200 s). While being a periodic stimulus to facilitate analysis, as suggested by our method, the variation in ON–OFF time intervals in the three experiments introduces variability in the presented stimulus. The maximum ON time for stimulus presentation was 20 s, and previous studies have reported that no habituation to the stimulus was observed in visual stimulus periods of 40 s [25].

The simplicity of our method allows for its replication in more research centers that have access only to f-TCD. The use of this non-invasive and more accessible method can assist in addressing clinical questions related to the predictive value of NVC in various pathologies, potentially providing new insights in this area because of its ease of use. The reduction in the need for equipment to mark the timing of the stimulus can facilitate its use in more challenging clinical settings. Future studies can use spectral analysis to evaluate outpatients, but also even acutely ill inpatients.

Limitations

The frequency domain analysis developed allows for the measurement of the amplitude of increased CBFV, but it could be a limitation in the time behavior of CBFV response to visual stimulus. The response time can be considered an indicator of the activation time required for an individual to respond to the presented stimulus. Previous studies have found differences in the response time parameter, and these values can be useful in characterizing the velocity of vasomotor reactivity in arterioles [19]. To overcome this limitation, we included stimuli with distinct ON–OFF times from rapidly changing (0.1 Hz) to longer periods of stimulation (0.025 Hz). The stimulus with 5 s ON and 5 s OFF allows for the observation of this rapid response and discrimination of the faster responders. It is expected that slower responses at 0.1 Hz will result in lower amplitudes as compared with those of faster responders.

The advantages of using frequency domain analysis apply only to periodic stimuli, stimuli that repeat in cycles with equal durations such as the visual stimulus presented. The stimulus given to the participant cannot vary in task between repetitions for frequency domain analysis to be applicable. Thus, it may limit its use in stimuli involving language or motor tasks that vary between repetitions.

Although with high temporal resolution, the use of f-TCD has low spatial resolution, which does not allow distinguishing gray for white matter response as in magnetic resonance imaging.

In this method, only the blood flow velocity in the PCA is measured, assessing only the NVC in the occipital region. Indeed, we intended to study the neurovascular unit as a whole and use it as marker of global dysfunction. As previously discussed, there is evidence that NVC is impaired globally, such as in patients with hypertension, diabetes and other cardiovascular and cognitive diseases [9–11]. Therefore, this method can be useful in studying these pathologies in more clinical contexts, across more research centers and with more patients.

A visual stimulus was used to assess NVC. The use of this method as an indicator of global dysfunction can be considered; however, cognitive function is much more complex than just visually evoked responses. Nevertheless, by restricting f-TCD to a simple stimulus, we also avoid potential confounders related to a disturbed cognitive state of the participant.

Conclusion

Neurovascular coupling can be quantified by the spectral amplitude of PCA CBFV at slower and higher frequencies of visual stimulation. This method represents an alternative to the classic overshoot for periodic stimuli, easily calculated and without the need for stimulus marking or synchronization. The classic overshoot method requires additional software for stimulus presentation and connection to an external device that marks the stimulus in the recording. In other words, temporal analysis of the signal requires marking the moment when the stimulus was presented in the recorded signal. The method we propose, on the other hand, uses only TCD and the monitoring software itself, as the signal will be analyzed in the frequency spectrum.

Conflict of interest

The authors declare no competing interests.

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Data availability statement

The data and SPSS syntax code that support the findings of this study are available from the corresponding author on reasonable request.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ultrasmedbio.2024.02.003.

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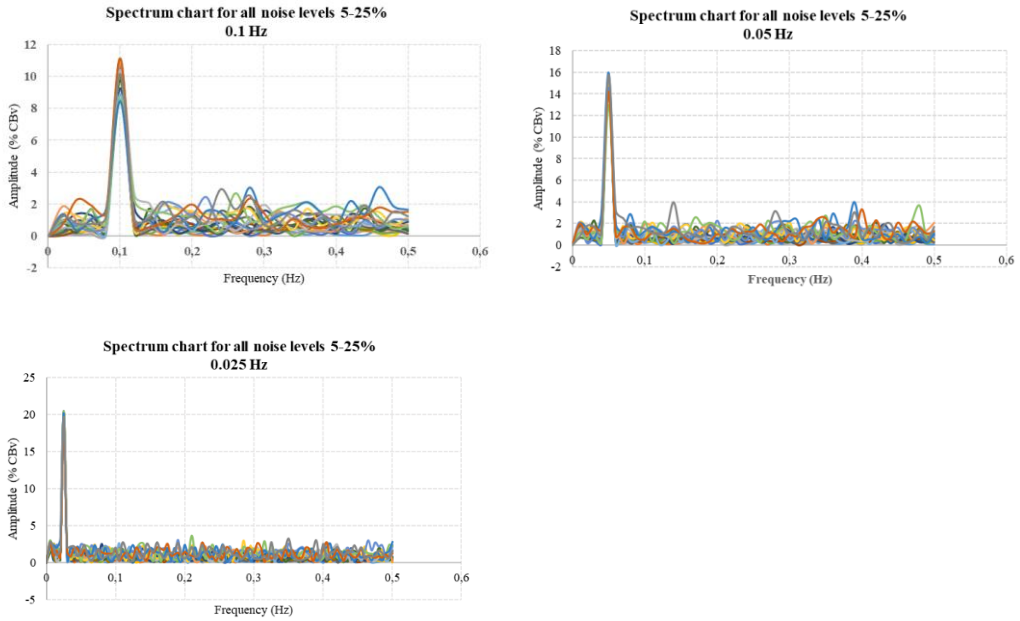
Supplementary Material

Supplementary Figure 1. Spectrum chart for all noise levels 5-25% with fixed distinct frequencies of 0.1, 0.05 and 0.025 Hz.



Hz: Hertz; CBv: Cerebral Blood Flow Velocity; THD: total harmonic distortion; s: seconds; SNR: signal-to-noise ratio; SINAD: Signal-to-noise and distortion ratio; THD: total harmonic distortion;

Supplementary Table 1. Artificial pure sinusoidal signal with fixed distinct frequencies of 0.1, 0.05 and 0.025 Hz with all noise levels 5-25%.

	0.1 Hz				0.05 Hz				0.025 Hz			
Noise (%)	Amp (%)	THD (%)	SNR (dB)	SINAD (dB)	Amp (%)	THD (%)	SNR (dB)	SINAD (dB)	Amp (%)	THD (%)	SNR (%)	SINAD (dB)
5	9.9	17.1	3.4	4.9	15.0	14.1	7.0	7.4	19.5	10.9	8.6	8.9
6	10.0	19.7	1.5	3.7	14.5	13.9	5.2	6.1	19.5	14.1	7.2	7.6
7	10.6	24.2	1.6	3.7	14.8	18.9	4.1	5.2	19.2	12.5	5.7	6.5
8	10.4	46.2	0.1	2.6	15.2	22.6	3.3	4.7	19.5	21.7	5.3	5.9
9	10.0	37.2	-1.9	2.0	14.6	29.2	1.9	3.7	20.2	21.6	4.2	5.2
10	10.1	30.6	-1.9	2.1	14.2	32.2	0.2	2.9	19.8	19.9	3.2	4.6
11	9.3	51.6	-4.3	1.3	14.9	29.3	0.2	2.9	19.3	20.4	2.2	4.1
12	10.6	52.0	-3.5	1.5	15.0	29.2	-0.9	2.4	19.3	32.0	1.4	3.4
13	10.5	51.3	-4.6	1.2	14.2	31.0	-1.9	2.1	19.1	30.7	0.6	3.1
14	9.8	58.5	-5.6	1.0	14.3	53.4	-2.5	1.7	19.2	25.7	0.0	2.9
15	10.2	57.7	-6.0	0.9	14.9	41.4	-2.4	1.8	20.5	26.7	-0.2	2.8
16	9.8	68.1	-6.8	0.8	15.1	39.0	-3.02	1.7	20.4	39.5	-0.4	2.6
17	9.0	60.5	-8.1	0.6	14.8	45.8	-3.7	1.4	20.2	31.5	-1.2	2.3
18	10.8	60.5	-7.2	0.7	15.0	59.9	-3.8	1.3	19.9	43.9	-1.4	2.1
19	10.4	76.6	-8.0	0.6	15.5	43.7	-3.7	1.5	18.9	34.2	-2.8	1.7
20	10.2	77.8	-8.5	0.5	14.8	53.3	-5.4	1.0	20.3	31.8	-3.7	1.4
21	11.1	56.4	-8.2	0.6	14.5	61.4	-5.9	0.9	18.5	52.9	-3.8	1.4
22	8.6	78.3	-10.7	0.3	13.0	76.1	-6.9	0.7	20.5	36.2	-3.7	1.4
23	8.4	90.7	-11.6	0.3	16.0	45.2	-6.1	0.9	20.2	52.2	-3.5	1.5
24	11.1	80.2	-9.13	0.5	14.2	79.5	-7.0	0.7	18.4	61.7	-4.7	1.1
25	10.2	96.3	-10.3	0.4	15.8	63.3	-6.3	0.8	19.7	50.4	-4.3	1.2

Amp: Amplitude; Hz: Hertz; dB: decibels; THD: total harmonic distortion; s: seconds; SNR: signal-to-noise ratio; SINAD: Signal-to-noise and distortion ratio; THD: total harmonic distortion;

CHAPTER V: NEUROVASCULAR COUPLING ASSESSMENT BY TRANSCRANIAL DOPPLER IN ACUTE STROKE COULD BE INFORMATIVE OF LONG-TERM COGNITIVE STATUS

Title: Neurovascular Coupling assessment by Transcranial Doppler in Acute Stroke could be Informative of Long-term Cognitive Status

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Supplemental material: Yes

Key-words: Neurovascular coupling, Cerebral blood flow, Stroke, Cognitive Impairment, Transcranial Doppler.

Word count: 8436

Abstract

Background and Aim: To assess whether Neurovascular Coupling (NVC) status in acute ischemic stroke predicts long-term cognitive impairment. **Methods:** This prospective study included consecutive ischemic stroke patients, without prior cognitive deficits, recruited between September 2021 and August 2022. Transcranial Doppler (TCD) monitoring was performed within 72 hours post-stroke to measure NVC, determined by peak relative increase in cerebral blood velocity (CBv) in the posterior cerebral artery (PCA) during a visual stimulus. Age-, gender-, and vascular risk factor-matched controls were included. The primary outcome was cognitive status at 12 months, evaluated using a 7-point cognitive scale. **Results:** A total of 144 acute stroke patients and 40 controls were included. NVC exhibited a U-shaped association with long-term cognitive outcomes. Patients in both the lowest (CI95% 2.29 [1.23–4.28], p<0.01) and highest (CI95% 0.53 [0.29–0.98], p=0.04) quintiles of NVC magnitude had significantly worse cognitive scores at 12 months compared to those in the reference quintile. **Conclusions:** NVC assessment via TCD in the acute phase of ischemic stroke may serve as a predictor of long-term cognitive impairment, suggesting that both diminished and exaggerated NVC responses are linked to poorer cognitive outcomes.

Main text

Introduction

Post-stroke cognitive impairment (PSCI) is a highly prevalent (15-70%) public health issue that impacts the quality of life of stroke survivors. This comes also with increased burden of economic for patient, relatives and society (Kalaria et al., 2016; Wang et al., 2021). Despite its significant impact, there are still gaps in our understanding of the complex interaction between the acute stroke event and PSCI. Understanding the factors that influence and increase the risk of PSCI is crucial for developing personalized prognostics. The development of biomarkers for earlier detection and monitoring are also crucial studying for the effects of preventive interventions, and for informing the design of future clinical trials (El Husseini et al., 2023; Rost et al., 2022).

After an ischemic insult and damage to a specific brain region, the ability of the surrounding brain areas to support recovery is strongly associated with better outcomes at 3 months post-stroke. This neuroplasticity-driven recovery is also linked to the integrity of the blood-brain barrier (BBB), which plays a crucial role in maintaining the brain's microenvironment and facilitating repair processes (Montagne et al., 2015; Sargento-Freitas et al., 2018). The BBB functions within the framework of the neurovascular unit, a highly integrated structure that facilitates communication between neurons and the cerebral vasculature (Kaplan et al., 2020; Wang et al., 2021). This unit, composed of neurons, astrocytes, smooth muscle cells, endothelial cells, pericytes, and the basal lamina matrix, plays a fundamental role in the progression of ischemic stroke by regulating the blood-brain barrier, preserving cells, and participating in the inflammatory response and neurovascular repair (Kaplan et al., 2020; Wang et al., 2021). The orchestrated functioning of the neurovascular unit is exemplified by the phenomena of neurovascular coupling (NVC).

Neurovascular coupling (NVC) represents the regional increase in cerebral blood flow in response to the metabolic demand of neurons and removes the resulting waste, being a fundamental aspect of brain health (Iadecola, 2017; Kaplan et al., 2020; Presa et al., 2020). A sensory stimulus or cognitive tasks are responsible for the increase in blood flow, controlled by various signalling pathways through NVC(Drew, 2022). NVC impairment has been documented in cerebral small vessel disease (Jokumsen-Cabral et al., 2019; Monteiro et al., 2021). Recent studies suggest a relationship between microcirculatory damage and cognitive impairment and dementia. Oxidative stress, mitochondrial dysfunction, and neuroinflammation contribute to cognitive processing impairment (Toth et al., 2017). The is also growing evidence that NVC is compromised

in neurodegenerative diseases such as Alzheimer's disease, frontotemporal dementia, amyotrophic lateral sclerosis, Parkinson's disease, and others (Iadecola, 2017). Therefore, the basal status of NVC could determine how well the patient recovers after a brain ischemic insult. The acute stroke will also affect neurovascular unit due to the accumulation of toxic material of disruption of BBB(Bernardo-Castro et al., 2020). Is it also documented that BBB damage extends beyond the ischemic area (Bernardo-Castro et al., 2020; Nian et al., 2020). Taken together, an intact neurovascular unit in the face of chronic vascular damage and after acute ischemic stroke could allow better changes of recovery and preserved brain health. Understanding how NVC correlates and is regulated in the post-stroke period is important for guiding therapies and interventions in conditions with cerebrovascular dysfunction.

NVC can be measured efficiently by Transcranial Doppler (TCD) and visual stimulus is the most studied paradigm (Azevedo E, 2007; Ball et al., 2024). This could offer the opportunity to evaluate the NVC status in challenging patients in acute stroke settings.

We aim to study NVC in the acute phase after stroke predicts cognitive function after 12 months (Clinicaltrials.gov ID: 06735274).

Methods

Ethics Considerations and Data availability

This study has been approved by the local institutional “Ethics Committee of Centro Hospitalar Universitário São João” (303/2020) and been registered to ClinicalTrials.gov (Clinicaltrials.gov ID: 06735274, https://www.clinicaltrials.gov/). In this study, written informed consents were obtained after a full description of the study to all participants and was performed in accordance with the Declaration of Helsinki's ethical principles. The data and SPSS syntax code and MATLAB script that support the findings of this study are available from the corresponding author upon reasonable request.

Participants

This is a prospective cohort study with the inclusion of consecutive ischemic stroke patients. Patients were recruited from the stroke unit, Centro Hospitalar Universitário São João, Porto, Portugal, between September 1, 2021, and August 31, 2022. Eligible patients had a previous Modified Rankin Score (mRs) <4 and able to be monitored with TCD within 72 hours after symptom onset. Exclusion criteria were: absence of adequate temporal bone window for TCD assessment; patients with a large cerebral infarct (greater than on third of the middle cerebral artery territory); advanced

kidney disease or hepatic diseases; active cancer patients; severe aphasia; severe hearing or visual loss; uncooperative patients; neurological diseases (including brain tumors, vascular or structural malformations and epilepsy); prior diagnosis of dementia, documented evidence of previous cognitive decline in the clinical record, or anti-demential drugs (e.g. anticholinesterase or memantine) or an Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) score >3.5 (Burton JK, 2021). A control group was recruited from our institution's professionals and relatives to match age, sex and vascular risk factors. They were assessed at a single time point, with NVC measured and sociodemographic and clinical information collected.

The follow-up assessment for the stroke patient group took place after 3±1 month and 12±1 month after onset symptoms, in outpatient. In the follow-up we excluded patients with kidney disease, active cancer patients, patients with severe aphasia and severe hearing loss that does not allow cognitive assessment.

Clinical, laboratorial and radiological assessment

Demographic and medical history were recorded at admission, including vascular risk factors, medications, previous cardiovascular disease, blood pressure (BP), serum glucose, cholesterol levels, reperfusion treatment (thrombolysis, endovascular procedures), and post-treatment grade 2b-3 according to the modified Thrombolysis in Cerebral Infarction scale (mTICI) (Higashida et al., 2003). National Institutes of Health Stroke Scale (NIHSS) scores were recorded at admission and 24 hours after onset. The presence of hemorrhage and infarct volumes were reviewed and calculated final infarct by planimetry, based on a head 24h-CT scan. Ultrasound studies were reviewed by experienced stroke physicians for the presence of carotid or middle cerebral artery ≥50% stenosis. At follow-up, were recorded blood pressure (BP), weight, height, abdominal circumference, medication, and new vascular events (Figure 1).

Neurovascular Evaluation

Cerebral blood flow velocity (CBv) was recorded at around 60 mm of depth the P2 segment of both segments of posterior cerebral artery (PCA) by transcranial Doppler (TCD) with 2-MHz TCD probes secured with a headframe (Doppler BoxX, DWL, Singen, Germany). Handedness was not considered in the assessment. NVC was determined by the peak relative increase in CBv during a three sessions of visual stimulus paradigm. Each paradigm was composed 5 repeated cycles ON- OFF (eyes closed) phases of similar periods. In the first session ON-OFF parts were faster with 5 seconds each; the next session ON-OFF periods were 10 seconds; and the last session, ON-OFF periods were slower with 20 seconds each.

This protocol allows the evaluation of the fast NVC responses (5 seconds) to more slower and maximum magnitude (20 seconds) CBv increases trough spectral analysis from the systolic PCA trends exported from the TCD device with excellent correlation against classic overhsoot (Ferreira et al., 2024) (supplementary figure 1 and supplementary figure 2). PCA 20 was considered the reference paradigm because classic overshoot occurs within this time frame (Castro et al., 2020).

Evaluations were carried out in a dim lighted room, ambient temperature and with bed inclined at 90º/sitting position. Subjects were refrained from caffeine, alcohol, exercise, or vasoactive drugs for at least 12 hours before evaluation. During visual stimulating ON period the patient was instructed to open his eyes and look at a flickering checkerboard at a frequency of 2 Hz, displayed in a monitor with resolution 2360 x 1640 pixels (iPad Air, 10.9 inches) at two meters of distance. The visual paradigm for assessing NVC is easier to use and reproduce in other studies, with less interference from the patient's cognitive abilities (Ferreira et al., 2024). The analysis was made offline in professional software tool (MATLAB, The MathWorks, Natick, MA, USA).

Cognitive and Functional Assessment

Previous cognitive decline was screening through the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) score (van Nieuwkerk et al., 2021). A score of < 3.5 was used as a cut-off to exclude patients.

The degree of disability or dependence in the daily activities of patients after a stroke was measured by the Modified Rankin Scale (mRS) at three- and 12-months post-stroke and by the Instrumental Activities of Daily Living (IADL) Scale (Araújo F., 2008; Justo-Henriques S, 2022; Lawton MP, 1969). Cognitive status at 12 months of follow-up was based in 7-scale cognitive score was operationalized from Montreal Cognitive Assessment (Supplementary Table 1) (Katz et al., 2021; Wardlaw et al., 2021; Wardlaw et al., 2023). The 7-level cognitive score is operationalized based on the MoCA and incorporates the functional impact on activities of daily living after stroke, including an assessment of multiple cognitive domains.

Clinical endpoints

The main outcome was the cognitive status at 12 months of follow-up which was based in 7-scale cognitive score was operationalized from Montreal Cognitive Assessment (Supplementary Table 1). As secondary endpoints, we compared NVC values over time (acute phase, three months, and 12 months after stroke), as well as the correlation of NVC in the chronic phase post-event with cognitive status at the time of NVC assessment, based on same a 7-scale cognitive score.

Statistical Analysis

The normality of continuous variables was assessed using the Kolmogorov–Smirnov test. Baseline characteristics between patients in the healthy control group and post-stroke patient group were compared using chi-square/Fisher exact tests for categorical variables and Mann-Whitney tests for continuous variables. To compare hemispheres in the post-stroke patient group, differences between hemispheres were obtained from a Paired Samples t-test for dependent samples (Table 1). To study the relationship of NVC with outcome We conducted a Mixed Model Analysis with adjustment for multiple comparisons to compare differences in hemodynamics characteristics across time in post-stroke patient group (Figure 3).

A Generalized Linear Mixed Model (GLMM) for Cognition Score 12 months after stroke was conducted (Table 2). A univariate model and two adjusted models were calculated. The adjusted GLMMs accounted for age, years of education, hypertension, diabetes, metformin use, statin use, heart failure, peripheral arterial disease, beta-blocker use, and prior coronary artery disease. Odds ratio and CI95% was calculated with ordinal output cumulative logit link functions. Statistical analyses were carried out with IBM SPSS Statistical version 28 and p-values<0.05 were taken as statistically significant.

Sample size calculation

Base on normative data(Ferreira et al., 2024; Madureira et al., 2017) and for an expected normal NVC of 15±5 and drop-off of 40% for technical reasons and considering a CI95% and marginal error of 5%, we estimate to include 40 controls as a reference group.

The sample size for the stroke cohort was calculated based on the prevalence of long-term cognitive impairment after stroke. Previous studies have shown that about 50 to 60% of patients develop cognitive impairment following a stroke (Exalto et al., 2023; Nijsse et al., 2017). Considering a 95% confidence interval, a margin of error of 5%, and an estimated 40% loss to follow-up, we aim to include 170 patients.

Sample size calculation was made with G*Power v.3.1.9.7.

Results

A total of 186 patients were recruited in the study. Sixteen patients were excluded due to poor-quality TCD recordings and an additional 26 were lost to follow-up (Figure 2). The final analysis involved a total of 144 acute stroke subjects and 40 controls.

The sociodemographic characteristics were similar between the stroke patient group and the control group, except for years of education, which were significantly higher in the control group. The stroke patient group comprised 67% anterior circulation. Regarding systemic hemodynamics, no differences were observed in systolic blood pressure (SBP), but differences were found in diastolic blood pressure (DBP), with a lower average in the stroke patient group (p-value=0.01) (Table 1).

Cerebral hemodynamics

The mean baseline PCA CBv was higher in the stroke patient group compared to the control group (p=0.03) (Table 1). There were no significant differences in the NVC between the hemisphere affected and the unaffected hemisphere in the stroke group. Considering the NVC responses and midrange, PCA10 was significantly higher than controls (13.7±3.1 versus 15.0±5.1, p<0.01). No differences were found a PCA5 (p=0.06) and PCA20 (p=0.21).

NVC responses tend to decrease overtime until 12-months follow-up after the event, although this was not significant with PCA5 only (p=1.00). Between time T0 and T2 there was also a decrease in the basal CBv (p<0.01) (Figure 3).

There is no association between the age of the stroke group and PCA5 (p=0.09), but an association is observed with years of education (p=0.01). The time from symptom onset to hospital admission is associated with PCA5 (p<0.01), as is the time from symptom onset to recanalization, having a history of coronary disease (p<0.01), peripheral vascular disease (p=0.03), beta-blockers use (p=0.04) and taking antidepressants (p<0.01). There is no association between PCA5 and sex (p=0.43), NIHSS at admission (p=0.15), NIHSS at 24 hours (p=0.89), hypertension (p=0.63), diabetes mellitus (p=0.55), dyslipidaemia (p=0.93), previous stroke (p=0.37), or the stroke circulation (p=0.70) (Supplementary Table 2).

