UNIVERSITY OF MOLISE

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Ph. D Course in Translational and Clinical Medicine– XXXI Cycle

White Matter Alterations and Cognitive Assessment in Hypertensive Patients Without Damage at Conventional Neuroimaging

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SOMMARIO

Il progetto di ricerca riportato nella presente tesi riguarda lo studio del danno cognitivo causato dall'ipertensione in relazione al danno della sostanza bianca sottocorticale. Scopo principale del progetto è stato quello di effettuare una valutazione di marcatori precoci di decadimento cognitivo in pazienti con ipertensione arteriosa attraverso tecniche di neuroimaging basate sul tensore di diffusione. Nello specifico, è stato condotto uno studio caso-controllo articolato selezionando pazienti ambulatoriali afferenti al Dipartimento di Angio Cardio Neurologia e Complicanze Neurologiche delle Malattie Internistiche presso I.R.C.C.S. Neuromed, che hanno acconsentito tramite consenso informato, di partecipare al progetto di ricerca. I pazienti ipertesi e il relativo campione di controllo normoteso sono stati sottoposti tutti alla medesima procedura: raccolta anamnesi prossima e remota, acquisizione dati di risonanza magnetica con particolari sequenze di DTI, misurazione pressoria, prelievi ematici, ecocardiogramma, ecocolordoppler TSA e valutazione cognitiva. Il fine è stato quello di individuare il più precocemente possibile segni di danno cerebrale causato da ipertensione non altrimenti individuabile attraverso una canonica RMN encefalica. Ciò è stato possibile tramite l'analisi di trattografia e alla relativa correlazione con i dati ottenuti dalla valutazione cognitiva. Dal punto di vista cerebrale e cognitivo gli ipertesi mostravano una performance significativamente peggiore nel Montreal Cognitive Assessment (MoCA), una batteria di test specificamente disegnata per la valutazione di molteplici funzioni cognitive e validata nelle patologie vascolari, nell'apprendimento e nella velocità di processamento delle informazioni. Mediante imaging cerebrale è stato possibile osservare che, sebbene gli ipertesi non avessero anomalie riscontrabili con metodiche convenzionali, gli indici di trattografia erano significativamente ridotti (FA, MD, RD, AD). In particolare, essi mostravano un'alterazione specifica nelle fibre di proiezione correlate alle funzioni mnemoniche (Anterior Thalamic Radiation), nelle fibre di associazione coinvolte nelle funzioni esecutive (Superior Longitudinal Fasciculus), nelle fibre del sistema callosale coinvolte in compiti di velocità di processamento delle informazioni (Forceps Minor). Correlando questi dati con i dati ottenuti dalle valutazioni cognitive è emerso come le fibre di proiezione e le fibre di associazione correlano con la valutazione della performance cognitiva globale del MoCA e come le fibre callosali correlano con la performance nello Stroop Test. Altresì i punteggi ottenuti nel MoCA correlano con la durata dell'ipertensione. Maggiore è la durata dell'ipertensione, minore è il punteggio ottenuto nel MoCA.

Dallo studio effettuato è possibile affermare che la metodica della trattografia rende possibile individuare segni precoci di danno cerebrale nell'ipertensione rendendo questo metodo prezioso per identificare nuovi approcci nella prevenzione della demenza vascolare.

SUMMARY

Hypertension is a public health problem affecting one third of world population according to the latest estimation of World Health Organization and these estimates are increasing considering diagnoses in non-industrialized countries. Hypertension has a long pre-symptomatic phase with initial damage of target organs: heart, kidney, vasculature and brain. Indeed, hypertension is the major risk factor for acute diseases such as cerebrovascular accidents and for chronic disease such as neurodegenerative pathologies first of all vascular dementia. An early phase that can likely lead to dementia, called vascular cognitive impairment, has been observed. Hypertension has been found to affect levels of attention and concentration (executive functions) and secondarily memory since early studies. In routinely clinical practice, assessing cognitive functions in hypertension is not usual and when this happens it is because patients or their families report deficits, an already evident phase of cognitive impairment.

The objective of the present research project was to assess cognition in hypertensive patients who did not report any damages in cortical white matter and to correlate the cognitive assessment with the MRI-DTI fiber tracking parameters, to identify biomarkers that can predict the future structural damage of the cortex.

In order to pursue this goal, subjects admitted at our outpatients' facility – Regional Excellence Hypertension Center of the Italian Society of Hypertension, affiliated to the Department of Neurological Complications of Cardiovascular Diseases of I.R.C.C.S. Neuromed - were recruited. All patients who did not report previous damage or neurological diseases and who had no cardiovascular problems except for arterial hypertension were included in the study.

After collecting the medical history and the informed consent, patients underwent MRI sequences to exclude those with cerebral cortex damages (Fazekas scale ≤ 1), then they underwent DTI sequences with the related parameters FA, MD, AD and RD. All recruited patients were subjected to the same procedures: blood pressure measurements, blood collection, ultrasonographic analyses for cardiac and vascular imaging, cognitive assessment. These procedures were necessary to include in this study only subjects with no overt signs of end organ damage or dementia. To this aim tests that could fit the need to better assess cognitive functions in the cardiovascular field were selected: first of all, MoCA battery of test, considered a diagnostic gold standard tool to evaluate cognition in

cardiovascular diseases. Along with this battery, each patient underwent a questionnaire for the autonomy in every day activity (IADL), a learning test, a semantic fluency test and the Stroop test.

Results in cognitive assessment showed that hypertensive patients perform worse in MoCA test, in anterograde learning test and in Stroop test compared to control group. Moreover, significant alterations in the parameters of associative (SLF), projection (ATR) and callosal (FMI) fibers emerged from the fiber tracking analyses. These alterations are implicated in executive functions, in memory and in processing information speed.

Correlation analyses pointed out the relation between MoCA and both SLF and ATR fibers, MoCA and hypertension duration and Stroop test and FMI fibers.