The age of the stroke group shows no association with PCA10 (p=0.98), nor do years of education (p=0.83), NIHSS at admission (p=0.97), NIHSS at 24 hours (p=0.10), hypertension (p=0.35), diabetes mellitus (p=0.87), dyslipidaemia (p=0.87), previous coronary artery disease (p=0.41), time from symptom onset to recanalization (p=0.08), or peripheral vascular disease (p=0.20). However, PCA10 in the stroke group is associated with statin use (p<0.01), time from symptom onset to hospital admission (p<0.01), antidepressant use (p<0.01), previous stroke (p<0.01), heart failure (p<0.01), glycaemia value at admission (p=0.01) and sex (p<0.01) (Supplementary Table 2).

Regarding PCA20, the stroke group shows no association with age (p=0.30), years of education (p=0.52), NIHSS at admission (p=0.76), NIHSS at 24 hours (p=0.62), hypertension (p=0.25), diabetes mellitus (p=0.89), dyslipidaemia (p=0.47), previous

stroke (p=0.07), previous coronary artery disease (p=0.93), heart failure (p=0.30), peripheral vascular disease (p=0.48), stroke circulation location (p=0.67), or time from symptom onset to recanalization (p=0.16). The time from symptom onset to hospital admission (p=0.02), statin use (p<0.01), antidepressant use (p=0.02), and sex (p=0.01) in the stroke group were associated with PCA20 (Supplementary Table 2)

NVC in acute phase after stroke and long-term cognition

At 12 months after stroke, only about 20% of our sample exhibited normal cognition (Figure 4). The distribution per group of cognitive score were from normal to higher, total class1 9.7%, class 2 18.1%, class 3 7.6%, class 4 31.3%, class 5 15.3%, class 6 14.6% and class 7 3.5%. Baseline factors associated with cognitive function were age (p<0.00), years of education (p<0.01), diastolic blood pressure (p=0.01), glycemia value (p=0.02) and NIHSS at admission (p<0.00), NIHSS at 24-hours after stroke (p=0.01), time from symptom onset to recanalization (p<0.01), hypertension (p<0.01), diabetes mellitus (p=0.02), previous stroke (p=0.04), heart failure (p<0.01) and depression (p=0.03).

The results of the GLMM analysis showed a significant association between the 7-level cognitive score variable and quintile of NVC. As compared to the refence quintile (2nd according to control data) PCA20 of 5th (CI95% 2.29 (1.23-4.28), p=0.00) and 1st quintiles of NVC (CI95% 0.53 (0.29-0.98), p=0.04) were significantly related to worse cognitive levels at 12 months in multivariate model (Table 2).

The PCA5 and PCA10 showed similar results, higher NVC values show a statistically significant association in the adjusted model with a worse outcome in the 7-level cognitive scores at 12 months post-stroke (quintile 5 versus quintile 2 CI95% 1.94 (1.03-3.63), p=0.03; CI95% 2.51 (1.33-4.70), p=0.00, respectively) (Table 2).

Discussion

In this prospective cohort study, we included 144 stroke patients and 40 matched controls, measured NVC with TCD with multiple visual paradigm sessions at baseline, 3 and 12 months and assessed the association with cognitive outcome at 12 months post-stroke. It was found that the NVC tended to be increased in acute stage of stroke, there after decreasing over time. Importantly, NVC exhibited a U-shaped association with long-term cognitive outcomes. Patients in both the lowest and highest quintiles of NVC magnitude had significantly worse cognitive scores at 12 months compared to those in the reference quintile and paradigm.

NVC after stroke and long-term cognition (first things first)

Our results indicate that extreme values of NVC, both the highest and the lowest, are associated with worse cognitive outcome at 12 months after the event (Table 2). PSCI is a prevalent condition but with few modifiable factors to intervene one specially in acute stage of stroke (Quinn et al., 2021; Stackhouse & Mishra, 2021). Our results support the fact that NVC deregulation at acute stroke can contribute to PSCI (Stackhouse & Mishra, 2021).

Impairment of NVU and NVC mechanisms as cause of cognitive impairment is well documented in literature (Iadecola, 2017; Schaeffer & Iadecola, 2021). Alterations in the NVU can result in cerebral hypoxia due to a decrease in cerebral blood flow below the level required for adequate brain oxygenation; they can lead to a limited supply of nutrients to the brain and impair the elimination of unwanted metabolites, resulting in the accumulation of molecules such as beta-amyloid and tau due to blood-brain barrier dysfunction. Additionally, the reduction in the production of trophic factors by the cells that comprise this unit may increase their vulnerability to diseases (Iadecola, 2017).

High and low NVC can have different causes. In acute stroke loss of BBB integrity occurs rapidly within 6-72hours (Bernardo-Castro et al., 2020). Even after reperfusion of ischemic cells, the oxidative stress resulting from this process is catastrophic for the neurovascular unit, leading to a failure in the normal functioning of ion channels. These changes in the ionic gradient cause excessive neuronal depolarization, excess glutamate, and excitatory neurotransmitter that increases the toxic level of calcium (Cai et al., 2017). Thus, hyperresponsive of NVC can be in line reperfusion injury. The neuroinflammatory response that occurs in the acute phase (6-72 hours after the event) (Ferro et al., 2021) triggers an exacerbation of the BBB, further increasing brain reperfusion damage (Bernardo-Castro et al., 2020). This is especially true in stroke patients which are general old. In the elderly population, there is a greater harmful immune response in the inflammatory phase and a poorer regenerative capacity in the chronic phase, explaining the worse cognitive outcome after the stroke(Bernardo-Castro et al., 2020; Cai et al., 2017; Shi et al., 2019).

The increased NVC response may not be merely compensatory but may also reflect repair processes occurring during the subacute phase, particularly angiogenesis. This mechanism restores blood flow, leading to oxygen supply and normal metabolism in the ischemic tissue. However, when it occurs too early after stroke, it may result in an increased risk of haemorrhagic transformation (Bernardo-Castro et al., 2020).

A low response of NVC might be related to poor brain reverse capacity has documented in patients with cerebral small vessel disease (Castro et al., 2020; Monteiro et al., 2024) although can be simply a manifestation of deregulated NVC.

The U-shaped response is resembling both blood pressure and cerebral autoregulation relation with outcome. These also follow the rule that extreme values (Tikhonoff et al., 2009) of deviation from optima BP are worse (Castro et al., 2021). As, depicted in figure 4, this represents a U-shaped curve, that is, very high or very values compared to normal control, will result in worse 7-level cognitive scores at 12 months post-stroke (Figure 4). We firstly show that NVC as a mechanism of cerebral vasomotor regulation has similar behaviour. It should be said that BP levels did not affect multivariate model results.

Cerebral blood flow is regulation is orchestrated by interlinked mechanism, not only NVC, but also dynamic cerebral autoregulation (dCA) maintaining constant cerebral blood flow despite fluctuations in BP, and chemical regulation to metabolites and gases in blood stream usually assessed by CO2 vasoreactivity (Azevedo & Castro, 2016). dCA has been shown to be a predictor of functional outcome three months post-stroke — that is, impaired dCA in the acute phase of stroke has been associated with poorer functional outcomes (Nogueira et al., 2021). A previous study investigating both dCA and NVC showed that CBv regulation is significantly impaired in acute stroke and becomes further compromised as stroke severity increases. Preservation of dCA and NVC was associated with better functional recovery at three months post-event (Salinet et al., 2019).

In the acute phase following stroke, preserved dCA may play a crucial role in restoring the penumbra area by buffering blood pressure fluctuations through the resistance vessels in the reperfused region. Additional ischemic damage, edema, and hemorrhagic transformation may be prevented by intact dCA (Castro, Azevedo, et al., 2017; Castro, Serrador, et al., 2017; Nogueira et al., 2021). Further studies are needed to better understand this interaction and to assess its prognostic value for long-term outcomes.

Overactive Neurovascular Coupling in Acute Phase of Stroke

The NVC showed higher responses in stroke group comparing to controls. This was evident in PCA10 (Table 1). The hyperreactivity of CBv could be related to generalized vasomotor deregulation and BBB leakage that occurs after stroke (Bernardo-Castro et al., 2020; Prakash & Carmichael, 2015). Previous studies have shown statistically significant differences in NVC values between the control group and patients with acute stroke, with NVC values being higher in the control group. However, it is important to note that this comparison is made between stroke patients and healthy individuals without cardiovascular risk factors (Salinet, Robinson, et al., 2013; Salinet et al., 2019).

In acute stroke is expected that NVU to be deregulated. The sudden hypoxia that occurs in the hyperacute phase causes cytotoxic edema, leading to increased permeability of the blood-brain barrier (BBB) (up to 6 hours after the event), which interferes with the dynamics responsible for maintaining the NVU and NVC status (Bernardo-Castro et al., 2020; Cai et al., 2017). This is in line with studies that show increased CBv in affected territory (Baracchini et al., 2019; Castro, Azevedo, et al., 2017). This study showed that the deregulation can be found widespread outside infarct area. Even with the lesion in another location, the NVC measured in the PCA shows this alteration, suggesting that focal ischemic stroke induces pathological disturbances in both the ipsilateral hemisphere and contralateral areas of the brain. Our data seem to support that microvascular damage in the subacute phase likely reveals ischemic diaschisis, which should be considered (Garbuzova-Davis et al., 2013).

Previous studies have shown that NVC remains decreased for at least a decade after the initial ischemic event, which corroborates our data showing a decrease in NVC values over time. This decrease in NVC was observed in a generalized manner throughout the brain (Stackhouse & Mishra, 2021), as observed in our sample. The fact that was no difference between hemisphere support the view (Table 1). This cannot be attributed to vascular risk and previous status, as this response decreased overtime and control were also match for this factors, particular arterial hypertension and diabetes (Monteiro et al., 2021; Morrison & Filosa, 2019).

NVC trends in time

In our sample, a decrease in NVC over time was observed in patients with stroke in both the anterior and posterior cerebral circulation, as assessed by % CBv in the P2 segment of the PCA. In Castro study (Castro, Azevedo, et al., 2017) cerebral autoregulation parameter also recovered until 3 months. The behaviour of NVC in the acute phase describes what occurs physiologically in the BBB and the components of the neurovascular unit in the acute phase and how these changes translate into impaired NVC, but the role of functional hyperaemia is still unclear (Bernardo-Castro S, 2023). These could be the general trend tower recovery of vasomotor function and BBB status. Finally, in the subacute phase (between 72 hours and 6 weeks), mechanisms of BBB repair such as neuroangiogenesis take place. After six weeks, in the chronic phase, there is a decrease in BBB permeability (Bernardo-Castro et al., 2020; Cai et al., 2017; Kaplan et al., 2020). We could not detect statistically the influence of post-stroke medication which could be responsible for the in NVC trends (Supplementary Table 2).

The metabolic signals from neurons do not completely dominate the control of cerebral vasculature, which raises questions about whether this metabolic activity is the

primary role of functional hyperaemia. Previous studies have shown that vasodilation itself is not necessarily required for increased brain oxygenation (Drew, 2022). did not significantly impair absolute cerebral blood volume, but they improved cerebrovascular reactivity (Webb, 2019). From our sample, 62% of patients had hypertension, and 22% were taking calcium channel blockers, while 44% were taking Angiotensin-converting enzyme or angiotensin receptor blockers inhibitors at the time of TCD monitoring (Table 1).

The behaviour of NVC following a stroke describes the physiological changes that occur in the BBB and the components of the neurovascular unit, as previously mentioned in this discussion. In our study, we found an association between the time elapsed from the onset of symptoms and hospital admission (Supplementary Table 2), indicating that the duration from the onset of the event influences the NVC values. This, in turn, reflects the variation observed during the acute phase (Bernardo-Castro S, 2023). A previous stroke also showed an association with the NVC value, supporting earlier studies that indicate a change in NVC in cerebrovascular diseases(Cai et al., 2017).

There is no association between the age and NVC value (Supplementary Table 2). Age significantly affects the integrity of the neurovascular unit, increasing vulnerability to brain injuries. The cellular damage caused by ageing can further exacerbate brain injury and accelerate its progression (Iadecola, 2017); however, in our sample, no correlation was found with the NVC value. Sex and age are two variables that have been associated with NVC in previous studies (Koep et al., 2023). In our study, age did not show an association, but the sex of the patients was associated with PCA20.

In our study, an association was found between prior statin use and the PCA10 and PCA20 values (Supplementary Table 2). Statins improve endothelial function and have antithrombotic and anti-inflammatory effects. Previous studies indicate an improvement in vasoregulation in patients undergoing this therapy(Rosengarten et al., 2007).

Previous coronary artery disease and heart failure showed an association with NVC values in our sample (Supplementary Table 2). Previous studies demonstrate that the neurovascular unit in individuals with chronically reduced cardiac pumping capacity is severely dysfunctional (Aires et al., 2020). An association was also found with the use of beta-blockers, a class of medication primarily used in the treatment of cardiovascular diseases, which has already been shown to influence NVC values (Farzam K, 2023).

The blood glucose level at admission was associated with the PCA10 value (Supplementary Table 2). Hyperglycaemia induces oxidative stress, and the adverse effects of hyperglycaemia on the neurovascular unit are well known. In our study, no association was observed with diabetes mellitus. Clinical studies report inconclusive

associations between cerebral blood flow in patients with type II diabetes mellitus, as evaluated by transcranial Doppler and functional MRI. The use of empagliflozin, which improves the neurovascular unit by inhibiting the sodium-glucose transporter, also did not show an association with NVC values in our study (Barloese et al., 2022).

Regarding antidepressants, previous studies show that serotonin influences cerebral blood supply and can have both vasoconstrictive and vasodilatory effects. Variations in its concentration affect vascular tone and, consequently, the vascular response to neuronal activity (Harris & Reynell, 2017). In our study, an association was found between the use of antidepressants and the PCA5, PCA10 and PCA20 values (Supplementary Table 2).

The location of the stroke did not show a relationship with PCA5, PCA10, or PCA20 (Supplementary Table 2). NVC value was measured in the P2 segment of the PCA, and the location of the stroke in either the anterior or posterior circulation did not show an association with the measured value. As discussed, these findings support the idea that microvascular damage in the subacute phase likely reflects ischemic diaschisis, meaning that regions not directly affected by injury exhibit metabolic and functional changes (Garbuzova-Davis et al., 2013).

Strengths

The main strength of the study lies in the follow-up of patients up to 12 months post-stroke and in comparing the NVC value with a control group with the same baseline cardiovascular risk factors as the post-stroke patient group. A rigorous control of potential confounders was implemented, as well as a validated easy to implement TCD monitoring protocol, adequate to acute stroke patients. This approach mitigated most of the confounding factors related to NVC assessment with TCD. Our cohort seems to have external validity, since it shows similar influence of specific baseline predictors of in cognitive impairment Years of education and age were variables considered in the GLMM (Table 2) and were shown to have an impact on cognitive function 12 months after the stroke (Heutz et al., 2023; Mukli et al., 2024; Ogoh, 2017). Also, cardiovascular risk factors also had statistic ant impact on cognition after stroke (Supplementary Table 3) in accordance with other (Heutz et al., 2023; Mukli et al., 2024; Ogoh, 2017). Our study contributes to clarifying the role of NVC, given its alterations in the acute phase of stroke, and its role in the long-term cognitive dysfunction observed in these patients. Experimental studies emphasize the need for clinical translation of findings. Understanding the mechanisms underlying NVC dysregulation could potentially help guide acute stroke care decisions and the development of new therapeutic targets.

Future studies may help clarify the correlation of increased NVC with patients' alertness status and how this may impair their long-term cognitive performance.

Limitations

Our study assessed the cognitive and functional performance of patients following stroke, but the previous evaluation of these performances was solely based on reports from family members. To exclude patients with pre-existing cognitive decline, we used the IQCODE and excluded patients with significant limitation mRS≥4. However, as mentioned, these scales are subjective and based on family reports.

The increase in blood flow during sleep is substantially greater than that which occurs with an alert brain, so regardless of the purpose of functional hyperaemia, it is greater during sleep (Drew, 2022). We cannot exclude that higher NVC results could be found in more severe stroke patients related to the state of alertness. However, all multivariate models were adjusted to stroke severity.

Given the absence of established normative values for NVC, we explored the relationship between categorized NVC (based on the sample distribution) and cognitive status. NVC was stratified into five classes corresponding to quintiles, and generalized linear mixed models were used to assess associations with cognitive function classes. Although using quintiles instead of the original continuous data may reduce statistical power and limit reproducibility, it allows for the detection of non-linear relationships and is considered a valuable approach in exploratory analyses. Continuous data, especially in the presence of outliers, can be heavily influenced by extreme values. Categorizing data into quantiles mitigates this influence by grouping the data, thereby making the analysis more robust to extreme observations that could otherwise skew the results in a continuous model. We also applied a log transformation to continuous variables, which yielded similar results; however, this approach has some limitations. This is particularly concerning in the context of hemodynamic data from acute stroke, where variability is high and results are strongly influenced by outliers.

Moreover, when comparing with normative data from the control group, we observed that stroke patients in quantile groups 2 and 3 (Q2–Q3) showed values similar to those of controls. These quantiles therefore served as excellent reference points for comparison. Due to the structure and non-normalized nature of the data, we believed that using continuous variables might introduce spurious findings and complicate clinical interpretation. Further studies are needed to validate these findings and refine thresholds for clinical application.

We assessed CBv in PCA territory outside affect territory as a marker of global impairment of NVC. Studies also show impairment in stroke affected territory but are less

standardized that PCA stimulus. The majority of our study sample (67%) consists of patients who suffered a stroke in the anterior circulation. We included patients with both anterior and posterior circulation strokes, and we studied NVC as a global marker. By assessing NVC globally, we avoided confounding effects related to the disturbed cognitive state of the participant and were able to evaluate NVC as an overall indicator of dysfunction. However, cognitive function is more complex than visually evoked responses alone. Therefore, future studies should be conducted in different vascular territories and using different paradigms to better explore NVC after stroke.

In our TCD assessment, we used a validated and easy-to-implement TCD monitoring protocol, suitable for acute stroke patients. Although TCD offers high temporal resolution, allowing us to capture fluctuations in CBv, it has spatial limitations. The CBv measurement is an indirect method that uses ultrasound waves to detect the linear velocity of red blood cells at a specific time and in a specific vessel. This measurement assumes that changes in CBv are proportional to changes in cerebral blood flow (Skow et al., 2022).

Regarding infarct imaging assessment, the presence of hemorrhage and infarct volumes was reviewed, and final infarct volume was calculated by planimetry based on a 24-hour follow-up CT scan. Although magnetic resonance imaging was used in some cases—particularly to confirm very small lacunar infarcts not visible on CT—the majority of assessments relied on CT.

Conclusions

NVC assessment with TCD in acute stroke could provide information on long-term cognitive status. Modulating CBv responses might be the focus of future trials to enhance post-stroke cognitive impairment.

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Figure Captions

Figure 1. Evaluation Protocol: 1 – Evaluation at acute phase (t0); 2 – Evaluation 3±1 months after onset (t1); 3 – Evaluation 12±1 months after onset (t2); (Created with BioRender.com)

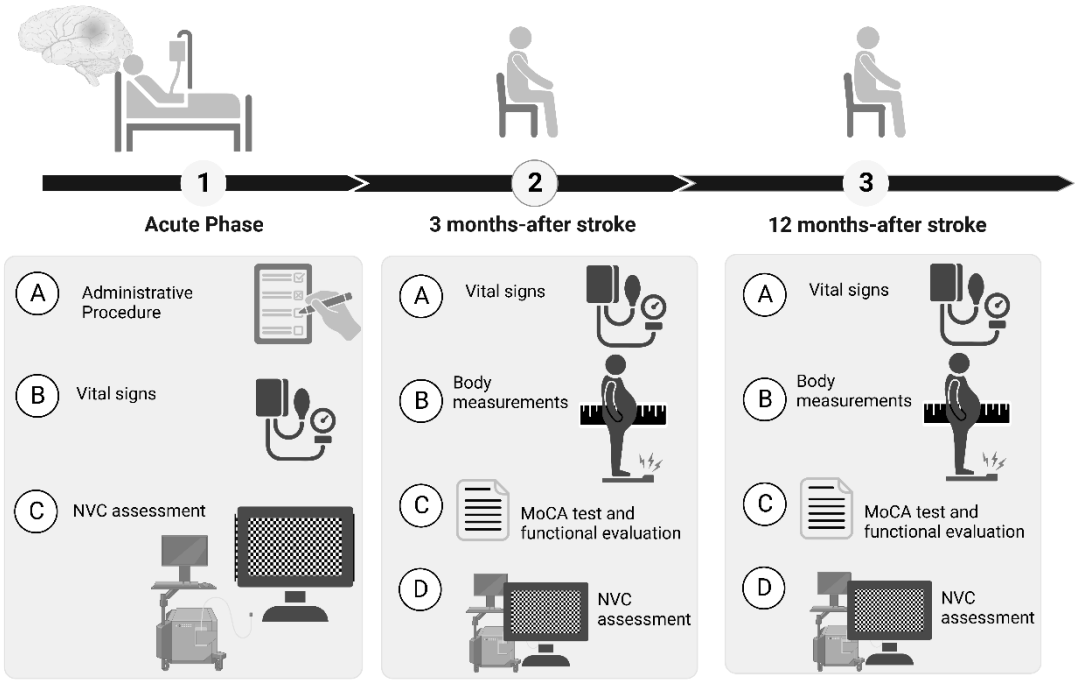


Figure 2. Flowchart of patient inclusion and exclusion after stroke

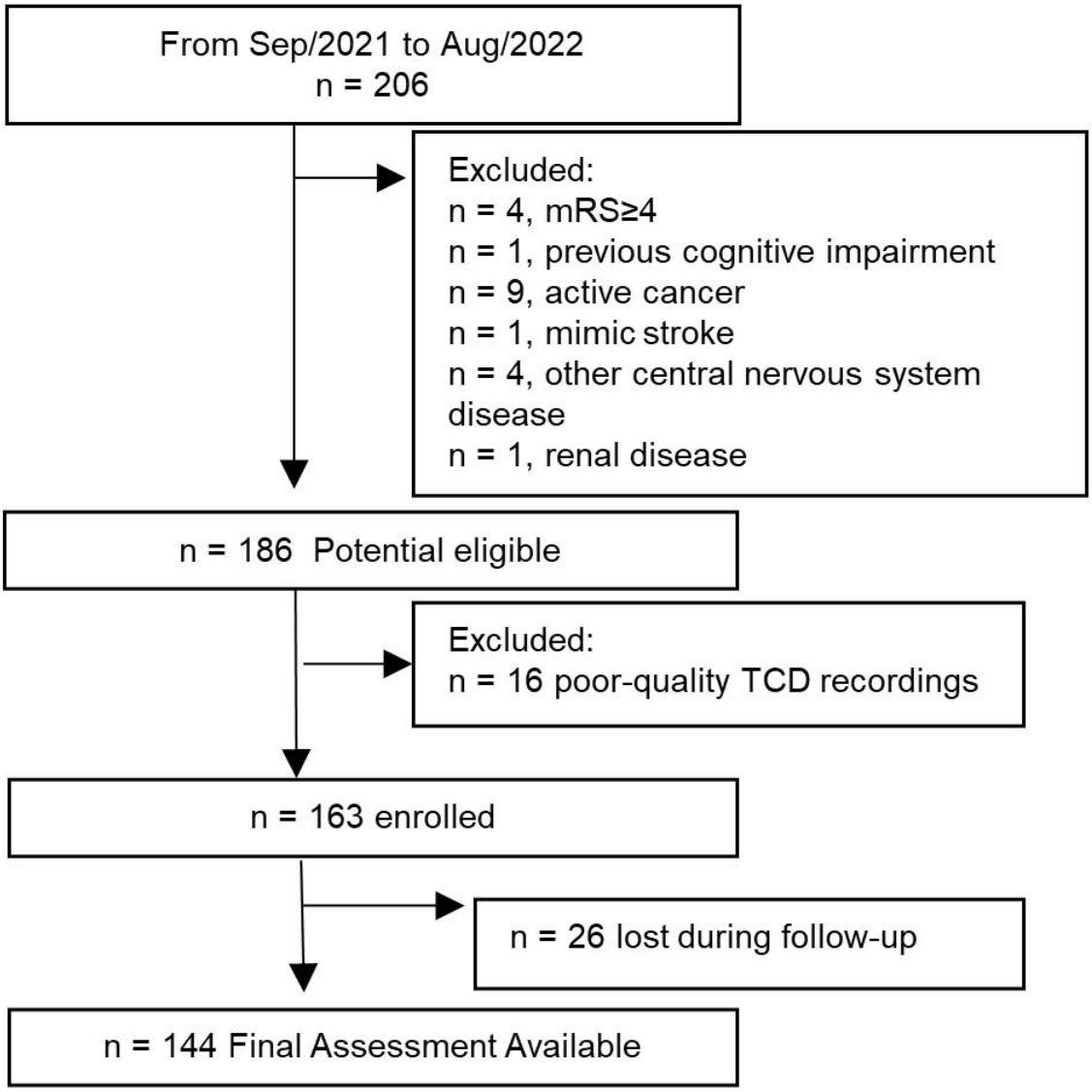


Figure 3. Differences in hemodynamics characteristics across time in stroke group. CBv = Cerebral Blood Flow Velocity; NVC = Neurovascular Coupling; t0 = Acute phase after stroke (until 72 hours after onset); t1=3±1 months after stroke; t2 = 12±1 months after stroke; *P-value obtained from Mixed Model Analysis with Adjustment for multiple comparisons: Bonferroni.