Overall, WM fiber-tracking on MRI make it possible to detect an early marker of damage in hypertension, and in perspective this method could be precious to identify new approaches in preventing vascular dementia.

CHAPTER 1. INTRODUCTION

1.1 Definition of Hypertension

Hypertension is a worldwide public health issue. Latest World Health Organization's data regarding epidemiology (2015), showed a remarkable blood pressure (BP) raising in the low-income countries and in the Eastern hemisphere while in high income countries, thanks to diagnosis, the percentage of people with hypertension decreased but increased the need of treatments (**WHO, 2015**; **Mills et al 2016**). This highlights that hypertension is a disease that affects the whole planet and preventing the related adverse events represents a challenge. Because of population aging, it is estimated that a third of worldwide population suffers or it will suffer from hypertension, (**Kearney et al. 2005; Novak & Hajjar 2010**), meaning that over one billion people are involved (**Muela et al. 2017**). This also explains the need of an increasing attention on the assessment, control and management of this pathology.

Hypertension occurs when the pressure of blood flowing through vessels is too high, causing cardiac and vasculature stress. The force of blood flowing is upraised when using a blood pressure device, BP values are upper than 120 mmHg for systolic blood pressure (SBP) and upper than 80 mmHg for diastolic blood pressure (DBP) (**Whelton et al. 2017**; **Chobanian 2017**).

BP evaluations need to be noted for few days prior to diagnose hypertension. It is suggested to record BP twice daily, preferably in the morning and in the evening. It takes two successive measurements, in sitting position and at least a minute apart between one measurement and another. Assessments recorded on the first day are excluded and the average value of all the other measurements is calculated to ascertain a diagnosis of hypertension (**WHO 2013**).

There are several recognized risk factors for high blood pressure, as stated both by the American Heart Association (AHA) and the American Stroke Association (ASA) (2014 and updated in 2017) (**Figure 1**). These are conditions that are known to increase the chance to develop hypertension. Risk factors fall in two categories: those that could be managed, and those that are uncontrolled.

- Manageable Risk Factors

Not conducting enough physical activity as well as following an unhealthy diet, especially one high in sodium, increase the probability to develop hypertension. A diet that is too high in salt consumption, along with calories, saturated fatty acid and sugar, carries an additional risk of BP increase, besides the fact that an incorrect nutrition can lead to overweight and obesity that raise the possibility to develop cardiovascular disease and diabetes. Another risk factor related to incorrect lifestyle is drinking alcohol. Alcohol abuse can cause many health problems related to the cardiovascular system, including heart failure, stroke, irregular heartbeats and higher BP.

These points mentioned above are all certain in contributing at the onset of cardiovascular problems. There are many other risk factors that may contribute to hypertension, concerning: smoking, stress and sleep apnea (**Konecny et al 2014**).

- Unmanageable risk factors

Family history, age, gender and ethnicity. With advancing age some of the elastic properties of the vessels are lost. Gender also is affected by ageing consequences: indeed, there are age periods in which men are more likely to develop hypertension than women and vice versa. Finally, Africans tend to develop high BP at younger stage more often and with severe form than Caucasians.