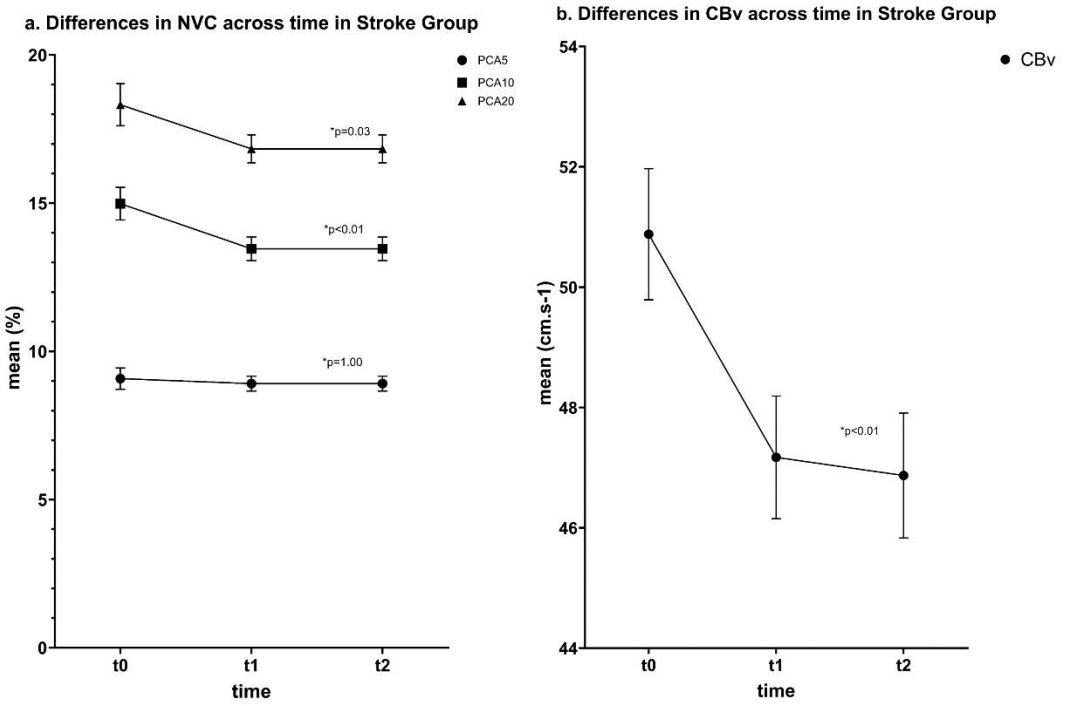
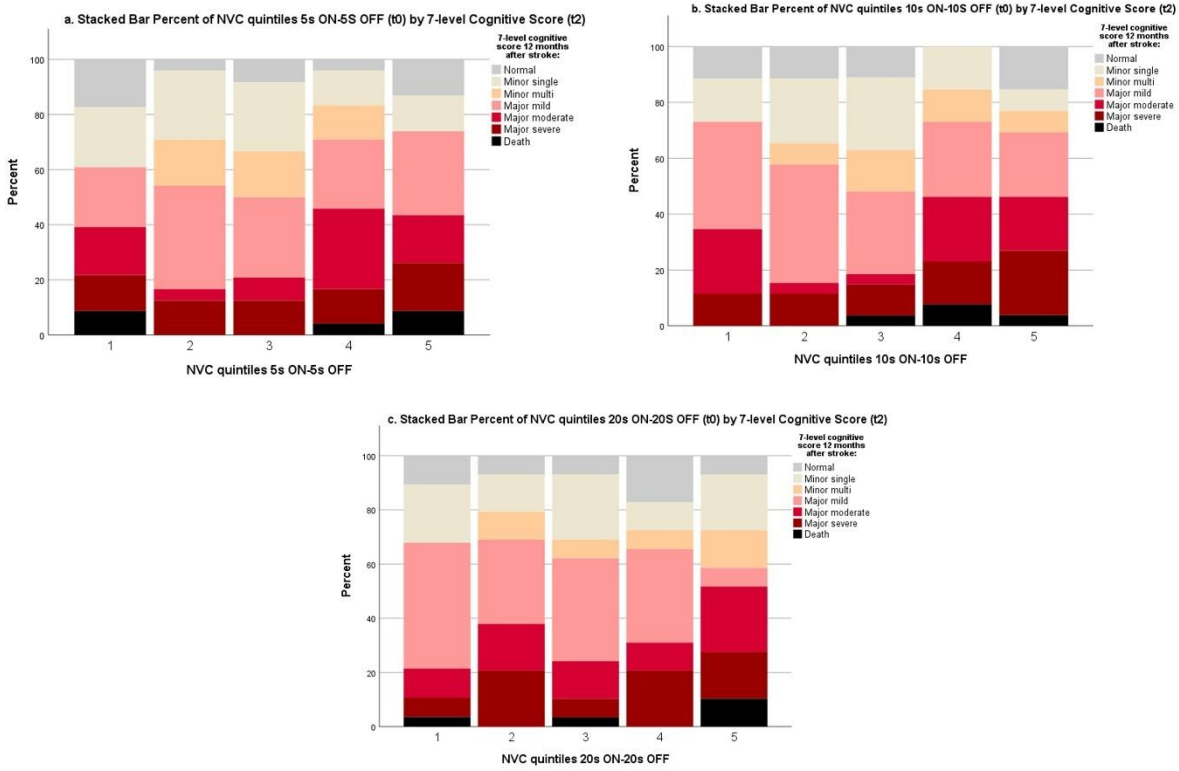


Figure 4. Cognitive Score Classes Distribution 12 months after onset by Quintiles of NVC



Tables.

Table 1. Demographic, clinical, and baseline hemodynamics characteristics of all participants and differences between group– - healthy control group and post-stroke patient group.

	Total	Controls	Stroke	*p-value
	N=184	N=40	N=144	
Demographic and clinical variables				
Age, years, median (range)	66 (58-76)	70 (60-76)	66 (58-75)	0.26
Male sex, n (%)	124 (67)	25 (63)	99 (69)	0.45
BMI, Kg.m^{-2}, median (range)	26.4 (24.2-29.8)	25.6 (23.1-29.4)	26.7 (24.6-30.1)	0.51
Years of Education, median (range)	6.0 (4.0-11.0)	9.0 (4.0-13.5)	5.0 (4.0-10.0)	0.00
Hypertension, n (%)	111 (61)	23 (58)	88 (62)	0.71
Dyslipidemia, n (%)	90 (49)	19 (48)	71 (50)	0.85
Diabetes, n (%)	39 (21)	6 (15)	33 (23)	0.38
Smoking, n (%)	40 (22)	10 (25)	30 (21)	0.66
Previous Stroke, n (%)	-	-	14 (10)	-
Previous Depression, n (%)	-	-	17 (12)	-
Coronary Disease, n (%)	-	-	6 (4)	-
Heart Failure, n (%)	-	-	6 (4)	-
Peripheral Artery Disease, n (%)	-	-	7 (5)	-
Glycemia, mg/dL, median (range)	-	-	118 (100-141)	-
Onset-To-TCD-Time, hours, median (range)	-	-	40 (26-62)	-
Medication				
Mono Antiplatelet Therapy, n (%)	91 (49)	8 (20)	83 (58)	0.00
Dual Antiplatelet Therapy, n (%)	41 (22)	0 (0)	41 (28)	0.00
Anticoagulants, n (%)	134 (73)	1 (3)	133 (92)	0.00
Low Statin, n (%)	21 (11)	20 (50)	1 (1)	0.00
High Statin, n (%)	135 (73)	0 (0)	135 (94)	0.00
Beta Blockers, n (%)	48 (26)	7 (18)	41 (28)	0.22
Calcium Channel Blockers, n (%)	31 (17)	0 (0)	31 (22)	0.00
ARBs or ACE inhibitors, n (%)	84 (46)	21 (53)	63 (44)	0.37

Sacubitril, n (%)	2 (1)	0 (0)	2 (1)	1.00
Antihypertensive Drugs, n (%)	20 (11)	4 (10)	16 (11)	1.00
Metformin, n (%)	29 (16)	5 (13)	24 (17)	0.62
DPP-4 inhibitors, n (%)	12 (7)	0 (0)	12 (8)	0.07
SGLT2 inhibitors, n (%)	6 (3)	0 (0)	6 (4)	0.34
GLP-1, n (%)	2 (1)	0 (0)	2 (1)	1.00
Other Antidiabetic, n (%)	4 (2)	1 (3)	3 (2)	1.00
Insulin, n (%)	-	-	5 (3)	-
Antidepressants, n (%)	-	-	14 (10)	-
Benzodiazepines, n (%)	-	-	39 (27)	-
Stroke Characteristics				
Baseline NIHSS score, median (range)	-	-	5 (2-8)	-
24 hours after onset NIHSS score, median (range)	-	-	3 (2-6)	-
Anterior Circulation, n (%)	-	-	97 (67)	-
Etiology - Large-artery atherosclerosis, n (%)	-	-	26 (18)	-
Etiology - Cardioembolism, n (%)	-	-	26 (18)	-
Etiology - Small-vessel disease, n (%)	-	-	52 (36)	-
Etiology - Stroke of other determined etiology, n (%)	-	-	14 (10)	-
Etiology – Dual cause, n (%)	-	-	3 (2)	-
Etiology - Stroke of undetermined etiology, n (%)	-	-	50 (35)	-
Infarct Largest Size, mm, median (range)	-	-	5 (3-15)	-
Onset-to-Recanalization-time, min, median (range)	-	-	358 (257-564)	-
Systemic hemodynamics				
SBP, mmHg, median (range)	-	133 (116-143)	133 (120-149)	0.45
DBP, mmHg, median (range)	-	79 (71-87)	74 (65-82)	0.01
HR, bpm, median (range)	-	65 (61-76)	67 (61-78)	0.65
Cerebral hemodynamics				

Basal CBv in affected hemisphere, $cm.s^{-1}$, mean±sd	-	-	52.4±16.9	0.11§
Basal CBv in unaffected hemisphere, $cm.s^{-1}$, mean±sd	-	-	50.7±13.7	
Basal CBv, $cm.s^{-1}$, mean±sd	-	46.7±9.7	58.8±13.4	0.03
Affected hemisphere NVC, % CBv 5s ON-5s OFF, mean±sd	-	-	8.8±2.8	0.08§
Unaffected hemisphere NVC, % CBv 5s ON-5s OFF, mean±sd	-	-	9.4±4.1	
Mean of both hemispheres NVC, % CBv 5s ON-5s OFF, mean±sd	-	9.8±3.9	9.1±3.1	0.06
Affected hemisphere NVC, % CBv 10s ON-10s OFF, mean±sd	-	-	15.7±6.2	0.36§
Unaffected hemisphere NVC, % CBv 10s ON-10s OFF, mean±sd	-	-	15.9±5.9	
Mean of both hemispheres NVC, % CBv 10s ON-10s OFF, mean±sd	-	13.7±3.1	15.0±5.1	<0.01
Affected hemisphere NVC, % CBv 20s ON-20s OFF, mean±sd	-	-	18.2±7.7	0.07§
Unaffected hemisphere NVC, % CBv 20s ON-20s OFF, mean±sd	-	-	19.2±7.1	
Mean of both hemispheres NVC, % CBv 20s ON-20s OFF, mean±sd	-	19.3±5.5	18.3±6.8	0.21

Continuous data presented as median (interquartile range) or mean ± standard deviation.

Abbreviations: BMI = Boy mass index; ACE= Angiotensin-converting enzyme; ARBs=Angiotensin receptor blockers; DPP-4 = Dipeptidyl Peptidase IV; SGLT2=Sodium-glucose cotransporter-2; GLP-1=Glucagon-like peptide-1; MCA=middle cerebral artery; NIHSS = National Institutes of Health Stroke Scale; TCD = transcranial Doppler; SBP= Systolic Blood Pressure; DBP= Diastolic Blood Pressure; HR= Heart Rate; NVC = Neurovascular Coupling; CBv = Cerebral Blood Flow Velocity; IQR indicates interquartile range;

Dashes (-) **=** value not available due to exclusion criteria for the control group, not applicable, and/or data not collected in the control group.

*P-values are shown for differences between groups obtained from chi-square test/ Fisher's exact test for categorical variables, or Mann-Whitney test for continuous

variables; §P-values are shown for differences between hemispheres obtained from Paired Samples t-test for dependent samples differences.

Table 2. Multivariable Model (GLMM) for 7-Level Cognition Score 12 months after stroke.

		Adjusted Model		Univariate Model	
Total N=144		aOR (95% CI)	P-value	OR (95% CI)	P-value
NVC, % CBv – 5s ON-5s OFF	4.64%-7.53%	1.62 (0.75-3.48)	0.21¥	1.32 (0.76-2.27)	0.30
	7.54%-9.1%	0.86 (0.49-1.51)	0.61	0.92 (0.56-1.51)	0.75
	9.2%-10.67%	2.62 (1.57-4.35)	0.00	2.06 (1.25-3.41)	0.00
	>10.67%	1.94 (1.03-3.63)	0.03	2.10 (1.23-3.58)	0.00
NVC, % CBv – 10s ON-10s OFF	6.1%-12.3%	1.14 (0.60-2.17)	0.66§	1.71 (1.05-2.80)	0.03
	12.4%-15.0%	0.91 (0.51-1.65)	0.77	0.93 (0.57-1.51)	0.78
	15.1%-17.7%	1.99 (1.04-3.81)	0.03	2.83 (1.73-4.64)	0.00
	17.8%-23.0%	2.51 (1.33-4.70)	0.00	2.38 (1.43-3.97)	0.00
NVC, % CBv – 20s ON-20s OFF	13.14 % - 16.06 %	0.53 (0.29-0.98)	0.04†	1.54 (0.91-2.61)	0.10
	16.06 % - 17.52 %	0.57 (0.30-1.07)	0.08	1.01 (0.60-1.70)	0.96
	17.52 % - 18.98 %	0.73 (0.40-1.32)	0.30	1.16 (0.68-1.98)	0.57
	18.98 % - 21.90 %	2.29 (1.23-4.28)	0.00	1.89 (1.09-3.28)	0.02

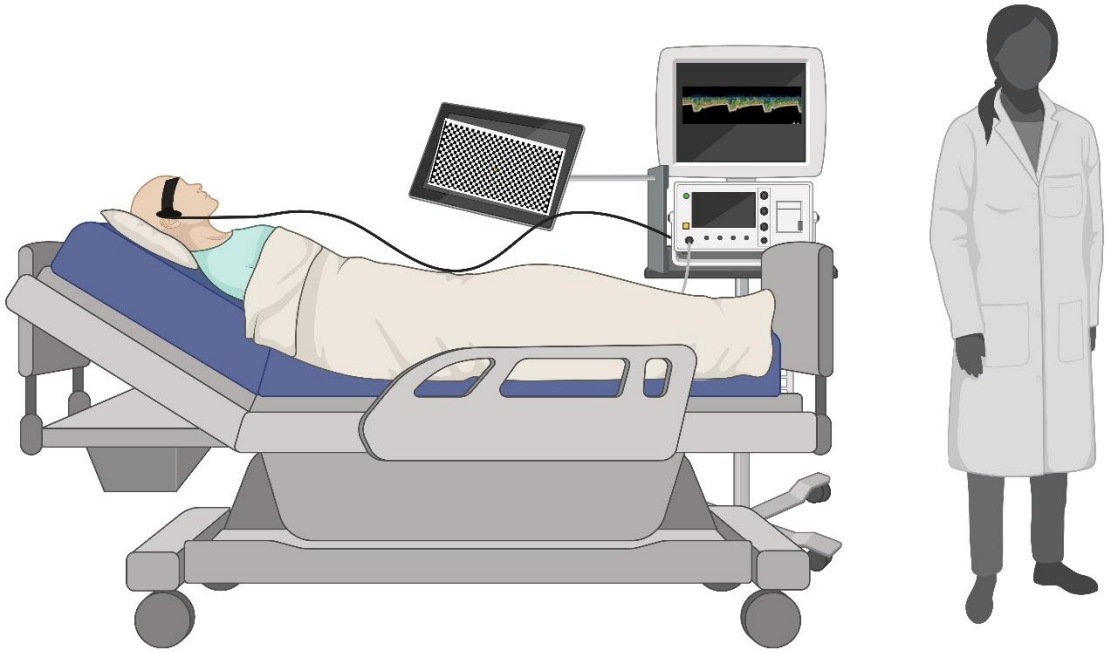
Abbreviations: NVC = Neurovascular Coupling; CBv = Cerebral Blood Flow Velocity.

¥All p values and odds ratio calculated by GLMM, adjusted for Age, Years of Education, Hypertension, Diabetes, Metformin, Statins, Heart Failure, and Peripheral Arterial Disease.

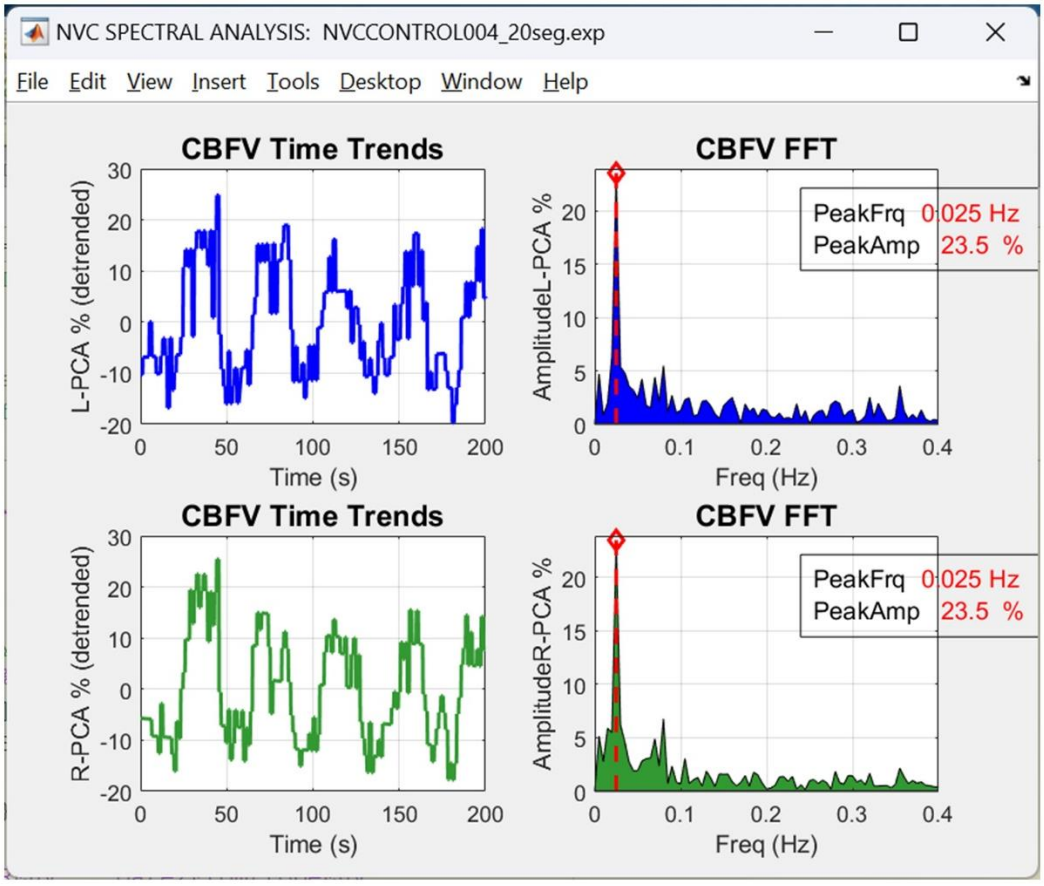
†§All p values and odds ratio calculated by GLMM, adjusted for Age, Years of Education, Hypertension, Diabetes, Metformin, Statins, Heart Failure, Beta-Blockers, and Previous Coronary Disease.

Supplementary Material

Supplementary Figure 1. NVC assessment in acute stroke setting. Created with BioRender.com.



Supplementary Figure 2. NVC spectral analysis in MATLAB software.



Supplementary Table 1. Cognition categories and operational for primary endpoint (Wardlaw et al., 2023)

Primary Category	Operationalization	Sub-category	Operationalization
Normal cognition	No evidence of cognitive impairment (MoCA: 30–29 OR IADL=8 for women/IADL≥5 for men)	Normal	
Minor Neurocognitive disorder (mild cognitive impairment)	Evidence of cognitive impairment (MoCA: 28–26 OR IADL: 7-6 for woman and IADL: 4-3 for men) AND No evidence of functional impairment (mRS < 2 OR no change in mRS if pre-stroke mRS > 1)	Single Domain	Scores are reduced by >1 point in only one cognitive domain of MoCA or IADL: 7-6 for woman and IADL: 4-3 for men
		Multi domain	Scores are reduced by >1 point in more than one cognitive domain of MoCA or IADL: 7-6 for woman and IADL: 4-3 for men
Dementia	Clinical diagnosis made independent of study -Any clinical diagnosis of dementia made by memory clinic (or equivalent, this would include primary care) -Any recording of dementia on death certification -Any prescription of cholinesterase inhibitor or memantine OR Pre-stroke dementia (Baseline assessment IQCODE > 3.6) OR In-study evidence of persisting multi-domain cognitive impairment (MoCA score ≤20 OR IADL≤5 for women/IADL≤3 for men on more than one annual follow-up) and Evidence	Mild	Cognitive impairments (MoCA 20-25 OR IADL ≤ 5 for women/IADL ≤ 3 for men) AND Minimal functional problems (mRS < 3)
		Moderate	More severe cognitive impairment (MoCA 14–19 OR IADL ≤ 5 for women/IADL ≤ 3 for men) AND more limiting function (mRS 3 or 4)
		Severe	Severest cognitive impairments (MoCA < 14 OR IADL ≤ 5 for women/IADL ≤ 3 for men) AND Most limited function Care-home admission OR mRS 4/5.

	of functional impairment (mRS ≥ 2 or IQCODE > 3.6 at final follow-up)		
Death		Death	

Abbreviations: IADL = Instrumental Activities of Daily Living; mRS = Modified Rankin Scale; MoCA test = Montreal Cognitive Assessment test; IQCODE = Informant Questionnaire on Cognitive Decline in the Elderly; Adapted by: 1. Wardlaw JM, Woodhouse LJ, Mhlanga, II, et al. Isosorbide Mononitrate and Cilostazol Treatment in Patients With Symptomatic Cerebral Small Vessel Disease: The Lacunar Intervention Trial-2 (LACI-2) Randomized Clinical Trial. *JAMA Neurol* 2023; 80: 682-692. DOI: 10.1001/jamaneurol.2023.1526.

Supplementary Table 2. Univariate Model for PCA5, PCA10 and PCA20 in acute phase.

	PCA5		PCA10		PCA20	
Total n=144	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Age	0.00 (-0.00-0.01)	0.09	0.00 (-0.00-0.00)	0.98	-0.00 (-0.00-0.00)	0.30
Sex	-0.05 (-0.18-0.08)	0.43	-0.23 (-0.34-(-0.11))	<0.01	-0.15 (-0.27-(-0.03)	0.01
Years of education	-0.01 (-0.03-(-0.00))	0.01	-0.00 (-0.01-0.00)	0.83	0.00 (-0.01-0.02)	0.52
Time from symptom onset to hospital admission	0.00 (0.00-0.00)	<0.01	0.00 (0.00-0.00)	<0.01	0.00 (0.00-0.00)	0.02
Time from symptom onset to recanalization	0.00 (0.00-0.00)	<0.01	0.00 (0.00-0.00)	0.08	0.00 (0.00-0.00)	0.16
Coronary disease	-0.27 (-0.36-(-0.17))	<0.01	-0.09 (-0.31-0.12)	0.41	-0.00 (-0.16-0.15)	0.93
Heart failure	0.32 (-0.15-0.80)	0.18	0.33 (0.13-0.53)	<0.01	0.08 (-0.08-0.25)	0.30
Peripheral vascular disease	0.25 (0.02-0.49)	0.03	-0.11 (-0.29-0.06)	0.20	0.06 (-0.11-0.24)	0.48
Beta-blockers	0.15 (0.00-0.30)	0.04	-0.03 (-0.16-0.09)	0.61	-0.02 (-0.17-0.11)	0.69
Statin	-0.20 (-0.47-0.06)	0.01	-0.16 (-0.27-(-0.05))	<0.01	-0.27 (-0.49-(-0.04))	0.02
Antidepressants	-0.25 (-0.31-(-0.18))	<0.01	0.40 (0.18-0.62)	<0.01	0.23 (0.03-0.42)	0.02
NIHSS at admission	0.00 (-0.00-0.01)	0.15	0.00 (-0.01-0.01)	0.93	0.00 (-0.01-0.01)	0.76

NIHSS at 24 hours	0.00 (-0.02-0.02)	0.89	0.02 (-0.00-0.04)	0.10	0.00 (-0.01-0.03)	0.62
Glycaemia value at admission	-0.00 (-0.00-0.00)	0.90	0.00 (0.00-0.00)	0.01	0.00 (-0.00-0.00)	0.38
Hypertension	0.02 (-0.09-0.15)	0.63	-0.05 (-0.17-0.06)	0.35	-0.07 (-0.19-0.05)	0.25
Diabetes mellitus	-0.04 (-0.19-0.10)	0.55	-0.03 (-0.18-0.11)	0.65	-0.01 (-0.15-0.13)	0.89
Dyslipidaemia	-0.00 (-0.12-0.11)	0.93	0.00 (-0.10-0.12)	0.95	0.04 (-0.07-0.16)	0.47
Previous stroke	0.09 (-0.11-0.31)	0.37	-0.21 (-0.35-(-0.06)	<0.01	-0.14 (-0.30-0.01)	0.07
Stroke Circulation	-0.02 (-0.14-0.99)	0.70	0.02 (-0.10-0.16)	0.67	0.02 (-0.10-0.16)	0.67

Supplementary Table 3. Univariate Model for 7-Level Cognition Score 12 months after stroke.

	Cognition Score 12 months after stroke	
Total n=144	OR (95% CI)	P-value
Age	0.40 (0.02-0.05)	<0.01
Sex	-0.03 (-0.41-0.34)	0.85
Years of education	-0.15 (-0.20-(-0.10)	<0.01
Time from symptom onset to hospital admission	0.00 (0.00-0.00)	0.82
Time from symptom onset to recanalization	-0.00 (-0.00-0.00)	<0.01
Coronary artery disease	-0.01 (-0.60-0.57)	0.96
Heart failure	0.84 (0.30-1.37)	<0.01
Peripheral vascular disease	-0.01 (-0.78-0.76)	0.98
Beta-blockers	0.41 (0.04-0.79)	0.03
Statin	1.15 (0.20-2.10)	0.01
Antidepressants	0.59 (0.06-1.12)	0.02
NIHSS at admission	0.04 (0.01-0.08)	<0.01
NIHSS at 24 hours	0.14 (0.03-0.25)	0.01
Glycaemia value at admission	0.00 (0.00-0.00)	0.02
Hypertension	0.69 (0.32-1.06)	<0.01
Diabetes mellitus	0.50 (0.06-0.94)	0.02
Dyslipidaemia	0.21 (-0.12-0.55)	0.20
Previous stroke	0.52 (0.02-1.03)	0.04

CHAPTER VI: DETECTION OF MICROEMBOLI IN PATIENTS WITH ACUTE ISCHAEMIC STROKE AND ATRIAL FIBRILLATION SUGGESTS POOR FUNCTIONAL OUTCOME

Research Article

EUROPEAN STROKE JOURNAL

European Stroke Journal
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Detection of microemboli in patients with acute ischaemic stroke and atrial fibrillation suggests poor functional outcome

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Abstract

Introduction: We investigated the burden of microembolic signals (MES) in patients with acute ischaemic stroke (AIS) and atrial fibrillation (AF), assessing their impact on functional outcomes.

Patients and methods: This multicentre international prospective cohort study involved patients with AIS and either a known or newly diagnosed anticoagulant-naïve AF. All centres utilised the same transcranial Doppler machine for 1-h monitoring with bilateral 2 MHz probes within 24 h of symptom onset. Recordings underwent MES analysis by a blinded central reader. The primary objectives were to ascertain the MES proportion and its association with functional outcomes assessed by the modified Rankin scale (mRS) score at 90 days.

Results: Between September 2019 and May 2021, we enrolled 61 patients, with a median age of 78 years (interquartile range 73–83) and a median stroke severity score of 11 (interquartile range 4–18). MES were observed in 14 patients (23%), predominantly unilateral (12/14, 86%), with a median rate of 6 counts/hour (interquartile range 4–18). MES occurrence was higher post-thrombectomy and among those with elevated brain natriuretic peptide levels ($p < 0.05$). A worse mRS score of 3–6 was more frequent in patients with MES, occurring in 11/14 (79%), compared to those without MES, 20/47 (43%), with an adjusted odds ratio of 5.04 (95% CI, 1.15–39.4), $p = 0.04$.

Conclusions: Nearly a quarter of patients with AIS and AF exhibited silent microembolization after the index event. Detecting MES within 24 h post-stroke (using transcranial Doppler) could signify a marker of poor functional outcomes. Subsequent trials will assess if very early antithrombotic treatment might enhance outcomes in this highly selective group of cardioembolic stroke patients. (Clinicaltrials.gov ID: NCT06018090).

Keywords

Infarction, embolism, atrial fibrillation, ultrasound, transcranial Doppler

Subject terms: ultrasound, ischaemic stroke, embolism, ultrasound, atrial fibrillation

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Introduction

In a patient with atrial fibrillation (AF), cerebral infarction typically arises from the embolisation of intracardiac thrombi, often formed within the left atrial appendage. The re-embolisation of residual thrombi likely contributes to the heightened recurrence of stroke in the initial hours following the index event.[1] In addition to the occurrence of new clinical events, it is conceivable that this factor contributes to new brain lesions, potentially diminishing the likelihood of achieving a favourable clinical recovery.

Recently, a large multicentre trial demonstrated a tendency towards a lower incidence of recurrent ischaemic stroke or systemic embolism within 30 days following a stroke with the early implementation of anticoagulation. However, certain limitations in the approach persist.[2] These limitations arise from the observation that stroke recurrence was not notably reduced, suggesting that additional factors might contribute to recurrent embolism. The coexistence of atherosclerosis doubles the risk of a subsequent stroke and might exhibit a more favourable response to antiplatelet therapy.[3] Other considerations persist due to potential individual variations in the effectiveness of different anticoagulants, which might account for some of the variability in outcomes.[4,5] The influence of recurrent embolism on the functional outcomes of post-stroke patients highlights the significance of investigating prevention strategies and enhancing long-term prognostic methods to stratify patient risk and improve functional outcomes.[6,7]

For these reasons, evaluating a patient's early embolisation activity would be beneficial in optimising management and preventing a poorer outcome. Transcranial Doppler (TCD) provides a unique opportunity to detect embolic material in the cerebral circulation at the bedside in a non-invasive manner.[8] Microembolic signals (MES) have been extensively studied in cerebrovascular disease, primarily in association with atherosclerotic conditions.[9] The estimation that MES are detectable in 15% of stroke patients due to AF is outdated, especially given the introduction of new acute treatments. Additionally, in this study, although patients taking anticoagulants at the onset were excluded, monitoring did not commence within the initial 24 h.[10]

Previous studies involving patients after non-cardioembolic aetiology stroke have indicated that the presence of MES during the acute phase and following endovascular treatment correlates with recurring events, increased mortality rates, and subsequently, poorer functional outcomes 90 days post-stroke.[11,12]

Hence, this multicentre international prospective study examined the incidence of MES and their effect on the clinical outcome in patients with acute ischaemic stroke caused by AF.

Methods

Data availability

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

Patients and participating centres

This is a multicentre international prospective cohort study involving central reading of MES events by an expert blinded to clinical data and outcomes. Five European comprehensive stroke centres participated in this study: University Hospital Centre of São João/Faculty of Medicine, University of Porto, Portugal; Hospital Centre Zagreb, Croatia; University of Padua, School of Medicine, Italy; Centre for Health Research, Faculty of Medicine, University of Ostrava, Czech Republic; and Justus-Liebig-University Giessen, Germany. The inclusion criteria comprised acute ischaemic stroke admitted to the Stroke Unit, a history of AF/flutter, or a new diagnosis of AF at the time of stroke, with the ability to be monitored within 24 h after symptom onset. Exclusion criteria included the use of anticoagulants at stroke onset, prosthetic valves, inadequate temporal bone window, and uncooperative patients.

Clinical, laboratory, and radiological assessment

Demographic and clinical data, encompassing vascular risk factors, medications, and prior cardiovascular disease, were documented upon admission. Additionally, blood pressure (BP), serum glucose, cholesterol levels, National Institutes of Health Stroke Scale (NIHSS) scores, reperfusion treatment (thrombolysis, endovascular procedures), and post-treatment grade 2b-3, as per the modified Thrombolysis in Cerebral Infarction scale (mTICI), were recorded.[13] Experienced stroke physicians reviewed radiological and ultrasound studies to identify the presence of carotid or middle cerebral artery stenosis $\geq 50\%$. All clinical data were stored in a decentralised electronic platform (Castor®'s).

Microembolic signals detection

The presence and rate of MES were assessed by 1 h of TCD monitoring (Doppler Box X, DWL, Sipplingen, Germany) within 24 h from the *last known well* time. Bilateral M1 segments of middle cerebral artery were insonated at a single depth with 2 MHz transducers secured by a probe-holder with a sample volume of 8 mm and low gain.[8] MES was defined according to previous consensus[8] as a signal with (1) duration < 300 ms, (2) amplitude > 3 dB with respect to underlying flow signal, (3) unidirectionality and (4) typical audible sound ('snap' and/or 'chirp'). Recordings were

analysed by a single experienced reader who was blinded to clinical data and outcomes. Patients with one or more MES detected were assigned to the MES-positive group.

Clinical endpoints

The primary objectives were to ascertain the proportion of patients with MES and to assess the association of MES with poor functional outcomes, evaluated using the modified Rankin scale (mRS) scores of 3–6 (indicating dependency or mortality) versus 0–2 (indicating independence) at 90 days. As secondary endpoints, we compared the functional outcomes between MES-positive and MES-negative groups across the entire scale using ordinal shift analysis. Additionally, we explored the relationship between MES and clinical, radiological, and laboratory characteristics.

We obtained approval from the local ethics committees of all participating institutions. Written informed consent was acquired from all participants or their guardians.

Statistical analysis

Sample size calculation. The largest study on non-valvular AF[10] estimated a 15% prevalence of MES within 24 h of stroke. However, more recent publications in the context of endovascular treatment reported higher proportions ranging from 31% to 61%.[11,12,14] We estimated a proportion of 35% of patients being MES-positive, with a power of 80%, $\alpha = 0.05$, and a marginal error of 10%. Additionally, based on the study by Farina et al.[11] 77% of MES-positive patients experienced long-term disability after stroke (mRS 3–6), compared to only 34% of MES-negative patients. Therefore, to achieve a primary functional outcome with an odds ratio of 6.5, $\alpha = 0.05$, and power of 80%, we calculated a sample size of 54. To account for a 10% loss to follow-up, we aimed to enrol a minimum of 60 patients. Sample size estimates were computed using G*Power version 3.1.9.7.

Descriptive and outcome analysis. The normality of continuous variables was assessed using the Kolmogorov–Smirnov test. Baseline characteristics between patients in the MES-positive and MES-negative groups were compared using chi-square/Fisher exact tests for categorical variables and Mann-Whitney tests for continuous variables. Clinical outcomes were compared between the MES-positive and MES-negative groups. Logistic regression was employed to generate odds ratios (OR) and 95% confidence intervals (CI) for poor outcome (mRS 3–6) versus good outcome (mRS 0–2) at 90 days. Additionally, outcomes were assessed using ordinal shift analysis modelling across all levels of the mRS scale after verifying conformity to the assumption of a common proportional odds.

We conducted two multivariate models to adjust analyses for all outcomes. The first model adjusted for independent variables associated with the outcome in univariate analysis, including age and NIHSS score, as major predictors of outcome. The second model adjusted for variables that exhibited imbalance between MES groups (Table 1 with $p < 0.30$) using a propensity score matching approach: a single propensity score between 0 and 1 was computed, encompassing brain natriuretic peptide, previous antiplatelet drugs, NIHSS, thrombectomy, anterior circulation, and carotid or middle cerebral artery $\geqslant$50% stenosis. We performed a sensitivity analysis excluding patients with large vessel lesions and adjusted for all outcomes in two multivariate models (Supplemental Table 2).

All statistical analyses were conducted using IBM SPSS Statistics for Windows, version 28. Statistical significance was determined at $p < 0.05$.

Results

Between September 1, 2019, and May 15, 2021, we screened 491 patients and excluded 283 patients who arrived more than24 h after symptom onset. The study period coincided with the COVID-19 pandemic, and therefore, the centres faced restrictions on the use of monitoring devices and longer intervals between symptom onset and entry into the unit to facilitate monitoring. Out of 208 potential eligible patients for the study, we excluded 15 due to anticoagulant use, three non-cooperative patients, five with prosthetic valves, and 52 without a temporal bone window. One patient with a stroke mimic (epilepsy) was excluded from the final analysis, and we enrolled 62 patients (Figure 1).

MES were present in 14 patients (23%), mostly unilateral (12/14, 86%), with a median count rate of six counts per hour (interquartile range 4–18). Among the unilateral MES-positive cases, eight occurred on the symptomatic side.

MES occurrence was significantly associated with thrombectomy and higher brain natriuretic peptide levels (Tables 1 and 2, $p < 0.05$). Among the 10 cases of MES-positive associated with thrombectomy, eight were on the same side as the stroke, one was bilateral, and one was detected contralateral to the stroke, $p = 0.034$. MES occurred more frequently with concomitant carotid or middle cerebral artery $\geqslant$50% stenosis, higher baseline NIHSS, and previous use of antiplatelet drugs, although these associations were statistically nonsignificant (Table 1).

Table 2 presents the outcome comparisons between the MES-positive and MES-negative groups. The distribution of patients in each mRS category according to MES detection is illustrated in Figure 2. Worse clinical outcomes, defined as mRS 3–6, were more frequent in patients with MES [11/14 (79%)] than in those without MES [20/47 (43%)], adjusted odds ratio = 5.04 (95% CI, 1.15–39.4), $p = 0.04$ (Table 2). The presence of MES was associated

Table 1. Demographic, clinical and radiological characteristics of all patients and differences between MES-positive and MES-negative groups.

	Total	MES-Negative	MES-positive	*p* Value,*
	N=61	*N*=47	*N*=14	
Demographic and clinical variables				
Age, years	78 (73–83)	78 (73–83)	76 (71–83)	0.494
Female sex, *n* (%)	31 (51)	23 (49)	8 (57)	0.736
Previous dependence (mRS score ⩾ 3), *n* (%)	8 (13)	7 (15)	1 (7)	0.668
BMI, $kg.m^{-2}$	26.6 (23.5–29.7)	23.5 (29.7–26.3)	29.7 (26.3–23.4)	0.393
Hypertension, *n* (%)	51 (84)	38 (81)	13 (93)	0.987
Dyslipidemia, *n* (%)	30 (50)	21 (46)	9 (64)	0.352
Diabetes, *n* (%)	18 (30)	12 (26)	6 (43)	0.434
Smoking, *n* (%)	18 (30)	14 (30)	4 (29)	0.531
Previous stroke, *n* (%)	10 (16)	8 (17)	2 (14)	1.000
Coronary disease, *n* (%)	10 (16)	7 (15)	3 (21)	1.000
Previous AP, *n* (%)	8 (13)	7 (15)	1 (7)	0.222
Previous statin, *n* (%)	31 (51)	24 (51)	7 (50)	0.499
GFR, $ml.min^{-1}.m^{-2}$	62 (47–75)	63 (50–76)	60 (42–74)	0.330
Fibrinogen, mg/dL	361 (288–406)	356 (299–393)	371 (262–459)	0.754
Onset-To-TCD-time, h	22 (14–23)	21 (14–23)	22 (15–23)	0.517
Stroke characteristics				
NIHSS score, median (range)	11 (4–18)	10 (2–18)	14 (10–16)	0.103
Anterior circulation, *n* (%)	21 (35)	15 (33)	12 (86)	0.109
Left side infarct, *n* (%)	26 (57)	18 (55)	8 (62)	0.669
Carotid/MCA stenosis ⩾50%, *n* (%)	10 (16)	6 (13)	4 (29)	0.223
Infarct largest size, mm, median (range)	38 (25–46)	38 (21–46)	38 (37–46)	0.661
Thrombolysis, *n* (%)	23 (38)	16 (34)	7 (50)	0.347
Thrombectomy, *n* (%)	26 (43)	16 (34)	10 (71)	0.029
Received AP, *n* (%)	18 (33)	12 (29)	6 (43)	0.647
Cardiac variables				
Permanent AF, *n* (%)	45 (76)	33 (73)	12 (86)	0.457
Mean BP, mm Hg	80 (62–88)	62 (88–77)	88 (77–61)	0.545
Troponin I, ng/mL	0.02 (0.01–0.05)	0.01 (0.01–0.06)	0.02 (0.02–0.04)	0.659
BNP, pg/mL	379 (145–835)	192 (123–389)	819 (534–1436)	0.004
Left atrial size, mm	43 (41–46)	44 (42–46)	41 (23–44)	0.349
CHA_2DS_2VASC score	4 (4–6)	4 (4–5)	6 (4–6)	0.349

AF: atrial fibrillation; AP: antiplatelets; BMI: body mass index; BNP: brain natriuretic peptide; BP: blood pressure; MCA: middle cerebral artery; MES: microembolic signals; NIHSS: National Institutes of Health Stroke Scale; TCD: transcranial Doppler.
Continuous data presented as median (interquartile range).
**p* Values are shown for differences between MES-positive and MES-negative groups obtained from chi-square test/ Fisher's exact test for categorical variables, or Mann-Whitney test for continuous variables.

with a poorer functional outcome at 90 days, even after adjusting for biased characteristics related to MES occurrence, age, and stroke severity (Table 2).

The ordinal shift analysis across mRS categories at 90 days also showed a significant difference between the MES-positive and MES-negative groups, indicating a shift towards worse functional classes at 90 days (adjusted OR = 3.59, 95% CI 1.12–36.2, $p = 0.02$) (Table 2).

Excluding patients with large vessel lesions, the presence of MES was still associated with a poorer functional outcome at 90 days, although without statistical significance in the multivariate model adjusted for unbalanced baseline differences between MES groups by a probability score (Supplemental Table 2).

Oral anticoagulation during follow-up did not independently associate with the outcome, but this could be due to our inability to access the timing of treatment initiation and potential interference with MES status and its consequences during follow-up.

Discussion

In this multicentre observational prospective study involving patients with acute ischaemic stroke and

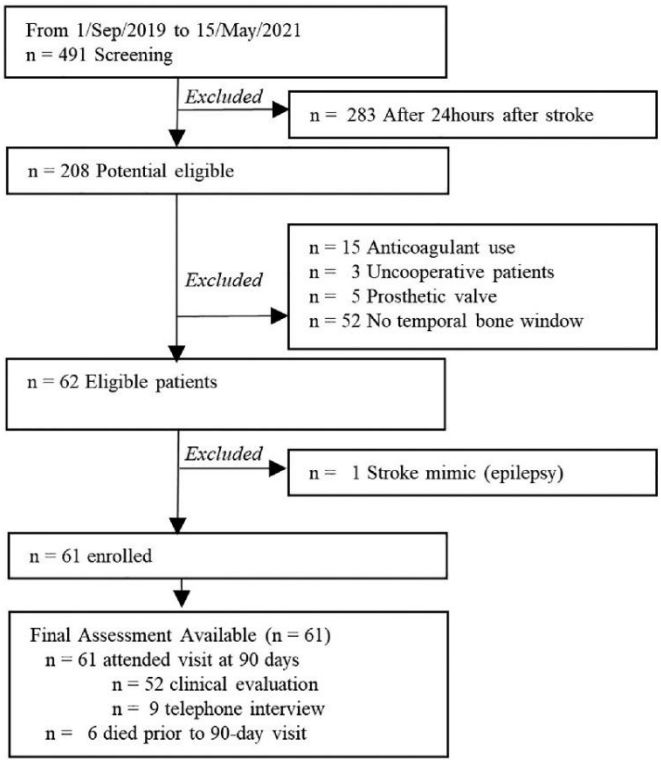

Figure 1. Study flow chart.

Table 2. Outcome measures based on MES positive or negative detection.

Outcome measures	MES- positive $n=14$	MES- negative $n=47$	Univariate Model	Multivariate Model 1,*	Multivariate Model 2†
			Unadjusted odds ratio (CI 95%); p value	Adjusted Odds ratio (CI 95%); p value	Adjusted Odds ratio (CI 95%); p value
mRS 3–6 at 90 days – n (%)§	11 (79)	20 (43)	4.95 (1.29–20.1); $p \leqslant 0.05$	4.34 (1.02–54.1); $p \leqslant 0.05$	4.34 (1.34–25.6); $p \leqslant 0.05$
mRS score shift – median (IQR)‡	2 (1–3)	4 (3–4)	3.59 (1.12–36.2); $p \leqslant 0.05$	2.98 (1.02–34.1); $p > 0.05$	3.02 (1.03–56.2); $p > 0.05$

MES: microembolic signals.
All p values and odds ratio calculated by logistic regression models.
*Multivariate model 1 = adjusted to Age, NIHSS and previous mRS.
†Multivariate model 2 = adjusted to BNP, previous antiplatelet drugs, NIHSS, thrombectomy, anterior circulation, previous mRS and carotid or middle cerebral artery $\geqslant$50% stenosis.
§Univariate and multivariate analysis with logistic regression to predict outcome.
‡Univariate and multivariate analysis with ordinal shift analysis to predict outcome.

anticoagulant-naive atrial fibrillation, MES were detected through transcranial Doppler monitoring in nearly one-quarter of patients within the initial 24 h after symptom onset. Notably, the presence of MES significantly increased the risk of experiencing a poor functional recovery by 5-fold at 90 days.