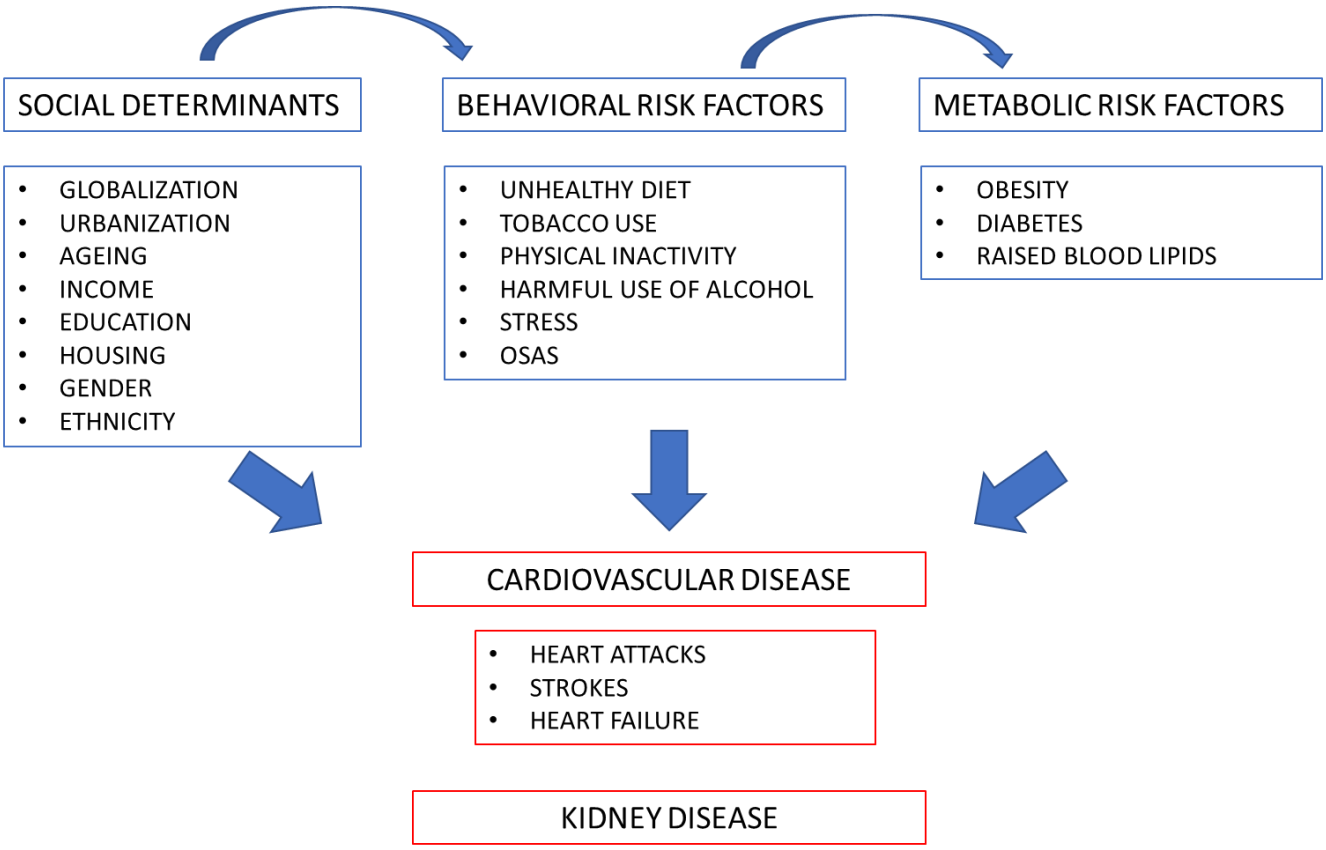


Figure 1. Risk factors for the development of hypertension. Adapted from: World Health Organization. A global brief on hypertension. Silent killer, global public health crisis. World Health Day 2013. Document number: WHO/DCO/WHD/2013.2

An important aspect that makes difficult to discover, and more importantly to prevent, hypertension is that the majority of people who suffer from hypertension reported no symptoms at all. Indeed, hypertension is also called “the silent killer”. There is a wrong belief that people with hypertension always show or report some kind of symptoms. Effectively the increase in BP only occasionally causes symptoms such as headache, chest pain, dizziness, shortness of breath, palpitations of the heart and nose bleeds.

When hypertension starts, patients must adopt lifestyle changes. The earlier the diagnosis is made, the sooner it is possible to reduce the risk of heart failure, heart attack, stroke or kidney damages (**WHO, 2013**).

1.1.1 Physiology of Hypertension

The physiology of hypertension follows physic laws and it involves several mechanisms that work together and are sensible to every change in homeostasis (**Lifton et al 2001**; **Seidel and Scholl 2017**). At the onset of hypertension different components and molecular pathways are involved such as: baroreceptors, natriuretic peptides, renin-angiotensin-aldosterone system, kinin-kallikrein system, adrenergic receptors system and blood vessels products such as nitric oxide or endothelin (**Lifton et al. 2001**). Hereby there is a brief explanation of these pivotal pathway's involvement.

Baroreceptors are neurosensory receptors placed in vessels and heart, particularly in the aorta and in the carotid sinus, that answer to BP variations. Principally, they react to wall vessel stretching and they are divided in: high-pressure arterial baroreceptors and low-pressure arterial baroreceptors (also known as cardiopulmonary or volume receptors). In normotensive patients as BP rises, there is an initial increase activity of baroreceptors aiming at restoring BP to baseline levels. Such modulation is known as baroreflex mechanism (**Navaneethan et al. 2009**). In chronic hypertensive patients, a prolonged exposition to high BP results in a diminished function of baroreceptors reflecting less sensitivity to BP fluctuations. This appears in changes in vascular distensibility and altered activity in the brainstem portion of the baroreflex (**Navaneethan et al. 2009**).

Natriuretic peptides are peptide hormones that control natriuresis. Their principal function concerns the modulation of sodium through kidney secretion. They are grouped in: Atrial Natriuretic Peptides (ANP), Brain Natriuretic Peptides (BNP) and C-type Natriuretic Peptides (CNP). ANPs are sensible to aorta vessel's stretch that occurs when BP starts to rise. BP variations increase ANP concentrations to restore normal BP values enhancing natriuresis while decreasing plasma renin and aldosterone (**Foëx and Sear, 2004**). BNPs are kept in ventricles and they respond to heart volume increase and cardiac stress (**Volpe et al. 2016; D'Elia et al.2017**). Along with ANP, BNP inhibits cardiomyocyte hypertrophy induced by both Ang II and ET-1, moreover it attenuates norepinephrine-induced growth of cardiac myocytes and fibroblasts. CNP is the most representative NP in the brain, but it is also produced by chondrocytes and endothelial cells. When a vascular insult occurs, CNP operates as a vasodilatory peptide and takes part in the paracrine action of other vasorelaxation mediators, such as nitric oxide (NO) and prostacyclin.

The Renin-Angiotensin-Aldosterone System (RAAS) is one of the main systems involved in the modulation of BP control, influencing body fluid homeostasis and sodium-potassium

balance (**Muñoz-Durango et al. 2016**). It is known that any changes in RAAS system could take part in the onset and progression of HT (**Te Riet et al. 2015**). Renin is firstly produced as an inactive form by the kidneys in answer to increased need of intratubular sodium, or as a consequence of hypotension in the afferent arterioles of renal glomerulus or sympathetic stimulation. Renin is synthesized as a preprohormone; mature (active) renin is gathered in granules of the juxtaglomerular cells and is emitted by an exocytic process involving stimulus-secretion coupling into the kidney and then into the systemic circulation. Beside this regulated pathway, it seems that the kidney also emits prorenin through a constitutive pathway (**Atlas 2007**). In the blood stream the active renin initiates the cleavage process of angiotensinogen generating angiotensin I (Ang I). Ang I is splitted by the angiotensin-converting enzyme (ACE) to realize angiotensin II (Ang II), which promotes vasoconstriction on cardiac and vascular tissues (**Muñoz-Durango et al. 2016**). Moreover, Ang II promotes production (and release) of aldosterone from the adrenal cortex, supporting the constriction of renal and systemic arterioles and the reabsorption of sodium in proximal segments of the nephron (**Atlas 2007**) (**Figure 2**).