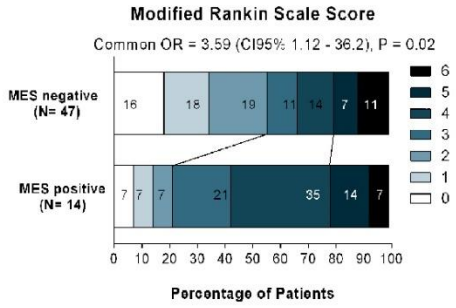

Figure 2. Distribution of disability scores at 90 days, as per the modified Rankin scale (range 0–6, higher scores indicating more severe disability), among patients in the MES-positive group compared to those in the MES-negative group. The figures within the bars denote the percentages of patients with each score. Statistical analysis details are provided in Table 2, including the Common Odds Ratio (OR) representing the shift analysis.

The study's primary strength lies in the meticulous patient selection criteria, specifically focusing on individuals with no anticoagulation, conducting recordings within 24 h after stroke onset, and excluding other stroke aetiologies. Rigorous control of potential technical confounders was implemented, including the use of a single type of ultrasound device, external offline reading, involvement of a single expert reader for MES recording, and pre-defined insonation parameters. This approach mitigated most of the confounding factors related to MES monitoring, particularly in patients with atrial fibrillation.[15]

Burden of microemboli in AF and outcomes

To the best of our knowledge, this is the inaugural study investigating MES detection after acute ischaemic stroke, specifically focusing on anticoagulation-naïve AF patients, with the primary endpoint being clinical outcome. Previous studies with diverse clinical cohorts and technologies exhibited a wide variability in positive MES counts, ranging between 17% and 40%[16] According to our approach, the MES burden accounted for 23% of the total cohort. This percentage aligns with the findings of a recent meta-analysis encompassing all MES studies in acute stroke.[17] However, it must be noted that the majority of previous studies involving patients with AF did not account for the use of anticoagulants, which significantly influences the occurrence of MES.

Our data provide evidence that MES indicate a poor functional prognosis after a stroke caused by AF (Table 2). This association was significant when considering dichotomised mRS and in shift analysis across the entire scale (Figure 2). Previous studies involving patients with acute stroke of various aetiologies and those undergoing thrombectomy also observed increased mortality in cases with MES activity.[18]

Several pathophysiological explanations might account for this association. Microembolic particles, as identified by ultrasound, represent the common final step in a complex sequence of preceding cellular and molecular pathways. They may indicate activated stages in the clotting system and highlight tissue surfaces vulnerable to thrombosis.[19] Indeed, MES not only serve as a robust surrogate marker for thrombogenicity but also accurately reflect actual embolic events. Interestingly, in our MES-positive cases, BNP values indicating cardiac insufficiency were significantly elevated. This may represent an additional crucial pathophysiological factor linking microembolism to a worse clinical outcome.[20]

Silent embolism occurring within the initial hours after an index stroke can be linked to new ischaemic lesions and/or an expansion of the final infarct area.[21] This often results from intracranial vessel obstruction, inadequate collateral flow, and decreased embolic washout.[22] For example, Kang et al. demonstrated that 23% of patients with AF exhibited silent new ischaemic lesions on MRI conducted 5 days after a stroke.[21] MES detected through TCD monitoring can serve as a surrogate for future ischaemic lesions and subsequent ischaemic events.[11,12,17] New cerebral ischaemic lesions, even if not linked to a sudden clinical event, predict new neurological deficits, including cognitive impairment.[23]

We must acknowledge that the size of the study cohort was relatively small, resulting in large confidence intervals for the calculated odds ratio. This limited number precluded more robust sensitivity analyses to complement the multivariate analysis.

Numerous studies have been unable to find evidence supporting the association between MES and poor outcomes following a stroke.[17] However, many of these studies encompassed various stroke types, often including patients with small vessel disease, which clearly doesn't align with the recording of microembolism in the primary stem of the middle cerebral artery. Most cases that tested positive for MES in prior studies were likely associated with carotid atherosclerosis, and the prognosis for these patients significantly differs from those with atrial fibrillation. Hence, research investigating the prognostic value of MES should distinctly differentiate between different stroke aetiologies and endeavour to minimise the inclusion of 'mixed' cohorts.

The source of MES and the cause of stroke

Our study was not intended to definitively identify the source of microemboli but could offer intriguing hypotheses. Among patients with atrial fibrillation, undissolved clots might persist within the atria and atrial appendages. A substantial presence of intracardiac clots could potentially cause blockages in major intracranial vessels, leading to more

severe strokes.[17] Hence, MES showed a significant association with a higher NIHSS and mechanical thrombectomy.

Among our sample of 61 patients, 26 underwent thrombectomy (43%) (Table 1) and conducting thrombectomy on the same side was significantly linked to unilateral MES, regardless of the final TICI score. The presence or absence of a successful TICI score did not show an association with the presence of MES in our cohort. While the connection between MES and recanalisation failure might be questioned, our study failed to establish this link. Past research indicated an association between TICI 2a and MES; however, a recent study involving only TICI 2b-3 cases did not find a similar association with MES.[11,12]

Elevated BNP values associated with MES support this idea (Table 1).[20] However, the size of the left atrium, a more reliable indicator of advanced atrial cardiopathy, did not show a significant association with MES activity. It's worth noting that a recent large multicentre trial demonstrated that anticoagulation provides no additional benefit over aspirin in patients with embolic stroke of undetermined source, despite the presence of atrial cardiopathy.[24]

The CHA_2DS_2VASC score, a well-validated risk stratification tool predicting cardioembolism, also showed no association with MES activity. This could be explained by the fact that the CHA_2DS_2VASC score is not designed for assessment in the context of an acute stroke event, whereas our study protocol required MES monitoring within 24 h after the index event.

The presence of concomitant atherosclerotic lesions along with endovascular treatment might serve as an alternative source of MES, distinct from the heart. This study reveals that MES were more frequently detected in patients who had undergone endovascular therapy (70%vs 34%, $p=0.029$, Table 1). The notably high rate of MES could be attributed to vascular/endothelial damage incurred during this procedure.[11,16,25,26] In our study, the rate of MES was higher among patients with co-existing atherosclerotic carotid or intracranial stenosis on the same side as the stroke. Interestingly, patients who were already using antiplatelet drugs at the time of the stroke tended not to exhibit MES. The documented efficacy of aspirin and clopidogrel in reducing emboli originating from atherosclerotic wall disease comes from extensive large-scale randomised and case-control trials.[27]

The risk of early recurrent embolism in stroke patients with AF is approximately 20 times higher than the risk of haemorrhagic transformation,[2] further emphasising the need for early treatment. Our findings support this conclusion and highlight the potential of MES monitoring to identify patients at a higher risk of a poor functional outcome.

Limitations

The commonly accepted monitoring time for detecting MES is 1 h.[8] Extended TCD monitoring might have increased detection rates, but we believe it wouldn't have significantly altered the observed relationship with the outcome. Only the M1 segment of the MCA was monitored, potentially missing embolisms to other vascular territories, although vertebrobasilar strokes tended to be less MES-positive (Table 1). Monitoring MES with TCD, a non-invasive method, relies on the accessibility of the temporal bone window. In our sample, 25% of eligible patients had an insufficient temporal window, which is a limitation of our study.

Differences in baseline characteristics between MES groups might have biased our ability to predict outcomes. The MES-positive group tended to exhibit higher NIHSS scores and were more frequently directed to endovascular treatment. Endovascular procedures encompass various complications, such as the previously mentioned vascular/endothelial damage, potentially influencing the recorded MES rate.[11,16,25,26] The variables NIHSS and thrombectomy were adjusted for in model 2 through propensity score matching to compensate for this bias (Table 2). Only 26 patients underwent thrombectomy, and a successful or unsuccessful TICI score was not found to be associated with the presence of MES in our cohort. Although adjusted in model 2 for the NIHSS of the sample, it is important to note that the average stroke severity in our sample was considerably high (NIHSS = 11). This needs to be considered when comparing it to the literature.

Conclusions

Nearly a quarter of patients with atrial fibrillation show evidence of microembolization within the first 24 h after an acute ischaemic stroke. The detection of MES within 24 h after a stroke, using transcranial Doppler, may serve as a marker for poor functional outcomes. Future studies will ascertain whether very early antithrombotic treatment can enhance the outcome in this highly selected group of cardioembolic stroke patients.

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Ethical approval

The ethics committee of Centro Hospitalar Universitário de São joão approved this study (REC number: 362/19)

Informed consent

Written informed consent was obtained from all subjects before the study.

Guarantor

P Castro

Contributorship

All authors should have made substantial contributions to all of the following:

P Castro: (1) the conception and design of the study, analysis and interpretation of data, (2) drafting the article and revising it critically for important intellectual content, and (3) final approval of the version to be submitted.

J Ferreira: (1) acquisition of data, analysis and interpretation of data, (2) drafting the article and revising it critically for important intellectual content, and (3) final approval of the version to be submitted.

B Malojcic: (1) acquisition of data, or analysis and interpretation of data, (2) revising it critically for important intellectual content, and (3) final approval of the version to be submitted.

D Bazadona: (1) acquisition of data, or analysis and interpretation of data, (2) revising it critically for important intellectual content, and (3) final approval of the version to be submitted.

C Baracchini: (1) acquisition of data, or analysis and interpretation of data, (2) revising it critically for important intellectual content, and (3) final approval of the version to be submitted.

A Pieroni: (1) acquisition of data, or analysis and interpretation of data, (2) revising it critically for important intellectual content, and (3) final approval of the version to be submitted.

D Skoloudik: (1) acquisition of data, or analysis and interpretation of data, (2) revising it critically for important intellectual content, and (3) final approval of the version to be submitted.

E Azevedo: (1) analysis and interpretation of data, (2) revising it critically for important intellectual content, and (3) final approval of the version to be submitted.

M Kaps: (1) acquisition of data, or analysis and interpretation of data, (2) revising it critically for important intellectual content, and (3) final approval of the version to be submitted.

Moreover, all authors have approved the submitted version of the manuscript.

Trial registration

Clinicaltrials.gov ID: NCT06018090

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Supplemental material

Supplemental material for this article is available online.

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Supplementary Material

Supplementary Table 1 Univariate analysis of the baseline characteristics of poor and good outcomes

	Total	Poor outcome (mRS 3-6)	Good outcome (mRS 0-2)		
	N = 61	N = 31	N = 30	odds ratio (95%)*	P value
Demographics and clinical					
Age, years)	**77 (73 - 83)**	**81 (75 - 84)**	**74 (68 - 83)**	**1.06 (1.00 - 1.12)**	**†0.05**
Female sex, n (%)	31 (50)	21 (68)	10 (32)	4.41 (1.52 - 12.8)	0.01
BMI, Kg.m-2	26.5 (24.1 – 29.1)	27.6 (23.8 - 31.1)	25.2 (23.4 - 27.4)	1.11 (0.96 - 1.28)	0.15
Hypertension, n (%)	52 (84)	28 (90)	24 (77)	2.72 (0.63 - 11.7)	0.18
Dyslipidemia, n (%)	30 (49)	17 (57)	13 (42)	1.81 (0.66 – 5.00)	0.25
Diabetes, n (%)	19 (31)	13 (42)	6 (19)	3.01 (0.96 - 9.42)	0.06
Smoking, n (%)	18 (29)	7 (23)	11 (35)	0.53 (0.17 - 1.62)	0.27
Previous Stroke, n (%)	10 (16)	6 (19)	4 (13)	1.62 (0.41 - 6.42)	0.49
Coronary Disease, n (%)	11 (18)	7 (23)	4 (13)	1.97 (0.51 - 7.56)	0.32
Previous AP, n (%)	10 (16)	6 (20)	4 (13)	1.69 (0.42 - 6.70)	0.46
Previous Statin, n (%)	32 (52)	17 (55)	15 (48)	1.30 (0.48 - 3.51)	0.61
GFR, ml.min-1.m-2	61.8 (47 - 74.9)	57.5 (42 - 66.05)	65 (57 - 84)	0.99 (0.97 - 1.01)	0.25
Fibrinogen, mg/dL	3.585 (2.93 - 4)	3.56 (2.99 - 3.93)	3.72 (2.88 - 4.39)	0.93 (0.50 - 1.74)	0.82
Onset-To-TCD-Time, min	22 (14 - 23)	16 (13 - 23)	22 (17 - 23)	0.95 (0.89 - 1.03)	0.20
Stroke Characteristics					
NIHSS	**10 (3 - 17)**	**14.5 (10 - 20)**	**5 (1 - 13)**	**1.21 (1.09 - 1.34)**	**†<0.01**
Anterior Circulation, n (%)	22 (36)	10 (33)	12 (39)	0.79 (0.28 - 2.26)	0.66
Left side infarct, n (%)	20 (43)	13 (46)	7 (37)	0.97 (0.33 - 2.85)	0.95
Carotid/MCA Stenosis ≥50%, n(%)	10 (16)	5 (16)	5 (16)	1.00 (0.26 - 3.87)	1.00
Infarct Largest Size, mm	41 (66)	26 (84)	15 (48)	5.55 (1.69 - 18.2)	0
Cardiac variables					
Permanent AF, n(%)	46 (77)	27 (90)	19 (63)	0.19 (0.05 - 0.78)	0.02
Mean BP, mm Hg	80 (70 - 90)	79.5 (62 - 87)	80 (70 - 96)	0.97 (0.94 – 1.00)	0.07
Troponin I, ng/mL	0.02 (0.01-0.06)	0.02 (0.01 - 0.07)	0.01 (0.01 - 0.04)	21 (0.01 - 69399)	0.46
BNP, pg/ml	343 (145 - 639)	428 (343 - 834)	145.2 (106 - 216)	1.00 (1.00 - 1.01)	0.02
Left atrial size, mm	43.0 (7.4 – 55)	41.8 (4.5 - 47)	44.5 (35.5 - 56.5)	0.98 (0.96 - 1.01)	0.25
CHA2DS2VASC score	4 (3 - 5)	4 (4 - 6)	4 (3 - 4)	2.04 (1.29 - 3.22)	<0.01
Acute treatments					
Thrombolysis, n (%)	23 (37)	11 (35)	12 (39)	0.87 (0.31 - 2.44)	0.79
Thrombectomy, n (%)	26 (42)	22 (71)	4 (13)	16.5 (4.47 - 60.8)	<0.01
Single AP, n (%)	18 (30)	7 (23)	11 (35)	0.55 (0.18 - 1.70)	0.3
Double AP, n (%)	1 (2)	0 (0)	1 (3)	-	1.00
Warfarin, n (%)	5 (8)	3 (10)	2 (6)	1.61 (0.25 - 10.3)	0.62
LMWH full, n (%)	17 (89)	8 (100)	9 (82)	-	1.00
LMWH low dose, n (%)	17 (89)	8 (100)	9 (82)	-	1.00
Xa Inhibitor, n (%)	12 (20)	6 (20)	6 (19)	1.04 (0.29 - 3.68)	0.95
IIa Inhibitor, n (%)	0 (0)	0 (0)	0 (0)	-	-
Follow-up treatments					
Single AP, n (%)	6 (30)	1 (3)	5 (17)	1.55 (0.89 - 2.70)	0.09
Double AP, n (%)	1 (2)	0 (0)	1 (3)	-	1.00
Warfarin, n (%)	1 (2)	1 (3)	0 (0)		1.00
LMWH full, n (%)	1 (2)	0 (0)	1 (3)	-	1.00
LMWH low dose, n (%)	0 (0)	0 (0)	0 (0)	-	1.00
Xa Inhibitor, n (%)	12 (20)	29 (94)	22 (74)	0.30 (0.07 - 1.19)	0.08

IIa Inhibitor, n (%)	1 (2)	0 (0)	1 (3)	-	1.00

Continuous data presented as median (interquartile range)
Abbreviations: AF = Atrial fibrillation; AP = Antiplatelets; BMI = Boy mass index; BNP = Brain natriuretic peptide; BP = blood pressure; LMWH = Lower Molecular Weight Heparin; MCA = middle cerebral artery; MES = microembolic signals; mTICI = modified Thrombolysis in Cerebral Infarction; NHISS = Scores on the National Institutes of Health Stroke Scale; TCD = transcranial Doppler; mRS = modified Rankin scale
* Odds ratio (95% CI) and P values calculated by logistic regression
† Remained significant with p<0.05 after backward stepwise regression model (highlighted in bold)

Supplementary Table 2 Sensitivity analysis: Outcome measures based on MES positive or negative detection excluding large vessel stenosis.

Outcome measures	MES-positive n = 10	MES-negative n=41	Univariate Model	*Multivariate Model 1	†Multivariate Model 2
			Unadjusted Odds ratio (CI 95%); P value	Adjusted Odds ratio (CI 95%); P value	Adjusted Odds ratio (CI 95%); P value
§mRS 3 – 6 at 90 days – n (%)	8 (80)	18 (44)	5.11 (1.001 – 31.1); P = 0.04	4.05 (0.98 – 39.4); P = 0.06	4.05 (0.97–20.4); P = 0.11
‡mRS score shift – median (IQR)	2 (1 – 3)	3 (3 – 4)	1.98 (0.72 – 26.2); P = 0.14	2.00 (0.56 – 17.2); P = 0.34	1.67 (0.03 – 45.0); P = 0.33

Abbreviations: MES = microembolic signals;
All p-values and odds ratio calculated by logistic regression models
* Multivariate model 1 = adjusted to Age and NIHSS
† Multivariate model 2 = adjusted to unbalanced baseline differences between MES groups by probability score
§ Univariate and multivariate analysis with logistic regression to predict outcome
‡ Univariate and multivariate analysis with ordinal shift analysis to predict outcome

CHAPTER VII: MICROEMBOLI DETECTION IN ACUTE ISCHEMIC STROKE IS AN EARLY MARKER OF POST-STROKE COGNITIVE IMPAIRMENT

Title: Microemboli Detection in Acute Ischemic Stroke could be an Early Marker of Poor Cognitive Outcome

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Key words: infarction, embolism, ultrasound, transcranial Doppler
Subject terms: ultrasound, ischemic stroke, embolism

List tables/figures:

Table 1 Demographic, clinical and radiological characteristics of all patients and differences between MES-positive and MES-negative groups.

Abstract

Introduction: We investigated the burden of microembolic signals (MES) in patients with acute ischemic stroke (AIS) and their impact on long-term cognitive function.

Methods: This prospective study included consecutive ischemic stroke patients, without prior dementia or severe neurological deficits recruited between December 2020 and May 2022. Transcranial Doppler (TCD) monitoring was performed within 72 hours of symptom onset for 60 minutes monitoring, with 30 minutes dedicated to each cerebral circulation - Bilateral M1 segments of middle cerebral artery (MCA) and bilateral segments P2 of posterior cerebral artery (PCA). Recordings were analyzed for MES by a blinded expert. The primary aim was to determine the association of MES with cognitive status at 12 months, evaluated using a 7-point cognitive scale.

Results: Between November 2020 and May 2022, we included 316 patients, with a median age of 67 years (interquartile range 59-76) with 68% males. 39 patients had MES (12.3%), with a median (IQR) of 3 (1–7) counts among those who were positive. The occurrence of MES was higher in patients who underwent thrombolysis, thrombectomy, those who were not previously on statins, and those with a higher NIHSS score ($p < 0.05$). The worse cognitive outcome occurred in patients with MES with an adjusted odds ratio of 2.04 (CI 95% 1.27-3.28), $p<0.01$.

Conclusions: Patients who are MES-positive in the acute phase have twice the likelihood of experiencing worse cognitive outcomes 12 months after stroke. The detection of MES using TCD could be considered in future studies as an early marker of poor cognitive performance, helping to stratify high-risk patients. Future trials should investigate whether very early antithrombotic treatment could improve long-term cognitive function in stroke survivors (Clinicaltrials.gov ID: 06735274).

Main text

Introduction

Microembolic signals (MES) are a consistent and stroke marker of stroke recurrence (Sheriff et al., 2020; Sudheer et al., 2021). This is particularly notable in carotid stenosis where the presence of MES increases by 1.5 (OR) the chance of a new event (Goessens et al., 2007). However, the relationship of MES with long-term functional recovery after stroke has not been consistent among studies (Das et al., 2020; Sheriff et al., 2020).

From the pathophysiological perspective, presence of MES could be linked to cognitive impairment. MES, as a marker of recurrent ischemic lesions in the brain should reduce the brain resilience overtime and the development of cognitive deficits (Gao et al., 2004). Evidence gathered from clinical, pathological, and neuroimaging studies shows that cognitive impairment arises because of cerebrovascular lesions or reduced cerebral blood perfusion (Clancy et al., 2024; Kalaria, 2012; Stefano Govoni et al., 2020). MRI studies show that cortical microinfarcts, presumably embolic in nature, independently contribute to dementia (Corrada et al., 2016). Interestingly, a recent trial shows that the MES burden during carotid intervention is associated with cognitive impairment at follow-up or endarterectomy (Hrbac et al., 2017; Skoloudik D. et al., 2023).

Post stroke cognitive impairment can affect up to 60% of survivors which presents as a major burden for patients and families. Of these patients, about 20% with mild PSCI fully recover their pre-stroke cognitive function; however, it is more common to maintain long-term cognitive impairment (El Husseini et al., 2023; Quinn et al., 2021). The role of MES in the cognitive decline process after stroke has been presented inconsistently in previous studies, and the practical significance of these findings remains unclear (Goldberg et al., 2012; C. J. Lin et al., 2022).

Assessing cognitive function in post-stroke patients requires careful attention, as it is necessary not only to evaluate the patient's cognitive symptoms but also to understand the impact on daily activities and monitor its progression over time (Biesbroek & Biessels, 2023; Quinn et al., 2021). Thus, our study will prospectively follow patients, assess the various cognitive domains, and evaluate their impact on daily living activities.

For better clinical management of patients, monitoring MES in the acute phase after stroke may be useful. Therefore, this prospective study investigated the burden of MES in patients with acute ischemic stroke and their impact on cognitive status at 12 months, evaluated using a 7-point cognitive scale.

Methods

Ethics Considerations

This study was approved by the local institutional "Ethics Committee of Centro Hospitalar Universitário São João" (303/2020) and was registered to ClinicalTrials.gov (Clinicaltrials.gov ID: 06735274, https://www.clinicaltrials.gov/). Written informed consents were obtained after a full description of the study to all participants/guardians.

Data availability

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

Participants

This is a prospective study with reading of MES events by one expert, blinded to clinical data and outcomes. Inclusion criteria were patients with acute ischemic stroke admitted to the Stroke Unit, a previous Modified Rankin Score (mRs) <4 and with possibility to be monitored within 72 hours after symptom onset. Exclusion criteria were: prosthetic valves; uncooperative patients; absence of adequate temporal bone window for TCD assessment; patients with a large cerebral infarct (greater than on third of the middle cerebral artery territory); advanced kidney disease or hepatic diseases; active cancer patients; severe aphasia; severe hearing or visual loss and prior diagnosis of dementia or documented evidence of previous cognitive decline in the clinical record or under anticholinesterase drugs or memantine, or an Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) score>3.5 (van Nieuwkerk et al., 2021).

The follow-up assessment took place after 12±3 month after onset symptoms, in outpatient. In the follow-up we excluded patients with kidney disease, active cancer patients, patients with severe aphasia and severe hearing loss that did not allow cognitive assessment.