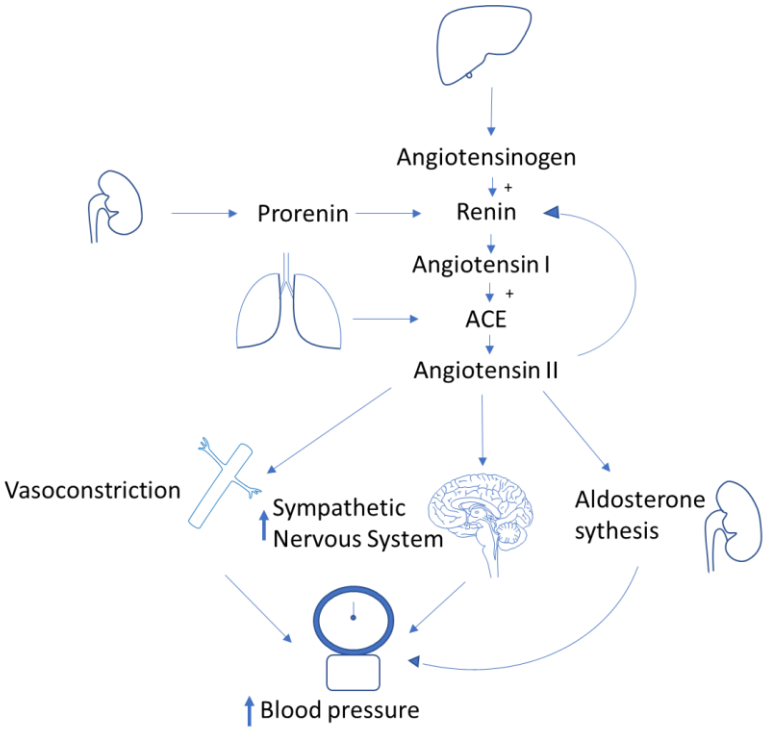


Figure 2: the RAAS system functioning and its damaging effects in malfunctioning conditions such as in arterial hypertension. Adapted from: Muñoz-Durango N. et al. Int. J. Mol. Sci. 2016; 17: 797.

The kinin kallikrein system (KKS) is a mechanism that works together with and in opposition of RAAS. Both systems are monitored by ACE that while produces Ang II, inactivates kinin proteins helping to maintain homeostasis (**Regoli & Gobeil 2017**). Kinins are autacoids, that are substances that proceed when they are requested and only in the district where they are needed (i.e. in inflammations, in traumatic lesions and in regions with damaging stimuli). The task of the kinin proteins is the activation of inflammatory processes to counteract potential damaging events and to provide for organ reparation. Kinin proteins deal with keeping the correct circulation of blood flow to ensure nutritional and metabolic needs of the organ (**Regoli & Gobeil 2017**). All these functions are possible thanks to a specialized and complex mechanism in which are involved endothelial cells and vascular smooth muscle cells.

The adrenergic receptors system is involved in the onset of hypertension because of its main neurotransmitters: Adrenaline and Noradrenaline. Adrenaline is at the basis of sympathetic nervous system involving two main receptors subfamilies, alpha and beta, that are dislocated

mostly in the heart and partially also in the vasculature (**Park & Lee, 2017**). In general, when alpha receptor's families are stimulated, there is a vasoconstriction effect, while when beta receptor's families are activated, there is an increase in cardiac output. Noradrenaline is released in the synaptic button in response to specific stimuli that require vasoconstrictor effects and the subsequent pressure rising. When hypertension starts, it produces a dysfunction in the balance between sympathetic and parasympathetic functions, inducing an increase in sympathetic tone and a reduction in the parasympathetic one (**Grassi and Ram, 2016**).

Blood vessels products refer to substances such as chemical compounds e.g. nitric oxide) or proteins (e.g. endothelin) produced in the endothelium, the inner layer of intima. In general, endothelial damage is a predictor of future problems related to atherosclerosis and coronary artery disease (**Agapitov & Haynes, 2002**). These products have several functions, including the balance of vasodilation and vasoconstriction in BP homeostasis (**Mordi et al. 2016**). In particular, Nitric Oxide (NO) deals with coagulation, inhibiting platelet aggregation and leukocytes adhesion to vessel wall. These functions make NO essential to prevent atherosclerosis (**Mordi et al. 2016**). On the other hand, endothelin's family has a pivotal role in increasing BP and vascular tone, possessing vasoconstriction functions (**Agapitov & Haynes, 2002**).

1.1.2 Damages of Hypertension

Hypertension is associated with the so-called Target Organ Damage (TOD), which involves specific organs in body districts that endure high BP. TOD includes left ventricular hypertrophy and dysfunction, renal failure, atherosclerotic vascular disease and vascular dementia (**Nadar et al. 2006**) (**Figure 3**).

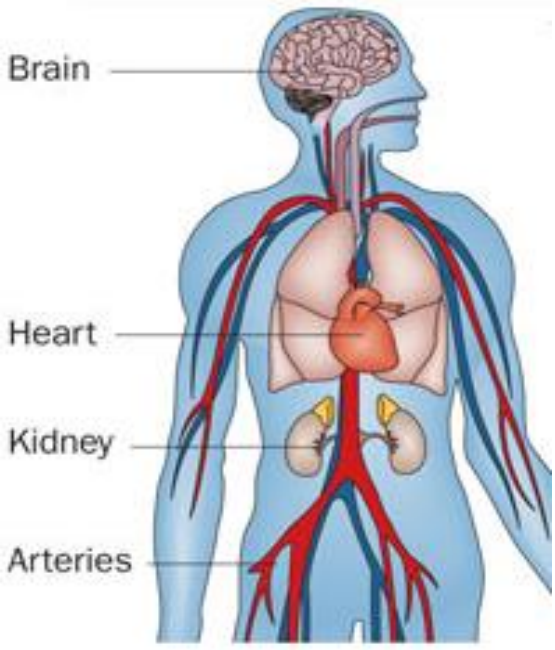


Figure 3. Target Organ Damage of hypertension.
From: Orthostatic hypertension - A new haemodynamic cardiovascular risk factor - Scientific Figure on ResearchGate. Available from: **https://www.researchgate.net/Systemic-haemodynamic-atherothrombotic-syndrome-risk-factors-and-clinical_fig3_258281551** [accessed 12 Sep., 2018]

Left ventricular hypertrophy is usually observed in patients with hypertension. In reaction to initial BP increase, heart adopts a compensatory strategy to preserve its efficiency consisting in shape adaptation to handle ventricular wall stress due to pressure overload (**Nadar et al. 2006**). This leads to heart remodeling. There are two main type of heart remodeling: concentric and eccentric (**Figure 4**). Pressure rising can cause a concentric hypertrophic phenotype, characterized by increased left ventricular wall thickness, whereas volume increasing can cause an eccentric hypertrophic phenotype characterized by dilatation of the left ventricular chamber. These two outcomes can originate from hypertension or resistance training in the first case, or from valvular defects or endurance training in the second case. Under both conditions a general increase of the dimensions of the cardiomyocytes can be observed (**Müller et al. 2013**).

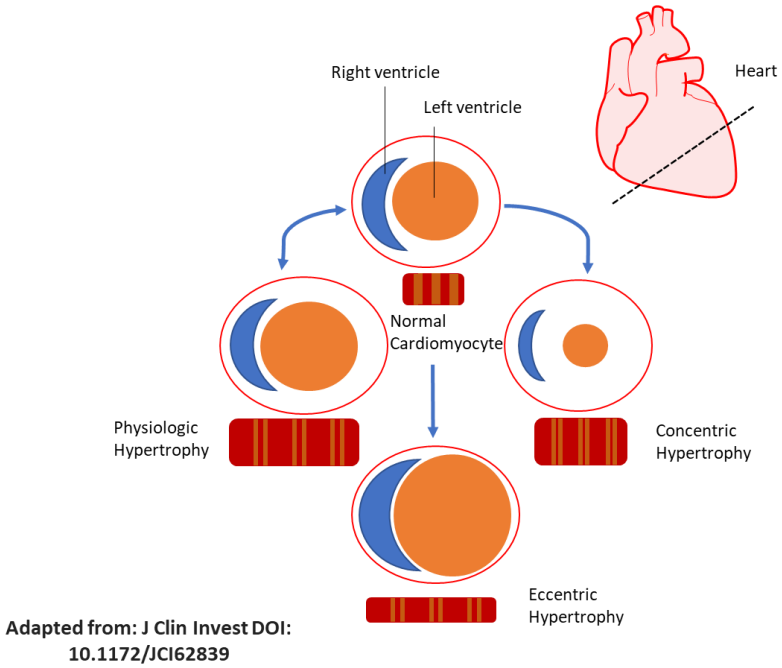


Figure 4: cardiac hypertrophy. In concentric hypertrophy, cardiomyocytes generally grow in width in comparison with length, giving rise to wall and septal thickening and to loss of chamber area. This situation can worsen into eccentric hypertrophy when there is an excessive extension of cardiomyocytes despite of their width. The consequence is a dangerous chamber expansion with loss of wall and septal thickness besides the increase of wall tension.

In the kidney, the pathogenesis follows a progressive pattern. Indeed, there is a system of autoregulation in the glomerular vessels determining vasoconstriction or vasodilatation on the basis of perfusion pressure to maintain the homeostasis. Protracted high perfusion pressures can bring to important vasoconstriction, which can then cause regional impairment to the glomeruli causing possible necrosis. This can lead to microalbuminuria and therefore to significant proteinuria. Renal deficiency could also be an effect of atherosclerosis of the renal arteries, causing underperfusion (**Nadar et al. 2006**).

Cerebrovascular system represents one of the major targets of hypertension.

1.1.3 Cerebrovascular System

Hypertension impacts several fields of human health, not only cardiovascular system per se. Bloodstream affects all body areas, and it particularly involves the nervous system and therefore brain par excellence.

Inflammation in the brain parenchyma can occur as a local process that can be triggered and sustained by activated glial cells, in the absence of immune cells recruited from the periphery, and it is thought to contribute to pathogenesis of several diseases (**Carnevale et al. 2010**).

Cerebrovascular system is a calibrate mechanism and brain blood vessels work together with brain cells to guarantee the right intake of oxygen and nutritional substances to neurons. It is known that, when there is an increasing of brain activities, the need of cerebral blood flow (CBF) also raises to ensure the energy intake. Indeed, the phenomenon called neurovascular coupling ensures the correct homeostasis between neuronal activity, the related energy need and the clearance of metabolic derivatives (**Iadecola et al**. **2016; Girouard &Iadecola 2006; Novak & Hajjar 2010**).

When hypertension starts, it produces several changes in CBF, disrupting neural homeostasis. This can happen because hypertension causes structural and functional modifications in vasculature such as atherosclerosis, increasing arterial stiffness, small vessels disease, loss of vessels contractile function, and interferes with blood brain barrier (BBB) operations (**Carnevale et al. 2012**; **Carmichael, 2014**). Hence, when particular cerebral areas are activated and need major metabolic activity, they suffer from hypoxia and reduce their efficacy (**Ahmed et al. 2017**).

Cerebrovascular system is divided in three main areas, supplied by anterior, middle and posterior arteries. There are two main arterial systems that supply the brain: anterior circulation which includes the anterior carotid system and posterior circulation which consists of the posterior vertebro-basilar system.

Regarding the anterior circulation, the internal carotid arises from the bifurcation of the common carotid in the lateral part of the neck, it reaches the base of the skull perforating it and achieving the subarachnoid space where it branches in ophthalmic artery and finally in anterior and middle cerebral arteries, anterior choroidal artery and posterior communicating artery (**Cambier et al. 2013**). They all principally supply the cerebrum and the deep structure such as thalamus, basal ganglia and internal capsule (**Cipolla 2009; Purves et al**. **2001**).

Regarding the vertebra-basilar circulation, vertebral arteries perforate the skull through the occipital foramen and they continue on the anterior surface of the medulla up to pons where they blend to form the basilar artery (**Cambier et al. 2013**). Branches of the vertebral and basilar arteries supply blood for the cerebellum and brain stem. Proximally, the basilar artery joins the two internal carotid arteries and other communicating arteries to form a complete anastomotic ring at the base of the brain known as the circle of Willis (**Purves et al**. **2001**) (**Figure 5**).