Clinical, laboratory, and radiological assessment

Vascular risk factors, medications, previous cardiovascular disease, blood pressure (BP), serum glucose, cholesterol levels and reperfusion treatment (Higashida et al., 2003) were recorded at hospital admission. National Institutes of Health Stroke Scale (NIHSS) scores were recorded at admission and 24 hours after onset. The infarct volumes were reviewed and calculated by planimetry, based on a head 24h-CT scan. At follow-up, BP, weight, height, abdominal circumference, medication, and new vascular events were recorded.

Microembolic signals detection

The presence and rate of MES were assessed by TCD monitoring (Doppler Box X, DWL, Sipplingen, Germany), within 72 hours from the last known well time. The presence and rate of MES were assessed over 60 minutes, with 30 minutes dedicated to each cerebral circulation - Bilateral M1 segments of middle cerebral artery (MCA) and bilateral segments P2 of posterior cerebral artery (PCA). Bilateral M1 segments of MCA and bilateral segments P2 of PCA were insonated at a single depth with 2-MHz transducers secured by a probe-holder with a sample volume of 8 mm and low gain (E. B. Ringelstein et al., 1998). MES was defined according to previous consensus (E. B. Ringelstein et al., 1998) as a signal with 1) duration <300 ms, 2) amplitude >3 dB with respect to underlying flow signal, 3) unidirectionality, and 4) typical audible sound ("snap" and/or "chirp") (Supplementary Figure 1). Recordings were analyzed by a single experienced reader blinded to clinical data and outcomes (GP). Patients with ≥1 MES detected were assigned to MES positive group.

Clinical endpoints

The main outcome was the cognitive status at 12 months of follow-up which was based on 7-scale cognitive score was operationalized from Montreal Cognitive Assessment.

The degree of disability or dependence in the daily activities of patients after a stroke was measured by the Modified Rankin Scale (mRS) at 12-months post-stroke and by the Instrumental Activities of Daily Living (IADL) Scale (Araújo F., 2008; Justo-Henriques S, 2022; Lawton MP, 1969). Cognitive status at 12 months of follow-up was based in 7-scale cognitive score was operationalized from Montreal Cognitive Assessment (Supplementary Table 1) (Katz et al., 2021; Wardlaw et al., 2021; Wardlaw et al., 2023). Cognitive status at 12 months of follow-up was based on a 7-level cognitive score, which was operationalized from the Montreal Cognitive Assessment. The 7-level score includes the functional impact on activities of daily living after stroke. As a secondary endpoint, we also studied cognitive impairment as defined by ESOG 2023 guideline (Quinn et al., 2021).

Statistical Analysis

Sample size calculation

In our study, we included patients with any etiology of ischemic stroke. A previous study shows that the percentage of MES varies between different etiologies, ranging from 14% in strokes of small-vessel cerebral etiology to 50% in embolic strokes of undetermined source (Higuchi et al., 2020). Across all etiologies, recent studies report

prevalence rates ranging from 15% to 61% (Farina et al., 2018; Nakajima et al., 2007; Sheriff et al., 2020).

We considered a 31% prevalence of MES-positive cases based on studies with diverse etiologies, with a power of 95%, α=0.05, and a margin of error of 10%. Additionally, we considered that about 60% of patients may have long-term changes in cognitive function, with Odds Ratio = 0.51 at 18 months (Aam et al., 2020).

Considering a 95% confidence interval, a margin of error of 5%, and an estimated 40% loss to follow-up, we planned to enroll a minimum of 350 participants. Sample size estimates were performed with G*Power version 3.1.9.7.

Descriptive and outcome analysis

The normality of continuous variables was inferred by the Kolmogorov–Smirnov test. The baseline characteristics of patients in the MES-positive and MES-negative groups were compared using chi-square/Fisher exact tests for categorical variables and Mann-Whitney tests for continuous variables (Table 1).

Clinical outcomes in the MES-positive and MES-negative groups were compared using an ordinal logistic regression model to generate odds ratios (OR) and 95% confidence intervals (CI). This model assessed the relationship between the presence of MES and cognitive outcomes, which were evaluated in ordered categories.

We used generalized linear mixed models (GLMMs) with an ordinal logistic link function to evaluate whether the presence of microembolic signals (MES) could predict the 7-Level Cognition Score 12 months after stroke. Analyses were adjusted using four multivariate models. In the Model 1 = Adjusted for age and years of education; Model 2 = Adjusted for age, years of education, hypertension, diabetes, and smoking; and Model 3 = Adjusted for age, years of education, NIHSS at admission, and systolic blood pressure; and Model 4 = adjusted for age, years of education, and medication use (statins, antiplatelets, anticoagulants, and antidepressants) (Table 2).

The ordinal outcome variable—the 7-level cognitive score—was modelled using a multinomial distribution with a cumulative logit link. For the binary outcome (cognitive impairment defined as MoCA<22; Supplementary Table 2), we used a binomial distribution with a logit link function. Random effects were specified for arterial site (MCA or PCA) and side (left or right) to account for potential clustering within these levels. Models were estimated using maximum likelihood with robust standard errors, and a 95% confidence level was applied for parameter estimates. Analyses were adjusted using four multivariate models with different sets of fixed factors. These factors were selected based on their association with MES, the outcome of interest in our exploratory analysis, and prior literature on known prognostic variables.

All statistical analyses were performed with IBM SPSS Statistics for Windows, version 28. Statistical significance was inferred at $p<0.05$.

Results

Between November 2020 and May 2022, we enrolled 316 patients (Figure 1), with a median age of 67 years (interquartile range 59-76) and a median National Institutes of Health stroke scale severity score of 5 (3-10) (Table 1).

MES were detected in 39 patients (12.3%), with a median (IQR) of 3 (1–7) counts among those who were positive. Patients with a higher number of microemboli did not show worse cognitive outcomes 12 months after the stroke (Figure 2). The occurrence of MES was significantly associated with a history of prior stroke, stroke severity, thrombolysis, and thrombectomy ($p<0.05$). Patients who were on statin therapy prior to the stroke were significantly more likely to be MES-negative (Table 1). MES were observed more frequently in patients with higher values of glycemia, High-Sensitivity Troponin T, BNP, and LDL cholesterol, although these associations were not statistically significant (Table 1) ($p>0.05$).

At follow-up 67% patients at least some degree of cognitive impairment. MES was significantly associated with worse cognitive outcomes, even after adjusting for age and years of education, adjusted OR 2.04 (95% CI 1.27–3.38), $p<0.01$. None of the MES-positive patients had normal cognition scores and 52% had at least moderate major cognitive impairment or death, against 40% in MES-negative groups (Figure 2). The presence of MES was associated with a worse cognitive outcome 12 months after stroke even after adjusting to the biased characteristics related to MES occurrence (hypertension, diabetes, smoking and medication) or age and stroke severity (Table 2). The burden of MES is not associated with worse cognitive outcomes 12 months after stroke ($p>0.05$) (Figure 3).

The results of the GLMM analysis also showed a significant association between the cognitive impairment variable (MoCA<22) and MES-positive ($p=0.04$). The results indicate that the presence of MES was significantly associated with cognitive decline 12 months after the stroke, even after adjusting for age and years of education, adjusted OR 0.50 (95% CI 0.26–0.93), $p=0.02$, and after adjusting for age, years of education, hypertension, diabetes, and smoking, adjusted OR 0.49 (95% CI 0.26–0.92), $p=0.02$ (Supplementary Table 2).

Patients with MES-positive and brain lesions in the anterior circulation had worse cognitive outcomes 12 months after stroke ($p<0.05$), as did those with MES-positive and brain lesions in the left hemisphere. No statistically significant differences were observed between MES detected in the M1 segment of the MCA or MES-positive detected in the

P2 segment of the PCA regarding cognitive outcomes (p=0.82) (Supplementary Table 3).

A sensitivity analysis by subgroups was conducted considering treatment (reperfusion vs. no reperfusion), sex (male vs. female), and pre-stroke functional disability (mRS 0–1) as subgroup variables. Statistically significant effects were observed for these variables ($p < 0.05$) (Supplementary Table 4).

Discussion

In this observational prospective study on patients with acute ischemic stroke, MES was detected by transcranial Doppler monitoring in 12% of patients, within the first 72 hours after symptom onset. Most importantly, the presence of MES was shown to have a negative impact on cognitive outcomes 12 months after stroke, regardless of stroke etiology. When adjusting for age and education, the effect of microembolic signals on cognitive outcomes remained significant, suggesting that the relationship is not solely explained by differences in age or educational level among patients. These findings indicate that the presence of microembolic signals may serve as an independent marker of worse long-term cognitive function. This information could be valuable for risk stratification and early intervention planning.

The major strength of this study is the sample size included and the rigorous control of potential technical confounding factors, such as having an external offline reading, one expert reader for MES recording and pre-defined insonation parameters. This obviates most of the confounding factors of MES monitoring (Cullinane et al., 2002).

Burden of microemboli in acute phase after stroke

Previous studies showed a large variability of positive MES counts between 17 and 40% (Ritter et al., 2008). In our study, the MES burden accounted for 12% of the total cohort (39/316), with a mean value of five microemboli. Patients with a higher number of microemboli did not show worse cognitive outcomes 12 months after the stroke (Figure 3).

We included strokes of various etiologies and an extended monitoring period during the acute phase. The median time between the last known well time and TCD monitoring was 35 (21–52) hours, which may explain the lower prevalence of MES-positive patients. A statistically significant difference was observed between the groups, with the MES-negative group including patients with a longer time interval between stroke onset and TCD monitoring (Table 1). Previous studies show that MES are more frequent in the first hours following an ischemic stroke and gradually decrease in the days after the event. A recent study with repeated TCD monitoring over time

demonstrated that patients who tend to be MES-negative within the first 24 hours are likely to remain so (Aarli et al., 2024).

Our results show that the occurrence of MES was significantly associated with a history of prior stroke, stroke severity, thrombolysis, and thrombectomy. Patients who were on statin therapy prior to the stroke were significantly more likely to be MES-negative. These findings suggest that pretreatment with statins appears to be associated with a lower incidence of MES in patients with acute cerebral ischemia (Safouris et al., 2018).

Endovascular treatment combined with thrombolysis and intravenous thrombolysis alone are commonly used treatments in the acute phase of ischemic stroke. The presence of MES has been described as a common occurrence following these treatments in the acute phase and is associated with monitoring by TCD shortly after the performance of thrombectomy and/or thrombolysis. The use of different recanalization strategies, with or without prior intravenous thrombolysis, has shown a similar embolic risk (Farina et al., 2018). In our results, the number of patients undergoing thrombectomy and/or thrombolysis was also higher in the group of patients who presented with MES.

Although without statistically significant differences, MES were observed more frequently in patients with higher values of glucose, High-Sensitivity Troponin T, BNP, and LDL cholesterol. Our findings support the role of MES as indicators of vulnerable tissue surfaces and reflect activated stages in the coagulation system. Interestingly, BNP and High-Sensitivity Troponin T values, which indicate cardiac insufficiency and cardiac damage, were significantly elevated in our MES-positive cases, as well as values of glucose and LDL cholesterol, which are associated with endothelial injury (Teo & Rafiq, 2021; Tomita et al., 2008). These characteristics highlight an additional important pathophysiological factor linking microembolism and worse clinical outcomes (Tomita et al., 2008).

Microembolism and Long-term Cognitive function after stroke

Our data provides evidence that the presence of MES in the acute phase after ischemic stroke predicts worse cognitive performance 12 months after the event, even after adjusting for variables that influence cognition: age, years of education, hypertension, diabetes, smoking, NIHSS at admission, SBP, and medication (statins, antiplatelets, anticoagulants, and antidepressants).

The subgroup sensitivity analysis suggested that both reperfusion treatment groups (treated vs. untreated), sex groups (male vs. female), and pre-stroke mRS group (0–1) influenced the cognitive outcome, with statistically significant differences observed

(Supplementary Table 4). Our data support a recent study showing that sex does not influence post-stroke cognitive impairment, indicating that being male or female does not affect cognitive outcomes (Exalto et al., 2023).

The assessment of cognitive function in post-stroke patients requires careful attention. It is essential to evaluate the patient's cognitive symptoms, understand their impact on daily living activities, and monitor their progression over time. Given the need for this assessment to cover multiple cognitive domains, the MoCA test is widely recommended and used in clinical settings (Biesbroek & Biessels, 2023; Quinn et al., 2021). In our study, to assess cognitive status at 12 months of follow-up, we used a 7-scale cognitive score operationalized from the Montreal Cognitive Assessment, which incorporates functional state (mRS) and independence in daily living activities (Supplementary Table 1).

We monitored MES in both the anterior and posterior circulation, regardless of the lesion location. A complementary analysis revealed that the presence of MES in patients with brain lesions in the anterior region or the left cerebral hemisphere was associated with worse cognitive outcomes (Supplementary Table 3).

Although patients with severe aphasia were excluded, brain lesions in areas responsible for language directly affect cognitive function, as do lesions in other anterior brain regions that impact cognitive performance. In this study, we included patients with lesions in the anterior and posterior regions of the brain, in both the left and/or right hemispheres.

Using the recommended cut-off score for cognitive impairment (MoCA<22) (Quinn et al., 2021), we conducted a complementary analysis based solely on this criterion. This analysis also indicated that the presence of microembolic signals (MES) in the acute phase after stroke predicted worse cognitive outcomes 12 months post-event. Although the univariate analysis suggested that being MES-positive in the acute phase was associated with a lower likelihood of cognitive impairment (MoCA < 22) at 12 months (OR = 0.55, 95% CI: 0.31–0.99), this association was confounded by stroke severity and other clinical characteristics (Supplementary Table 2, models 3 and 4). Importantly, this endpoint does not consider functional status after stroke, and poorer functional outcomes—including death—are not necessarily linked to cognitive impairment. Therefore, the use of multidimensional scales to assess cognitive impairment is advisable (Supplementary Table 2) (Wardlaw et al., 2023).

Silent embolism occurring during the first hours after a stroke may be associated with new ischemic lesions and/or enlargement of the final infarct area, which can worsen the cognitive outcomes of patients (D. W. Kang et al., 2016; Lee et al., 2016). Most importantly, the presence of MES was shown to have a negative impact on cognitive

outcomes 12 months after stroke. MES presence varies according to stroke etiology (Castro et al., 2024; Sheriff et al., 2020), but the low number of cases prevents definitive conclusions about the interaction between etiology and MES influence on outcome (Supplementary Table 5).

Limitations

To cover the monitoring of both the posterior and anterior circulation, regardless of the lesion's location, patients were monitored for only 30 minutes per segment. Monitoring for more than one hour is the recommended duration to increase the probability of detecting microemboli and measuring the embolic load (Aarli et al., 2024). However, in our study, the burden of microemboli was not related to any of the outcomes.

The differences in baseline characteristics between MES groups may introduce bias, as the MES-positive group had a higher NIHSS. Patients with more severe strokes are more frequently referred to for endovascular treatments, which, in turn, are associated with various complications, such as vascular/endothelial damage. Endothelial damage not only increases the recorded MES rate but also directly impacts cognition (Farina et al., 2018; Ritter et al., 2008; Sliwka et al., 1997; Spence, 2016).

In this study, the actual dropout rate (21%) was substantially lower than the 40% anticipated in the sample size calculation. Given that the study was conducted during the COVID-19 pandemic and in a mothership, centre serving remote areas of northern Portugal, a higher rate of loss to follow-up at 12 months had been expected.

Conclusions

Even with the best medical treatment, 12% of patients experience microembolism within the first 72 hours after an ischemic stroke. The detection of microembolic signals with transcranial Doppler was associated with worse cognitive function 12 months after stroke. Future studies will investigate whether specific strategies, such as anticoagulants and antiplatelet agents in patients with cardiovascular risk factors, can reduce the frequency of microembolism and improve cognitive outcomes.

Tables

Table 1 Demographic, clinical and radiological characteristics of all patients and differences between MES-positive and MES-negative groups.

	Total	MES-Negative	MES-positive	P value*

	N = 316	N = 277	N = 39	
Demographic and clinical variables				
Age, years	67 (59-76)	67 (59-76)	66 (54-75)	0.39
Male sex, n (%)	215 (68)	195 (70)	20 (51)	**0.02**
BMI, $Kg.m^{-2}$	26.7 (24.4-29.4)	26.9 (24.5-29.5)	26.0 (24.2-29.2)	0.43
Years of Education	5 (4-10)	5 (4-10)	4 (4-9)	0.27
SBP, mm Hg	133 (120-148)	134 (120-149)	124 (103-143)	**0.02**
DBP, mm Hg	72 (64-82)	73 (65-82)	67 (55-80)	0.04
Glycemia, mg/dL	118 (100-144)	117 (100-144)	121 (104-145)	0.52
High-Sensitivity Troponin T, ng/L	9.6 (4.0-18.0)	9.2 (4.0-18.0)	13.4 (4.3-28.7)	0.16
Creatinine, mg/dL	0.8 (0.7-1.0)	0.8 (0.7-1.0)	0.8 (0.6-1.0)	0.50
BNP, pg/mL	72.8 (27.0-228.4)	66.4 (26.8-223.3)	88.1 (43.1-310.8)	0.10
HDL-chol, mmol/L	45.0 (38.0-55.0)	45.0 (38.0-55.0)	49.0 (42.0-56.0)	0.24
LDL-chol, mmol/L	98.0 (73.0-128.0)	97.0 (72.0-127.0)	106.0 (76.0-144.0)	0.32
HbA1c, %	5.8 (5.5-6.4)	5.8 (5.5-6.4)	5.8 (5.5-6.1)	0.66
Hypertension, n (%)	203 (64)	180 (65)	23 (59)	0.47
Dyslipidemia, n (%)	169 (54)	149 (54)	20 (51)	0.86
Diabetes, n (%)	81 (26)	73 (26)	8 (21)	0.55
Smoking, n (%)	71 (23)	60 (22)	11 (28)	0.41
Previous Stroke, n (%)	49 (16)	38 (14)	11 (28)	**0.03**
Coronary Disease, n (%)	23 (7)	19 (7)	4 (10)	0.50
Atrial Fibrillation, n (%)	59 (19)	49 (18)	10 (26)	0.27
Heart Failure, n (%)	19 (6)	15 (5)	4 (10)	0.27
Peripheral Arterial Disease, n (%)	16 (5)	15 (5)	1 (3)	0.70
Depression, n (%)	41 (13)	36 (13)	5 (13)	1.00

Onset-To-Door-Time, min		205 (88-690)	229 (91-691)	162 (78-620)	0.30
Onset-To-Recanalization-Time, min		400 (268-614)	378 (260-622)	419 (297-606)	0.39
Onset-To-TCD-Time, hours		35 (21-52)	37 (22-54)	25 (19-40)	**0.01**
Previous Medication					
Antidepressants, n (%)		39 (12)	34 (12)	5 (13)	1.00
Benzodiazepines, n (%)		81 (26)	72 (26)	9 (23)	0.84
Antihypertensives, n (%)		61 (19)	52 (19)	9 (23)	0.51
Betablockers, n (%)		90 (28)	78 (28)	12 (31)	0.70
Calcium channel blockers, n (%)		42 (13)	41 (15)	1 (3)	**0.04**
ACE inhibitors or ARB		127 (40)	114 (41)	13 (33)	0.38
Metformin, n (%)		63 (20)	59 (21)	4 (10)	0.13
DPP-4 inhibitors, n (%)		33 (10)	30 (11)	3 (8)	0.78
SGLT2 inhibitors, n (%)		19 (6)	17 (6)	2 (5)	1.00
GLP-1 receptor agonists, n (%)		3 (1)	3 (1)	0 (0)	1.00
Other Antidiabetic, n (%)		5 (2)	5 (2)	0 (0)	1.00
Insulin, n (%)		11 (3)	9 (3)	2 (5)	0.63
Anticoagulants, n (%)		208 (66)	182 (66)	26 (67)	1.00
Previous Statin, n (%)	Low	4 (1)	3 (1)	1 (3)	**0.01**
	High	219 (69)	200 (72)	19 (49)	
Previous AP, n (%)	Mono AP therapy	166 (53)	144 (52)	22 (56)	0.11
	Dual AP therapy	88 (28)	82 (30)	6 (15)	
Stroke Characteristics					
Stroke Etiology	Large Vessel Atherothromboembolic Etiology, n (%)	54 (17)	44 (16)	10 (26)	0.26
	Cardioembolic Etiology, n (%)	59 (19)	49 (18)	10 (26)	
	Small Vessel Disease Etiology, n (%)	63 (20)	59 (22)	4 (10)	
	Stroke of Other Determined Etiology, n (%)	15 (5)	14 (5)	1 (3)	
	Dual Cause of Stroke, n (%)	7 (2)	7 (3)	0 (0)	
	Stroke of Undetermined Etiology, n (%)	112 (36)	98 (36)	14 (36)	

Admission NIHSS score	5 (3-10)	5 (3-9)	8 (5-15)	**<0.01**
24-hours NIHSS score	3 (2-6)	3 (2-6)	7 (3-11)	**<0.01**
Infarct Largest Size, mm, median (range)	7 (3-16)	8 (2-17)	7 (2-14)	0.45
Thrombolysis, n (%)	86 (27)	69 (25)	17 (44)	**0.02**
Thrombectomy, n (%)	80 (25)	62 (22)	18 (46)	**<0.01**

Continuous data presented as median (interquartile range)

Abbreviations: BMI = Boy mass index; SBP= Systolic Blood Pressure; DBP= Diastolic Blood Pressure; HbA1c = hemoglobinA1c; HDL-chol = high-density lipoprotein cholesterol; LDL-chol = low-density lipoprotein cholesterol; AP = Antiplatelet; ACE = Angiotensin converting enzyme inhibitors; ARB = angiotensin receptor blockers; DPP-4 inhibitors = Dipeptidyl Peptidase-4 inhibitors; SGLT2 inhibitors = Sodium-glucose Cotransporter-2 Inhibitors; GLP-1 receptor agonists = Glucagon-like peptide-1 receptor agonists; MES = microembolic signals; NIHSS = National Institutes of Health Stroke Scale; TCD = transcranial Doppler

* P values are shown for differences between MES-positive and MES-negative groups obtained from chi-square test/ Fisher's exact test for categorical variables, or Mann-Whitney test for continuous variables.

Table 2 Multivariable Model (GLMM) for 7-Level Cognition Score 12 months after stroke.

	Univariate Model		Multivariate model 1*		Multivariate model 2†		Multivariate model 3§		Multivariate model 4‡	
Total n=316	OR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value
MES-positive	1.78 (1.12-2.84)	0.01	2.04 (1.27-3.28)	<0.01	2.09 (1.30-3.35)	<0.01	1.75 (1.07-4.28)	0.02	1.67 (1.03-2.70)	0.03

Abbreviations: MES = microembolic signals; SBP= Systolic Blood Pressure; NIHSS = National Institutes of Health Stroke Scale; OR=Odds Ratio; aOR=Adjusted Odds Ratio;

All p-values and odds ratios were obtained from generalized linear mixed models (GLMMs), using logistic regression for binary outcomes and ordinal logistic regression (ordinal shift analysis) for ordinal outcomes.

* Multivariate model 1 = adjusted to Age and years of education

† Multivariate model 2 = adjusted to Age, years of education, hypertension, diabetes and smoking.

§ Multivariate model 3 = adjusted to Age, years of education, NIHSS at admission and SBP.

‡ Multivariate model 4 = adjusted to Age, years of education and medication (Statins, antiplatelets, anticoagulants, and antidepressants).