From the circle of Willis origin three pairs of arteries, the anterior, middle and posterior cerebral arteries, which are progressively divided into smaller vessels that cover the brain surface until they penetrate the brain tissue to supply blood to the corresponding regions of the cerebral cortex (**Purves et al**. **2001**). This system of vessels guarantees correct balance of blood-flow between the two brain hemispheres and it allows anastomotic circulation, that is a collateral way to let the blood flow if a part of the circulation is occluded for some reasons. (https://radiopaedia.org/articles/circle-of-willis)

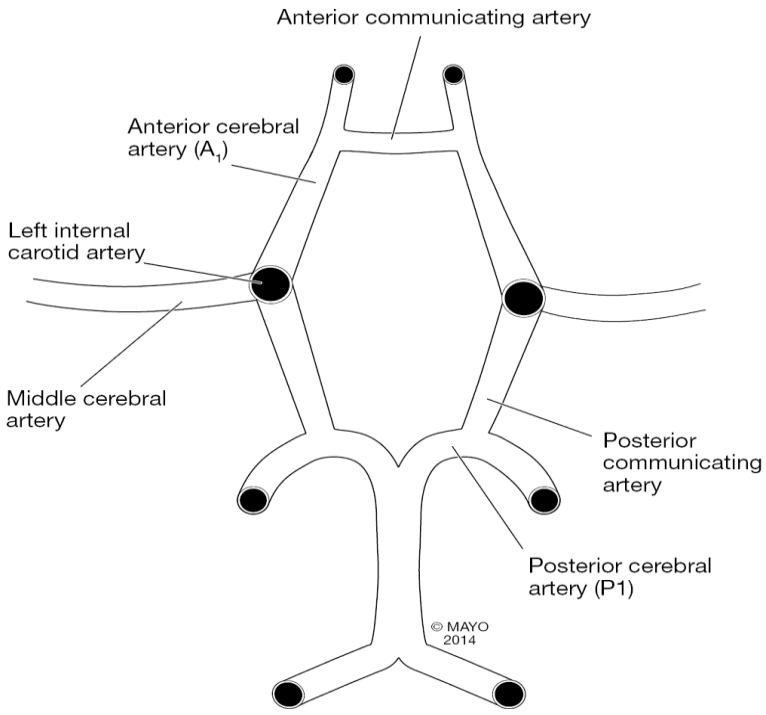


Figure 5. Representation of the Circle of Willis.

Chapter: Cerebrovascular Anatomy and Pathophysiology. Author: Kelly D. Flemming. From: Mayo Clinic Neurology Board Review.

Arteries are made of three layers, called tunica intima, tunica media and tunica adventitia (**Figure 6**). Intima-Media Thickness (IMT) is a routinely non-invasive ultrasound procedure to measure hypertension TOD (**Ferreira et al 2016**). Arterial stiffness is a measure of vessel wall stress induced by dysregulation in CBF (in the sense of augmented BP) and it is related to cerebral damage (also in cognitive sense).

In literature, it has been observed that in patients with Alzheimer's Disease (AD) biomarkers (e.g. APOE ε4), the presence of arterial stiffness along with plaque burden, worsen cognitive performance and interferes with cognitive aging (**Gardener et al. 2017**). In normal condition vessels adapt to BP fluctuations, but when there is a chronic vessels stress condition due to high BP levels, walls are under strain and they develop a damage, symptomatic of cardiovascular diseases (**Magnussen, 2017**; **Cromwell et al.2016**; **Ferreira et al 2016**).

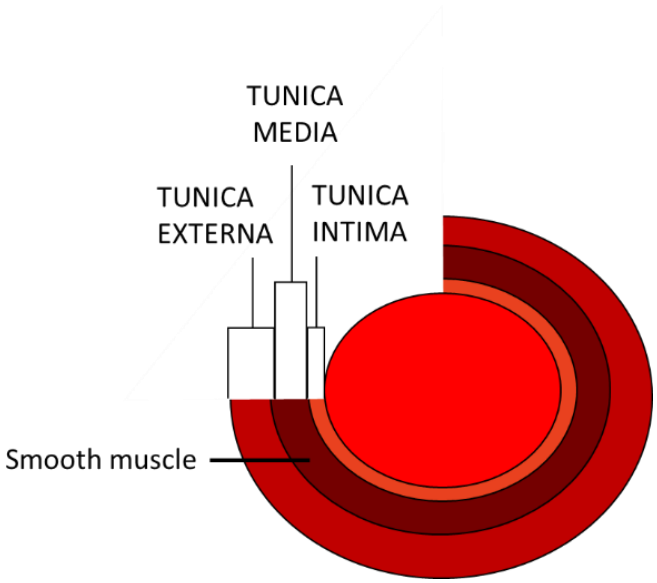


Figure 6. Representation of artery layers. The outermost layer is the tunica externa or adventitia. This layer is made of loose connective tissues and of an external elastic membrane. The tunica media is the middle layer. This layer is built of collagen, elastic fibers, and smooth muscle fibers. The tunica intima is the innermost layer. It is composed of an internal elastic membrane and connective tissue. The intima also includes endothelium, a layer that is directly exposed to blood flow within the vessel.

CBF through the mechanism of autoregulation impedes the chance of cerebrovascular accident due to unexpectedly increasing of BP thanks to myogenic tone and vasocontractile function that intrinsically are activated to maintain the constant CBF (**Iadecola et al**. **2016**).

BP fluctuations betray on neurovascular coupling and on the related cerebral areas activation. These mechanisms have been studied through neuroimaging techniques, particularly through magnetic resonance imaging (MRI) and perfusion MRI (**Carmichael, 2014**), showing how hypertension and increasing arterial stiffness are associated with major risk of silent infarcts, micro-hemorrhages, deficits in cerebral perfusion and in carotid plaques charge (**Carmichael, 2014**).