Figures

Figure 1 Study flow chart

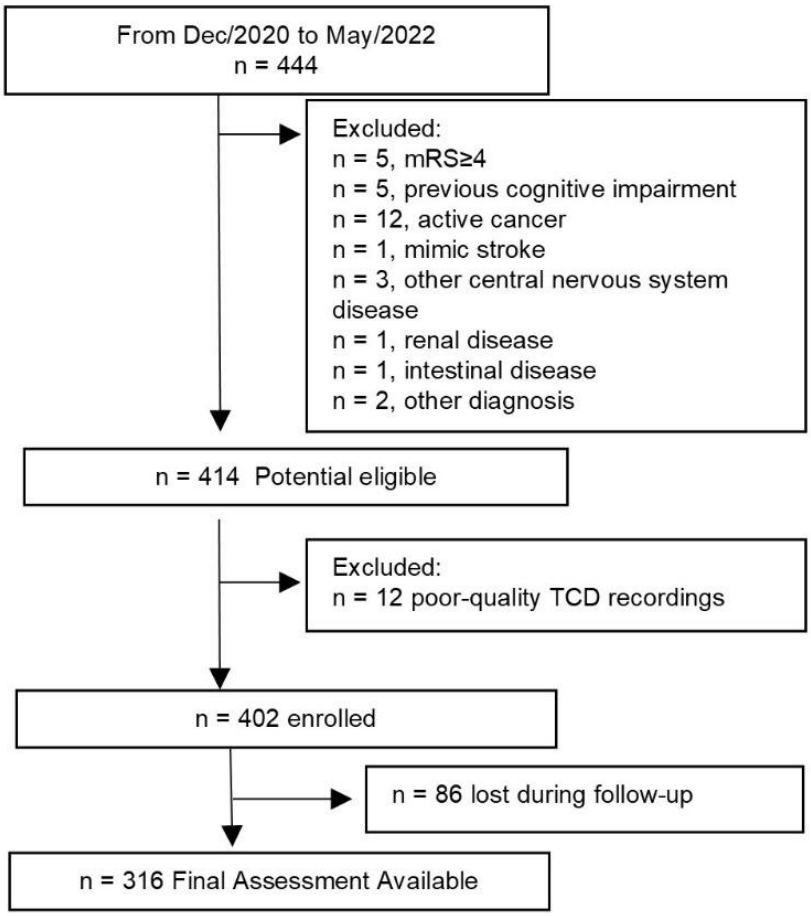


Figure 2 Presence of MES by 7-Level Cognition Score 12 months after stroke.

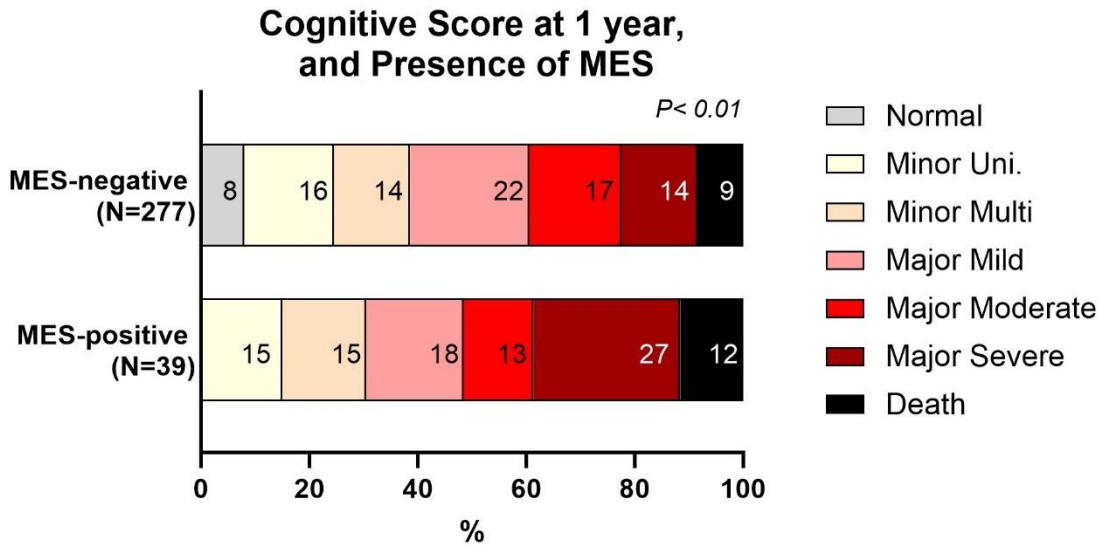
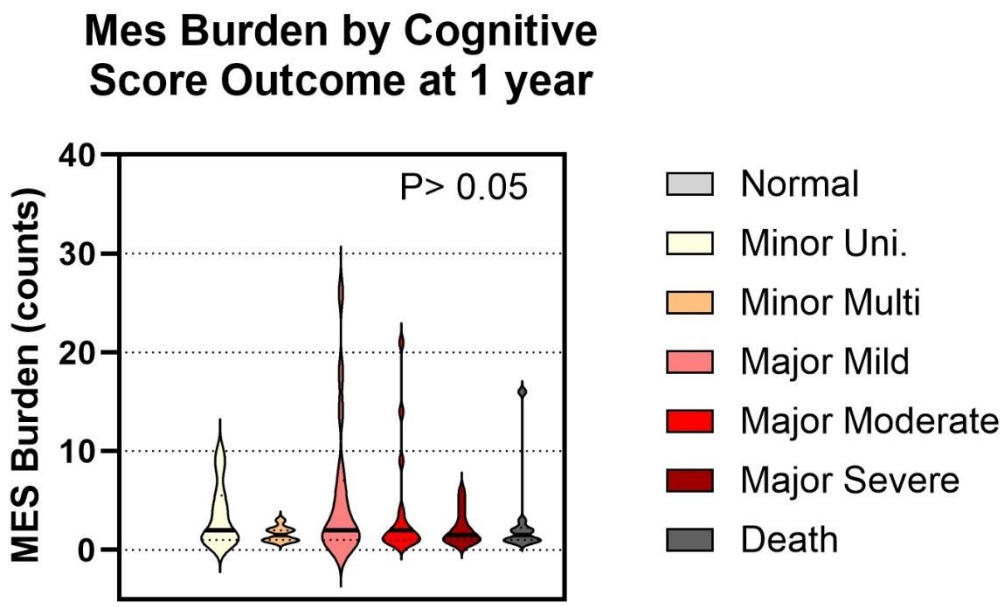


Figure 3 MES burden by 7-Level Cognition Score 12 months after stroke



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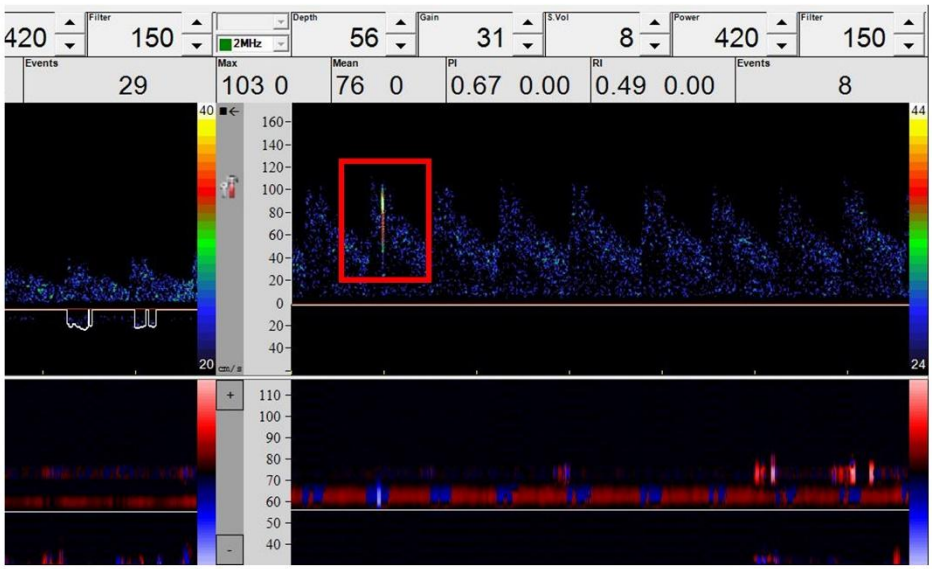
Supplementary Figure 1. Microembolic signal recorded by transcranial Doppler.



Supplementary Table 1. Cognition categories and operational for primary endpoint (Wardlaw et al., 2023)

Primary Category	Operationalization	Sub-category	Operationalization
Normal cognition	No evidence of cognitive impairment (MoCA: 30–29 OR IADL=8 for women/IADL≥5 for men)	**Normal**	
Minor Neurocognitive disorder (mild cognitive impairment)	Evidence of cognitive impairment (MoCA: 28–26 OR IADL: 7-6 for woman and IADL: 4-3 for men) AND No evidence of functional impairment (mRS < 2 OR no change in mRS if pre-stroke mRS > 1)	**Single Domain**	Scores are reduced by >1 point in only one cognitive domain of MoCA or IADL: 7-6 for woman and IADL: 4-3 for men
		Multi domain	Scores are reduced by >1 point in more than one cognitive domain of MoCA or IADL: 7-6 for woman and IADL: 4-3 for men
Dementia	Clinical diagnosis made independent of study -Any clinical diagnosis of dementia made by memory clinic (or equivalent, this would include primary care) -Any recording of dementia on death certification	**Mild**	Cognitive impairments (MoCA 20-25 OR IADL ≤ 5 for women/IADL ≤ 3 for men) AND Minimal functional problems (mRS < 3)
		Moderate	More severe cognitive impairment (MoCA 14–19 OR IADL ≤ 5 for women/IADL ≤ 3 for men) AND more limiting function (mRS 3 or 4)

	-Any prescription of cholinesterase inhibitor or memantine OR Pre-stroke dementia (Baseline assessment IQCODE > 3.6) OR In-study evidence of persisting multi-domain cognitive impairment (MoCA score ≤20 OR IADL≤5 for women/IADL≤3 for men on more than one annual follow-up) and Evidence of functional impairment (mRS ≥ 2 or IQCODE > 3.6 at final follow-up)	**Severe**	Severest cognitive impairments (MoCA < 14 OR IADL ≤ 5 for women/IADL ≤ 3 for men) AND Most limited function Care-home admission OR mRS 4/5.
Death		**Death**	

Abbreviations: IADL = Instrumental Activities of Daily Living; mRS = Modified Rankin Scale; MoCA test = Montreal Cognitive Assessment test; IQCODE = Informant Questionnaire on Cognitive Decline in the Elderly; Adapted by: Wardlaw, J. M., Woodhouse, L. J., Mhlanga, II, Oatey, K., Heye, A. K., Bamford, J., Cvoro, V., Doubal, F. N., England, T., Hassan, A., Montgomery, A., O'Brien, J. T., Roffe, C., Sprigg, N., Werring, D. J., Bath, P. M., & Lacunar Intervention Trial-2 Investigator, G. (2023). Isosorbide Mononitrate and Cilostazol Treatment in Patients With Symptomatic Cerebral Small Vessel Disease: The Lacunar Intervention Trial-2 (LACI-2) Randomized Clinical Trial. *JAMA Neurol*, *80*(7), 682-692. https://doi.org/10.1001/jamaneurol.2023.1526

Supplementary Table 2. Multivariable Model (GLMM) for Cognitive Impairment 12 months after stroke (MoCA<22).

	Univariate Model		Multivariate model 1*		Multivariate model 2†		Multivariate model 3§		Multivariate model 4‡	
Total n=316	OR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value

MES-positive	0.55 (0.31-0.99)	0.04	0.50 (0.26-0.93)	0.02	0.49 (0.26-0.92)	0.02	0.61 (0.31-1.18)	0.14	0.69 (0.35-1.34)	0.27

Abbreviations: MES = microembolic signals; SBP= Systolic Blood Pressure; NIHSS = National Institutes of Health Stroke Scale; OR=Odds Ratio; aOR=Adjusted Odds Ratio;

*All p-values and odds ratios were calculated using multinomial logistic regression models. A multivariate analysis with a generalized logit model was employed to predict outcome. Multivariate model 1 = adjusted to Age and years of education

† Multivariate model 2 = adjusted to Age, years of education, hypertension, diabetes and smoking.

§ Multivariate model 3 = adjusted to Age, years of education, NIHSS at admission and SBP.

‡ Multivariate model 4 = adjusted to Age, years of education and medication (Statins, antiplatelets, anticoagulants, and antidepressants).

Supplementary Table 3. Sensitivity Analysis: GLMM for 7-Level Cognition Score at 12 Months After Stroke

	Univariate Model	
Total n=316	OR (95% CI)	*P-value
MES-positive in anterior circulation stroke	1.67 (1.00-2.78)	**0.04**
MES-positive in posterior circulation stroke	1.95 (0.60-6.28)	0.26
MES-positive in left side stroke	1.90 (1.02-3.53)	**0.04**
MES-positive in right side stroke	1.75 (0.72-4.22)	0.21
MES-positive in P2 segment of the posterior cerebral artery	1.02 (0.83-1.24)	0.82

Abbreviations: MES = microembolic signals.

*All p-values and odds ratios were derived from multivariable generalized linear mixed models (GLMMs) using ordinal logistic regression (ordinal shift analysis) to predict the 7-level cognitive score at 12 months post-stroke.

Supplementary Table 4: Sensitivity Analyses by Subgroups

	Univariate Model		Multivariate model 1*		Multivariate model 2†		Multivariate model 3§		Multivariate model 4‡	
With Reperfusion (n = 129)										
	OR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value

MES-positive	1.85 (1.02–3.36)	0.04	2.10 (1.12–3.95)	0.02	2.05 (1.08–3.88)	0.03	1.78 (1.03–3.86)	0.03	1.74 (1.03–3.60)	0.04
Without Reperfusion (n = 187)										
	OR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value
MES-positive	1.72 (1.01–2.94)	0.04	1.95 (1.09–3.52)	0.02	2.00 (1.12–3.59)	0.02	1.68 (1.05–3.45)	0.04	1.61 (0.01–3.22)	0.05
Previous mRS 0–1 (n = 274)										
	OR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value
MES-positive	1.80 (1.11–2.91)	0.02	2.08 (1.27–3.39)	<0.01	2.12 (1.29–3.48)	<0.01	1.76 (1.01–3.55)	0.04	1.71 (1.00–3.01)	0.05
Previous mRS 2–3 (n = 42)										
	OR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value
MES-positive	1.65 (0.68–3.99)	0.27	1.80 (0.72–4.47)	0.20	1.75 (0.70–4.38)	0.22	1.54 (0.62–4.12)	0.31	1.51 (0.59–3.95)	0.33
Male (n = 215)										
	OR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value
MES-positive	1.85 (1.11–2.91)	0.04	2.08 (1.03–2.45)	0.01	2.12 (1.10–3.23)	<0.01	1.24 (1.01–3.55)	0.02	1.35 (1.02–2.02)	0.02
Female (n = 107)										
	OR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value
MES-positive	1.81(1.21–3.76)	0.02	1.78 (1.27–3.39)	<0.01	2.01 (1.05–3.67	0.04	1.76 (1.08–4.96)	0.04	1.53 (1.001–5.39)	0.05

Abbreviations: MES = microembolic signals; OR=Odds Ratio; aOR=Adjusted Odds Ratio;

All p-values were calculated using generalized linear mixed models (GLMMs). For multivariate analysis, we applied an ordinal mixed-effects model (ordinal GLMM).

* Multivariate model 1 = adjusted to Age and years of education

† Multivariate model 2 = adjusted to Age, years of education, hypertension, diabetes and smoking.

§ Multivariate model 3 = adjusted to Age, years of education, NIHSS at admission and SBP.

‡ Multivariate model 4 = adjusted to Age, years of education and medication (Statins, antiplatelets, anticoagulants, and antidepressants).

Supplementary Table 5: MES-positive number according to stroke etiology

	MES-positive	*p-value
Large vessel atherothromboembolic, median (IQR)	3 (2-7)	0.55
Cardioembolic, median (IQR)	3 (1-6)	
Small vessel disease, median (IQR)	4 (1-14)	
Stroke of other determined etiology, median (IQR)	3 (3-3)	
Stroke of undetermined etiology, median (IQR)	2 (1-3)	

*p-value was obtained using the Kruskal-Wallis test.

CHAPTER VIII: GENERAL DISCUSSION

This research project investigated the potential utility of transcranial Doppler (TCD) markers of microvascular dysfunction as tools for predicting cognitive and functional outcomes in post-stroke patients, as well as their usefulness for patient monitoring from the acute phase to the medium- and long-term post-event. The study aimed to assess whether these markers could reliably indicate early signs of cognitive decline and functional impairment, thereby contributing to a deeper understanding of patient recovery trajectories and facilitating more tailored interventions throughout the rehabilitation process.

We selected participants who had suffered a stroke and were admitted to the stroke unit (n=512), along with an age-, gender-, and vascular risk factor-matched control group (n=40). We also included a healthy control group without vascular risk factors (n=20). Our study focused on post-stroke patients, as stroke is the most recognised type of cerebrovascular disease and is highlighted as a significant cause of disability and cognitive impairment. The high prevalence of cognitive and functional impairment following a stroke has a negative impact on public health, contributing to economic strain on countries and reducing the quality of life for stroke survivors. The prediction of these outcomes for risk stratification and the subsequent adoption of preventive measures still presents knowledge gaps. In addition to the evidence of cognitive and functional impairment, there is also evidence of cerebral haemodynamic changes after a stroke, although knowledge of their long-term impact remains limited.

TCD was chosen as the method for studying cerebral hemodynamics and understanding its correlation with cognitive and functional outcomes due to its non-invasive nature, excellent temporal resolution, and portability, which allowed us to conduct evaluations during hospitalisation (acute phase) and later during long-term follow-up in an outpatient setting. TCD is a cost-effective tool with prior studies supporting its reliability in assessing cerebral hemodynamics.

Neurovascular coupling (NVC) is a marker of healthy brain function, and our study aimed to determine if it could be used as a predictor of long-term cognitive and functional outcomes post-stroke. The presence of microembolic signals (MES) has been associated with poorer clinical outcomes in previous studies, though its relationship with cognitive impairment has shown some inconsistencies.

To achieve our objectives, we conducted a longitudinal study of NVC and investigated MES in a cohort of post-stroke patients during the acute phase, and then three- and 12-months post-event. Studying NVC in the acute phase posed an additional methodological challenge due to the hospital environment, which is why we described

and developed a method for NVC assessment using spectral analysis with TCD. For a better understanding of the predictive value of NVC, we studied a control group.

The aim was to collect TCD data during the acute phase after stroke and at medium- and long-term intervals, identify differences with the control group, observe interhemispheric differences (affected vs. non-affected), and analyse various subgroups. MES monitoring was conducted simultaneously on the right and left middle cerebral arteries (MCA) for 30 continuous minutes. NVC measurement was based on the variation of cerebral blood flow velocity (CBv) following a visual stimulus, recorded in the P2 segments of the posterior cerebral arteries (PCA) on both sides.

Functional and cognitive assessments of participants were carried out using various scales validated for European Portuguese and widely used in different studies: the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE), Montreal Cognitive Assessment (MoCA), Modified Rankin Scale (mRS), and Lawton Instrumental Activities of Daily Living Scale (IADL). The IQCODE was used at the time of patient inclusion to ensure participants did not have significant cognitive decline prior to the stroke, and the MoCA test was administered at three- and 12-months post-stroke.

To better understand the impact of vascular risk factors, the severity of brain lesions, sociodemographic factors, and medication on cognitive and functional outcomes, as well as on cerebral hemodynamics, patient data were recorded continuously.

1. MAIN FINDINGS

Assessment of the Neurovascular Coupling by Spectral Analysis

NVC has been widely studied in various neurological conditions as a measure of proper brain functioning. Changes in CBv have been measured by numerous techniques, including TCD (Girouard & Iadecola, 2006; Iadecola, 2017; Monteiro et al., 2021).

The advantages of TCD are widely known in the study of cerebral hemodynamics (see 3. TRANSCRANIAL DOPPLER AND FUNCTIONAL STUDIES). The goal of our work, which focuses on studying the role of TCD in predicting cognitive decline and using this method in a more challenging context — the acute stroke unit — led us to consider an alternative and simpler method for assessing NVC using TCD.

This study presents an alternative method for TCD-measured NVC analysis using fast Fourier transformation (FFT) to perform spectral analysis of CBv responses to light stimulation in the PCA and MCA, as measured by TCD. A simpler, non-invasive method may enable its use in more centres and diverse clinical settings, making it a valuable tool

for studying NVC in different pathologies and contributing to the advancement of research in this field.

What is the advantage of this alternative method compared to the methods currently used? Additional instruments can deter many researchers from using these methods, particularly in clinical labs equipped only with TCD. Some TCD devices do not allow for the inclusion of the stimulus signal for further analysis, and in devices that do include it, stimulus marking can be prone to synchronization errors with the collected signal (Hage, et al. 2021, Rosengarten, et al. 2001, Shabir, et al. 2018).

Our study shows that NVC can be quantified by the spectral amplitude of CBv, presenting an alternative to the classic overshoot for periodic stimuli, easily calculated and without the need for stimulus marking or synchronization. It is important to highlight that this method was developed to provide a simpler way to assess NVC and, therefore, only applies to periodic stimuli, which repeat in cycles with equal duration, such as the presented visual stimulus. Thus, this may limit its use in stimuli with varying time intervals between repetitions, for example, language tasks (e.g., evoking words that start with a specific letter).

Assessments of NVC where the task used in the experiment is repeated over time, gradual adaptation may occur. This gradual adaptation can lead to a different vascular response compared to the initial response to the task, potentially reducing this vascular response. However, it is important to consider the nature of the task, experimental conditions, and the complexity of the vascular and neuronal systems involved. The visual stimulus presented in our study was arranged in increasing order of ON-OFF time intervals. Specifically, Experiment I consisted of 5 cycles of 5s ON – 5s OFF (total of 50s), followed by Experiment II with 5 cycles of 10s ON – 10s OFF (total of 100s), and finally, Experiment III with 5 cycles of 20s ON – 20s OFF (total of 200s).
While being a periodic stimulus to facilitate analysis, as suggested by our method, the variation in ON-OFF time intervals in the three experiments introduces variability in the presented stimulus. The maximum ON time for stimulus presentation was 20s, and previous studies have shown that no habituation to the stimulus was found in visual stimulus periods of 40s (Rosengarten & Kaps, 2010).

To describe our method, we followed the recommendations from the CARnet White Paper (ref) which has been recently updated. Evaluations were carried out in a dim lighted room, ambient temperature, sitting position and participants were refrained from caffeine, alcohol, exercise, or vasoactive drugs for at least 12 hours before evaluation. The assessments were always conducted in the same room and during daytime hours.

The procedure was explained to the participants in advance, at the time their consent was obtained, and any questions regarding the materials to be used, the duration of the experiment, and the nature of the visual stimulus were clarified to reduce participants' anxiety (Panerai et al., 2023).

In our method, the peak systolic beat-to-beat data was calculated in the machine software (DWL, Singen, Germany) and exported. Before the analysis, the signals were visually inspected: Data points that exceed 300% of precedent beat were excluded as outliers. We linearly interpolate the outliers with spline interpolation. We did not compare with and without spike artefacts, but this is known to distort the FFT in CBv analysis (Panerai et al., 2023).

In the CARnet White Paper, it was recommended to use the Welch method, with overlap between segments to help improve spectral resolution and reduce errors due to random fluctuations in the signal analysis. However, this paper primarily pertains to cerebral autoregulation signals (Panerai et al., 2023). The Welch method was not used in our analysis; instead, we applied the FFT directly to the signal. This is particularly useful when segmenting a signal for FFT application, as frequency components from signals outside the analysis window might appear in the spectrum as artifacts. As mentioned, the removal of artifacts in our signal was carried out by excluding data points that exceeded 300% of precedent beat. After excluding the outlier values, the FFT was applied to the signal.