Hypertension influences not only mechanisms involved in CBF but also in the Blood Brain Barrier (BBB) activity. BBB is a membrane made of endothelial cells that protects brain from harmful substances circulating in the blood and it functions as a filter between vascular and nervous system (**Carnevale et al. 2012**). In particular, endothelial cells of the BBB have several characteristics that do not allow free transfers in neurons. The chief property is the tight junction that restricts the passage unless particles have certain features. Secondly, transcytosis is lower in BBB, diminishing transport activity and the risk of toxins entrance. Third, expression of several receptors is reinforced to admit nutritional substances. Lastly BBB has low LAMs (Leukocyte Adhesion Molecules) to preserve immunity mechanism in brain. (**Andreone et al 2015**). Therefore, one of the main features of BBB is coordination of transport activity in neurons, hence the role in the clearance in amyloid β (Aβ) (**Carnevale et al. 2012**). Aβ is the main metabolic waste responsible for neurotoxicity in the etiopathology of AD along with neurofibrillary tangles. The disposal of metabolic end-products is mediated by several mechanisms in the BBB: passive diffusion, active transport and receptor-mediated transport (**Banks 2009; Jain 2012**). Literature has individuated several receptors implicated in the transport and in the clearance of Aβ: low-density-lipoprotein receptor (LDLR), LDLR related protein 1 (LRP1), LRP2, formylpeptide receptor-like-1 (FPRL1), ATP-binding cassette (ABC) transporter–A1 (ABCA1), ABCC1,ABCG4, ABCB1, CD36, insulin-degrading enzyme (IDE), and the receptor for advanced glycation end product (RAGE) (**Ueno et al 2016**). Particles that elude BBB and pass through CBF and vice versa are exiguous and they could be fat-soluble substances, particles that brain needs or molecules that have special affinity with BBB receptors (**Jain 2012**). Vasculature variations due to HT can be expected to trigger neurovascular unit signaling, finally inducing the activation of microglia, the brain resident macrophages, and the neuroinflammation. In this way, HT could induce the onset or the progression of late-life dementia or sporadic AD by promoting neuroinflammatory processes (**Carnevale et al. 2010**).

1.1.4 Hypertension and Neurodegeneration

That said, it is evident that hypertension interferes with Aβ clearance, one of the leading contributing factors for neurodegeneration (**de La Torre 2002; de la Torre2004**; **Carnevale et al. 2010; de Roos & Mitchell 2017**). Neurodegeneration is a chronic detrimental process that affects the person in its entirety. Signs and symptoms hit brain areas and the related cognitive functions (**Figure 7**).

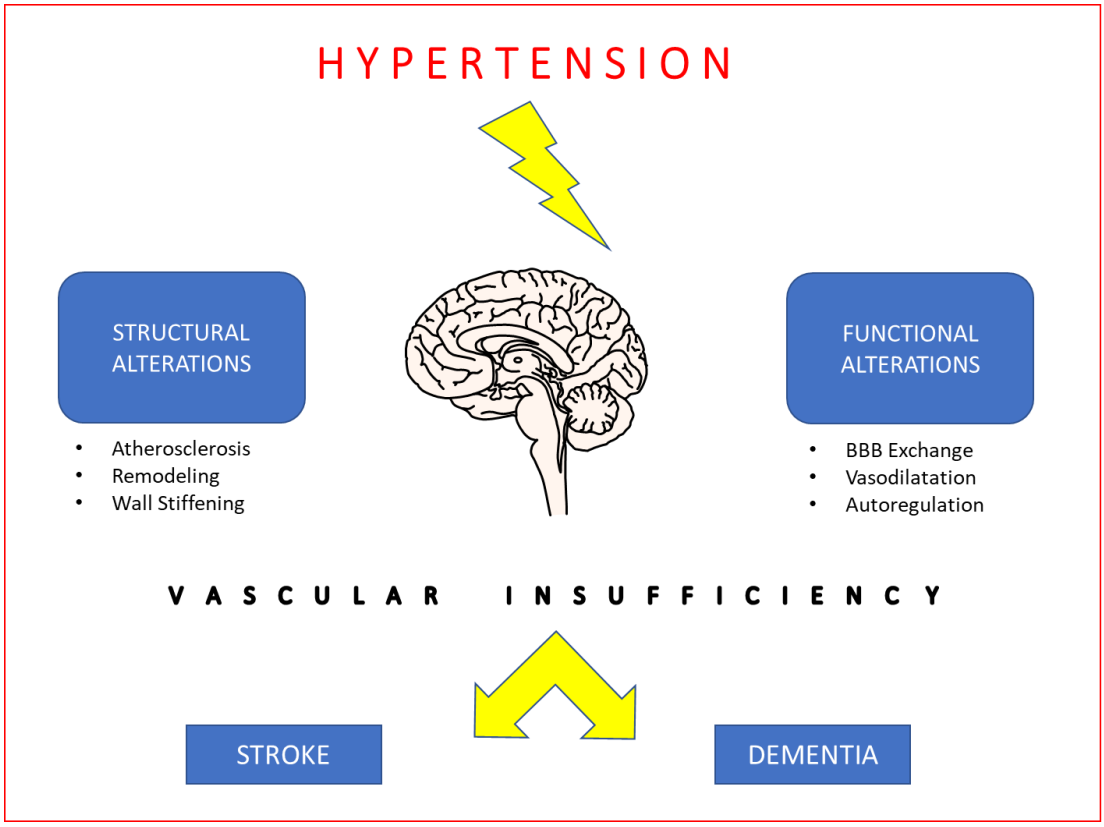


Figure 7. Hypertension origins structural and functional modifications in cerebrovascular system which compromise the blood provision to the brain and rises the risk of stroke and dementia.
Adapted from: Hypertension and cerebrovascular dysfunction. 2008. Cell Metabolism 7, (6)476-84. Authors: Iadecola C. and Davisson R. L.