The FFT was applied to the collected signals resulting from three experiments with time intervals of 200 s, 100 s, and 50 s, our goal was not to calculate spectral power in frequency bands, but rather at precise frequencies - 0.025 Hz, 0.05 Hz, and 0.1 Hz - where the maximum CBv peaks were expected. Given that Hz = 1/s, in the (5s ON - 5s OFF) experiment, the peak is reached at 0.1 Hz = 1/10; in the (10s ON - 10s OFF) experiment, at 0.05 Hz = 1/20; and in the (20s ON - 20s OFF) experiment, at 0.025 Hz = 1/40.

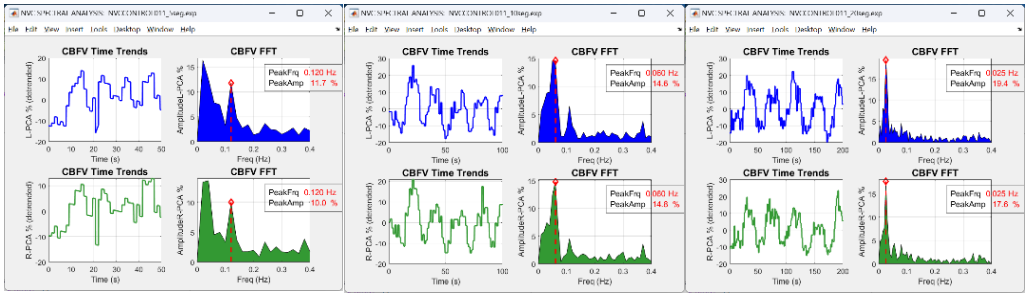


Figure 1: Example of an individual analysis in MATLAB (0.1 Hz, 0.05 Hz, and 0.025 Hz, respectively).

How does the spectral method correlate with the overshoot? Our study shown that the spectral amplitude and classic overshoot were highly correlated and linearly related with 1.04 for beta value ($p<0.0001$).

Considering that the parameters SNR (Signal-to-Noise Ratio) and SINAD (Signal-to-Noise and Distortion Ratio) are essential in signal quality analysis, although within acceptable and clinically relevant limits, the 0.1Hz experiment corresponding to the 5s ON-5s OFF visual stimulus appears to be the least reliable for assessing NVC. Among the three experiments, the 0.1Hz experiment shows lower SINAD and SNR values in the PCA signal analysis, indicating that this signal is of lower quality compared to the others. This experiment was designed to distinguish patients who are quicker to increase CBv after visual stimulation, but it shows lower quality for assessing the CBv peak in comparison with the others. Physiologically, it is understandable that this experiment may not be as sensitive in distinguishing the maximum CBv peak, as during the ON phase, the overshoot (peak maximum) occurs around 8 to 12 seconds, and in the 0.1Hz experiment (10s ON/OFF cycle) it may not capture the CBv maximum in some patients (Klingelhöfer, 2016; Rosengarten et al., 2001).

How would new insights be provided with our method? A simpler, non-invasive method may enable its replication in more centres and various clinical contexts, making it a valuable tool for studying NVC in different pathologies and contributing to the advancement of research in this field.

NVC has been described as altered in many clinical conditions and has been identified as a predictive measure of good neuronal performance (Aires et al., 2020; Iadecola & Gottesman, 2019; Jokumsen-Cabral et al., 2019; Monteiro et al., 2021; Mukli et al., 2024; Owens et al., 2022; Ruan et al., 2023; Schaeffer & Iadecola, 2021; Yang & Webb, 2023). Therefore, simplifying its assessment may enable more frequent evaluation in patients with various pathologies and in more demanding contexts, such as intensive care units. More frequent and standardized assessments, achieved through the simplification of the materials used, can contribute to new insights in the field of NVC across different pathologies.

Neurovascular Coupling assessment by Transcranial Doppler in Acute Stroke could be Informative of Long-term Cognitive Status

NVC assessment with TCD in acute stroke could provide information on long-term cognitive status. NVC was assessed using TCD during the acute phase, at 3 months, and at 12 months post-stroke. The results revealed that NVC initially increased during

the acute phase of stroke but progressively decreased over time. Notably, a U-shaped relationship was observed between NVC and long-term cognitive outcomes. Our results indicate that extreme NVC values, both higher and lower, are associated with worse cognitive outcomes 12 months after the event. Our findings support the hypothesis that NVC dysregulation during the acute phase of stroke may contribute to PSCI (Stackhouse & Mishra, 2021).

As mentioned in the literature review (see 1.2.4. CEREBRAL BLOOD PERFUSION, subsection NEUROVASCULAR COUPLING), NVC is considered a marker of healthy brain function (Schaeffer & Iadecola, 2021). There is growing evidence of the association between NVC and vascular cognitive impairment observed in patients with small vessel disease (Monteiro et al., 2024; Yang et al., 2022; Yang & Webb, 2023).

Changes in hemodynamic responses are observed in studies with stroke patients compared to healthy individuals (Claassen et al., 2021; Schaeffer & Iadecola, 2021). Previous studies have shown that increased cerebral blood flow in regions near the brain lesion and in the contralateral hemisphere can improve brain function. However, there are still gaps in understanding the regulation of cerebral blood flow after stroke, and many inconsistencies exist in the behaviour of cerebral blood flow over time and between the hemispheres (Salinet, Haunton, et al., 2013; Salinet et al., 2019; Wang et al., 2021).

Our prospective study, which included 144 patients in the acute phase of stroke, showed that patients with NVC magnitudes in the lowest and highest quintiles had significantly worse cognitive performances at 12 months, compared to those in the reference quintile. High and low NVC values may have different causes.

In the acute phase of stroke, the loss of blood-brain barrier integrity occurs rapidly, within 6 to 72 hours (Bernardo-Castro et al., 2020) As mentioned in the literature review (see 2. MECHANISMS OF COGNITIVE DECLINE IN CEREBROVASCULAR DISEASES), oxidative stress following ischemia is devastating for the neurovascular unit, even after reperfusion therapy.

NVC hyperresponsiveness may be aligned with reperfusion injuries. The neuroinflammatory response that occurs during the acute phase (6–72 hours after the event) contributes significantly to this process (Ferro et al., 2021) it worsens blood-brain barrier dysfunction, further exacerbating brain damage caused by reperfusion (Bernardo-Castro et al., 2020) and the low NVC response may be related to limited cerebral reserve capacity, as documented in patients with small vessel disease (Castro et al., 2020; Monteiro et al., 2024), although it may also simply be a manifestation of dysregulated NVC.

Our study also included a control group of 40 patients matched for age, sex, and vascular risk factors. They were assessed at a single time point, with NVC measured and sociodemographic and clinical information collected. The NVC showed higher responses in stroke group comparing to controls. Previous studies have shown statistically significant differences in NVC values between the control group and patients with acute stroke, with NVC values being higher in the control group. However, it is important to note that this comparison is made between stroke patients and healthy individuals without cardiovascular risk factors (Salinet, Robinson, et al., 2013; Salinet et al., 2019). Previous studies have demonstrated NVC dysfunction in patients with hypertension, age-related NVC dysfunction, persistent reduced ejection fraction and cardiac output and diabetes mellitus (Aires et al., 2020; Iadecola & Gottesman, 2019; Monteiro et al., 2021; Mukli et al., 2024; Owens et al., 2022; Schaeffer & Iadecola, 2021).

We consider that CBv hyperreactivity may be related to generalized vasomotor deregulation and increased BBB permeability that occurs after a stroke (Bernardo-Castro et al., 2020; Prakash & Carmichael, 2015). This dysregulation of NVC observed in our study was found to be generalized, meaning that NVC was altered even outside the infarcted area. Thus, our findings suggest that focal ischemic stroke induces dysregulation of this mechanism in both the ipsilateral and contralateral hemispheres, supporting studies that point to ischemic diaschisis in the subacute phase (Garbuzova-Davis et al., 2013).

It is important to highlight that our study shows a global decrease in NVC values over time across the brain. The influence of vascular risk factors is a critical consideration; therefore, our control group was matched for age and vascular risk factors. These changes may represent a general trend of recovery in vasomotor function and the status of the BBB.

And regarding medication? In our study, we found an association between the previous use of statins and NVC values. Statins improve endothelial function, have antithrombotic and anti-inflammatory effects, and our results were consistent with previous studies (Rosengarten et al., 2007). We also found an association with the use of beta-blockers, a class of medications widely used in the treatment of cardiovascular diseases, which has been shown to influence NVC values (Farzam K, 2023). Regarding antidepressants, an association was found between the use of antidepressants and NVC values. Previous studies indicate that serotonin influences cerebral blood supply and can have both vasoconstrictive and vasodilatory effects (Harris & Reynell, 2017).

Detection of microemboli by TCD suggests a poor functional outcome

Nearly a quarter of patients with acute ischaemic stroke and atrial fibrillation (AF), who had not received prior anticoagulant treatment, exhibited silent microembolisation following the initial event. The detection of MES within the first 24 hours after stroke may serve as a marker of poor functional outcomes. Our results indicate that the occurrence of MES was associated with worse outcomes (mRS>3).

This study was designed to address the knowledge gaps identified in previous studies, such as the need to investigate haemodynamic variables by subgroups and the methodological variation between studies from different research centres. In this multicentre study, several centres replicated the methodology, and the counting of MES, which is operator-dependent, was doubly validated across different centres. Previous studies show significant variability in the positive MES count, ranging from 17% to 40%. In this study, which focused on patients with stroke and atrial fibrillation without prior anticoagulant treatment, a positive MES count of 23% was obtained.

The increased mortality in patients with positive MES after stroke has been identified in several previous studies. It is important to reflect on what the formation of these microembolic particles represents – namely, a common final step in a complex cellular and molecular sequence and an indicator of thrombogenicity. The presence of MES, monitored in real-time, has implications and significance in clinical practice, but what exactly does it indicate? Could it be a marker of clinical prognosis? Could it serve as a marker for risk stratification?

Silent embolism occurring in the early hours after a stroke may be linked to new ischaemic lesions and/or the expansion of the final infarct area. These lesions, even if not associated with an acute clinical event, predict new neurological deficits, including cognitive impairment (D. Kang et al., 2016).

Our results showed a significant association between the presence of MES and patients with a high NIHSS score, as well as with patients undergoing thrombectomy. Of the 43% of patients who underwent thrombectomy, a unilateral positive MES was found on the side of the procedure, regardless of the final TICI score. The presence or absence of a successful TICI score did not show an association with the presence of MES in our cohort, although the relationship between MES and unsuccessful recanalization could be questioned, and the results in previous studies are not consistent (Farina et al., 2018; Kumral E et al., 2001) This study reveals that MES were detected more frequently in patients who had undergone endovascular therapy (70% vs 34%). The high MES rate may be attributed to vascular/endothelial damage occurring during the procedure.

Regarding the study hypothesis, patients positive for MES showed worse outcomes after 90 days, regardless of the best medical treatment. This confirms the utility of MES detection as a relevant clinical marker for outcome prognosis, meaning they represent a powerful marker for systemic and/or local thrombogenicity, although they are not primarily direct causes of cerebral ischaemic lesions.

In our study, patients who were already using antiplatelet drugs at the time of the stroke tended not to have MES. Oral anticoagulation during follow-up was not independently associated with the outcome (Table 1). However, as we were unable to access the time of treatment initiation, we considered the potential interference with MES status and its consequences during follow-up.

Table 1. Oral anticoagulation in patient follow-up and its association with functional outcome.

Follow-up treatments	Total	Poor outcome (mRS 3-6)	Good outcome (mRS 0-2)	Odds ratio (95%)*	P value
	N = 61	N = 47	N = 14		
Single AP, n (%)	6 (30)	1 (3)	5 (17)	1.55 (0.89 - 2.70)	0.09
Double AP, n (%)	1 (2)	0 (0)	1 (3)	-	1.00
Warfarin, n (%)	1 (2)	1 (3)	0 (0)	-	1.00
LMWH full, n (%)	1 (2)	0 (0)	1 (3)	-	1.00
LMWH low dose, n (%)	0 (0)	0 (0)	0 (0)	-	1.00
Xa Inhibitor, n (%)	12 (20)	29 (94)	22 (74)	0.30 (0.07 - 1.19)	0.08
IIa Inhibitor, n (%)	1 (2)	0 (0)	1 (3)	-	1.00

The effect of recurrent embolism in patients with atrial fibrillation who have suffered a stroke on long-term functional outcomes still needs to be studied. Based on what we know, the main cause of post-stroke hospitalisation is related to the recurrence of events, both ischaemic and haemorrhagic. Population studies indicate that, compared to the general population, an acute stroke reduces life expectancy by approximately 5 years (Abreu et al., 2021; Peng et al., 2022). These findings reinforce the need for coordinated efforts to improve acute stroke care and secondary prevention, aiming to enhance long-term prognosis.

Regarding the recurrence of ischaemic events, only one patient had a recurrent stroke, three were not questioned, and three could not be assessed. The recurrence of stroke was exploratory, but these data cannot be analysed. However, the 3% stroke recurrence rate is within the 1-3% range found in recent studies on patients with atrial fibrillation and stroke (Fischer et al., 2023).

This was the first study on MES detection after acute ischaemic stroke, specifically focusing on patients with atrial fibrillation who had never used anticoagulants, with clinical outcome as the main objective. However, the interpretation of our results should

consider some limitations. The MES-positive group tended to have higher NIHSS scores and were more frequently referred for endovascular treatment, making them more prone to various complications, such as the mentioned vascular/endothelial damage. Our study involved several reference stroke centres, and as a result, the average severity of stroke in our sample was considerably high (NIHSS=11). In our study, 15 out of 61 patients (25%) had minor strokes (NIHSS ≤4), a proportion similar to those observed in recent trials on atrial fibrillation (Fischer et al., 2023).

Our results only show an association with functional outcome and not a direct causal relationship. The occurrence of MES is merely a marker and does not exert a direct causal effect. To confirm this, a more detailed study with magnetic resonance imaging would be needed to document recurrent ischaemic lesions. The results must be interpreted considering the exploratory nature of the study, the small sample size, the lack of perfect adjustments, as well as the non-significant results when analysing the change in mRS in the adjusted models.

The results suggest a possible association between the presence of MES in stroke patients and a poor functional outcome at 90 days, although the adjusted models reduce statistical significance. This suggests that MES could be a marker of poor functional outcome, but the data are insufficient to confirm this relationship conclusively, especially after the adjustments (Table 2).

Table 2**.** Sensitivity analysis: Outcome measures based on MES positive or negative detection excluding large vessel stenosis.

Outcome measures	MES-positive n = 10	MES-negative n=41	Univariate Model	*Multivariate Model 1	†Multivariate Model 2
			Unadjusted Odds ratio (CI 95%); P value	Adjusted Odds ratio (CI 95%); P value	Adjusted Odds ratio (CI 95%); P value
§mRS 3 – 6 at 90 days – n (%)	8 (80)	18 (44)	5.11 (1.001 – 31.1); P = 0.04	4.05 (0.98 – 39.4); P = 0.06	4.05 (0.97–20.4); P = 0.11
‡mRS score shift – median (IQR)	2 (1 – 3)	3 (3 – 4)	1.98 (0.72 – 26.2); P = 0.14	2.00 (0.56 – 17.2); P = 0.34	1.67 (0.03 – 45.0); P = 0.33

Abbreviations: MES = microembolic signals;
All p-values and odds ratio calculated by logistic regression models
* Multivariate model 1 = adjusted to Age and NIHSS
† Multivariate model 2 = adjusted to unbalanced baseline differences between MES groups by probability score
§ Univariate and multivariate analysis with logistic regression to predict outcome
‡ Univariate and multivariate analysis with ordinal shift analysis to predict outcome

Detection of microemboli by TCD in Acute Phase after Stroke and Long-term Cognitive Impairment

Microembolic signals are an independent marker of recurrent embolic events within 90 days, but the long-term term impact on functional outcome and cognitive status is not well understood (Das et al., 2020; Sheriff et al., 2020). Although transcranial Doppler detects MES in numerous settings, the practical significance of such findings remains unclear.

A previous study showed a correlation between the results obtained in neuropsychological tests and the presence of MES in patients undergoing cardiovascular surgery, but without brain injury (C. J. Lin et al., 2022). Previous studies have shown the association of cerebral microembolism as a cause of silent ischaemic cerebral infarcts, and consequently, contributing to cognitive decline (Goldberg et al., 2012). What is the role of MES in the process of cognitive decline?

In our study, the definition of cognitive impairment was based on MoCA Test scores validated in studies with the Portuguese population. (Freitas et al., 2011). In 2023, these values were updated, with the number of age groups increased. Specifically, the ≥65 years category was divided into 60-69, 70-79, and ≥80 (Gonçalves, 2023). The average age of our sample is 72±12 years, and this change could influence our results.

The adaptation of scores in the Portuguese population and the use of these measures in numerous studies raises the question of what the best way would be to characterise cognitive decline. Is it sufficient to assess different areas of cognition, or will activities of daily living provide important information? Since the MoCA Test is widely used in studies and clinical trials, what cut-off score for the MoCA Test should be used to define post-stroke cognitive impairment? Some studies suggest that scores below 26 should be considered indicative of cognitive decline, while more recent reviews suggest a lower cut-off of between 21/22 for patients after a stroke (Wei X. et al., 2023). Regarding the inclusion of functional parameters, recent clinical trials consider the patient's functionality after a stroke, particularly their independence in activities of daily living (Wardlaw et al., 2020).

Our results showed that MES-positive patients had worse cognitive outcomes, with an adjusted odds ratio of 2.04 (95% CI 1.27-3.28, $p<0.01$). A total of 316 patients were included, with a median age of 67 years and a median stroke severity score of 5. Of these, 39 patients (12.3%) had MES, with a mean of 5.15±8.34 MES events. MES was more frequent in patients who underwent thrombolysis or thrombectomy, those not on

statins, and those with higher NIHSS scores ($p<0.05$). MES prevalence did not vary significantly by stroke etiology ($p=0.55$).

This prospective study included consecutive ischemic stroke patients without prior cognitive deficits. Transcranial Doppler (TCD) monitoring was performed within 72 hours of symptom onset for 30 minutes using bilateral 2-MHz probes, with recordings analyzed for MES by a blinded reader. The primary goal was to assess the proportion of patients with MES and its association with cognitive status at 12 months, evaluated using a 7-point cognitive scale. In conclusion, MES-positive patients in the acute phase were twice as likely to experience worse cognitive outcomes 12 months post-stroke. TCD detection of MES could serve as an early marker of poor cognitive performance, helping to identify high-risk patients. Future research should explore whether early antithrombotic treatment could improve long-term cognitive function in stroke survivors.

The assessment of cognitive function in post-stroke patients requires careful attention. Given the need for this assessment to cover multiple cognitive domains, the MoCA test is widely recommended and used in clinical settings (Quinn et al., 2021). In our study, to assess cognitive status at 12 months of follow-up, we used a 7-scale cognitive score operationalized from the Montreal Cognitive Assessment, which also incorporates functionality in daily living activities (Wardlaw et al., 2023).

To determine whether the difference was solely due to the inclusion of variables related to functionality and independence in daily living activities, we conducted a complementary analysis using MoCA<22 as a cutoff for cognitive impairment. This analysis also showed that the presence of MES in the acute phase after stroke was a predictor of worse cognition 12 months after the event.

The main differences relate to the inclusion of strokes with various etiologies, a significantly larger cohort, and, according to statistical power calculations, patients who underwent endovascular treatments versus those who did not.

Thus, our results suggest a relationship between the presence of MES in the acute phase after stroke and worse long-term cognitive outcomes for stroke survivors.

CHAPTER IX: FUTURE PERSPECTIVES

Our study highlighted the role of cerebral haemodynamics in functional and cognitive recovery after a stroke. The results of this research project open several other promising avenues for understanding the implications of cerebral haemodynamics in the acute phase after a stroke, over the first year following the event, as well as in the management of stroke patients, assisting in risk stratification. The description of the spectral analysis method of NVC demonstrates the potential use of TCD as a non-invasive, low-cost resource that can be applied in various clinical contexts.

I. **Long-term Understanding of the Effects of Acute Phase Cerebral Haemodynamics Post-Stroke:**

Our results show an association between the presence of MES and the functional outcome in patients with AF after a stroke. The occurrence of MES is a marker for poorer long-term functional recovery. Recognising the exploratory nature of the study, this marker could be used in future studies, providing a complementary marker in clinical trials with this population. In long-term cognitive recovery, MES-positive in the acute phase did not prove to be a marker of poorer long-term cognitive performance.

The detection of microembolic signals with transcranial Doppler was associated with worse cognitive function 12 months after stroke. Additional studies in PSCI should investigate whether specific strategies, such as anticoagulants and antiplatelet agents in patients with cardiovascular risk factors, can reduce the frequency of microembolism and improve cognitive outcomes.

The NVC data in the acute phase demonstrated in our study help to clarify its role, considering its changes in the acute phase of stroke and its relationship with long-term cognitive dysfunction observed in these patients. Future studies could help elucidate the correlation between increased NVC and patient alertness, as well as how this may compromise long-term cognitive performance.

II. **Risk Stratification**:

Our results suggest that patients who are MES-positive in the acute phase may have poorer long-term functional recovery, and therefore, may constitute a high-risk group. Similarly, patients with significantly increased or decreased NVC in the acute phase post-stroke may be at risk for developing long-term cognitive decline following the event.

III. **Personalised and Preventive Therapeutic Interventions:**

Understanding the mechanisms underlying NVC dysregulation in the acute phase post-stroke can potentially help guide decisions in acute stroke care and the development of new therapeutic targets.

Reducing the presence of MES in the acute phase appears to be important for the functional recovery and cognitive function of stroke patients. Future studies will investigate whether very early antithrombotic treatment can improve outcomes.

IV. **Implications for the Study of Neurovascular Coupling:**

Our method of spectral NVC assessment using a periodic stimulus could facilitate NVC evaluation in more challenging contexts and encourage further studies. Future studies may help clarify the correlation between increased NVC and patient alertness, as well as how this may impair their long-term cognitive performance.

NVC assessment with TCD in acute stroke can provide information about long-term cognitive status. Modulations in CBv responses could be the focus of future clinical trials to improve post-stroke cognitive impairment. The method described in our study could encourage the study of this haemodynamic measure in other pathologies, increasing insights and knowledge about NVC.

CHAPTER X: GENERAL CONCLUSIONS

Our study aimed to investigate cerebral haemodynamics in the acute phase of stroke, examining the parameters of MES and NVC, their progression over the first year following the event, and their impact on the functional and cognitive recovery of these patients. Our study also used a control group matched by age, sex, and cardiovascular risk factors to compare NVC values.

Our method of spectral NVC assessment using a periodic stimulus does not show correlation with predictive variables of cognitive function and therefore may be a useful tool for future studies of post-stroke cognitive performance. Our study showed that spectral amplitude and classic overshoot were highly correlated and linearly related.

Assessing NVC with TCD in the acute phase of stroke may offer insights into long-term cognitive health. Future trials might focus on adjusting CBv responses to improve cognitive outcomes after a stroke.

Our results show an association between the presence of MES and functional outcomes, but not a direct causal relationship in a subgroup of patients with AF. To study the predictive capacity of MES-positive status in relation to long-term cognition, we conducted a prospective study. Our data shows that patients who are MES-positive in the acute phase have twice the likelihood of experiencing worse cognitive outcomes 12 months after stroke. The detection of MES using TCD could be considered in future studies as an early marker of poor cognitive performance, helping to stratify high-risk patients.

This study aimed to provide insights into the haemodynamic parameters in the acute phase of stroke and stimulate discussion on their potential as markers to be used in clinical practice. While the findings highlight significant associations and potential therapeutic targets, the inherent limitations of the study invite additional research.

Regarding acute phase NVC, it seems to provide insights into long-term cognition, and our study showed its variation over time, providing data on both its temporal variation and its relationship with cognition. The practical applications of this research could pave the way for more personalised treatment strategies for stroke patients, and risk stratification will allow the identification of groups more vulnerable to poorer functional and cognitive recovery after stroke.

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