To guarantee right working of mental processes, cerebral vasculature should be in right conditions. Hypertension induces stress to wall vessels and the allover mentioned structural and functional variations. In this way, also cognitive functions are altered (**Manolio et al.**

2003). Cognitive functions are major target of neuropsychological assessment aimed at evaluating signs of cognitive and functional deficits characteristics of neurodegenerative disorders.

Hypertension has long been known as one of the major risks for stroke, but it has been showed that mid-life hypertension is a main risk factor for cognitive decline, mild cognitive impairment (MCI) and later dementia (**Tariq and Barber 2017**). It was suggested that a high part of late life dementia is due to cardiovascular and psychosocial risk factors. These aspects are modifiable and therefore the targets of effective prevention. In Tariq and Barber review (2017) seven modifiable risk factors that are implied in the onset of late life dementia are listed. They are: low education, mid-life hypertension, mid-life obesity, diabetes, physical inactivity, smoking and depression (**Table 1**).

	Modifiable risk factors	PAR (95% CI)
CardiovascularRisk Factors	Diabetes	2.9% (1.3-4.7)
	Midlife obesity	2.0% (1.1-3.0)
	Midlife hypertension	5.1% (1.4-9.9)
	Hyperlipidemia	Inconsistent/ No definitive data
Lifestyle Risk Factors	Smoking	13.9% (3.9-24.7)
	Physical inactivity	12.7% (3.3-24.0)
	Diet	No definitive data
	Cognitive inactivity	No definitive data
Other Risk Factors	Low education	19.1% (12.3-25.6)
	Traumatic brain injury	No definitive data
	Depression	7.9% (5.3-10.8)
	Sleep disturbances	Inconsistent/ No definitive data

Table 1. Modifiable risk factors for late life dementia as listed in Tariq and Barber (2017). For each item is shown the related Population Attributable Risk (PAR)with the Confidence Intervals.

From: Dementia risk and prevention by targeting modifiable vascular risk factors. J Neurochem. 2018 Mar;144(5):565-581. 10.1111/jnc.14132. Authors: Tariq S., Barber P.A.

In the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM 5) (**American Psychiatric Association 2013**) these risk factors are described, and further factors are added: high homocysteine levels, atherosclerosis and arteriolosclerosis, atrial fibrillation, conditions increasing the risk of cerebral emboli, cerebral amyloid angiopathy and the hereditary condition cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, or CADASIL.

Since the relationship between hypertension and cognitive dysfunctions was evidenced, there were an exponential interest in studying this issue (**Elias et al. 2012**). In last years, several studies have focused attention on techniques in delaying or in hindering the detrimental effect between hypertension and cognitive dysfunctions. These approaches include cognitive training, physical activity interventions, nutraceutical interventions (e.g. **Giugliano et al. 2018**), diet interventions, multimodal interventions, hormone therapy interventions, vitamins interventions, antihypertensive treatment, lipid lowering treatment, nonsteroidal anti-inflammatory drugs (NSAIDs), antidementia treatments, diabetes medication treatment, other interventions (**Kane et al. 2017**).

1.2. Cognition in Hypertension

1.2.1 Organization of the Brain

The central nervous system contains the brain and the spinal cord. The pivotal function of the spinal cord is to transmit neural motor inputs from the brain to the periphery, and sensory communications from the periphery to the brain.

The deeper sections of the brain are designated for more elementary functions: the medulla monitors breathing, ingestion, digestion and heart rate. The hypothalamus controls the expression of basic needs. The cerebellum plays a central task in coordination and voluntary movement. The thalamus acts as a relay station for motor and sensory inputs from deeper areas to the cortex. Thalamus and cerebellum have also roles in higher human cognitive functions. An essential group of subcortical units is the basal ganglia (**Figure 8**). The basal ganglia handle both in elementary motor regulation and in the regulation of complex cognition. They receive projections from major part of the cortex and have projections to the frontal cortex.

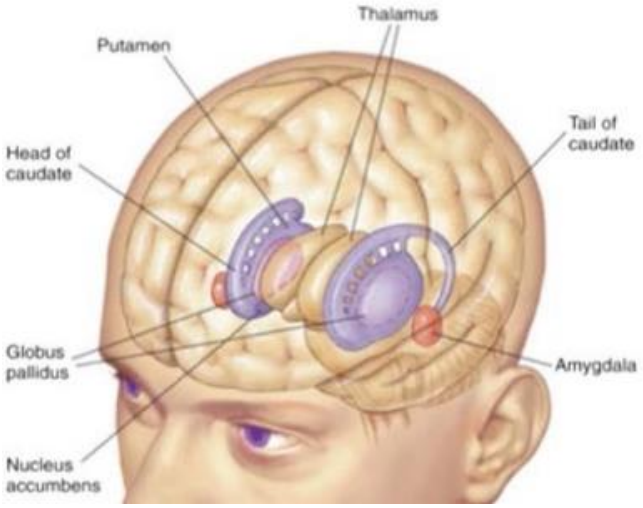


Figure 8. Basal ganglia system. The cerebral cortex projects to the caudate and putamen of the dorsal striatum, and nucleus accumbens of the ventral striatum of the basal ganglia. The output of the basal ganglia from the globus pallidus projects to the thalamus, which then projects back to the cortex, forming a loop.

From: Prospective Optimization. *Proceedings of the IEEE Institute of Electrical and Electronics Engineers*. 2014;102(5):10.1109/JPROC.2014.2314297. Authors: Sejnowski TJ, Poizner H, Lynch G, Gepshtein S, Greenspan RJ.

The cerebral cortex is evolved later. It is divided in two hemispheres: left and right. The left part of the brain controls mostly the right part of the body and vice versa. Cerebral cortex is divided by major folds (or sulci) in four lobes per hemisphere: frontal, parietal, temporal and occipital (**Figure 9**).

Summarizing the occipital lobe includes the primary visual cortex. The parietal lobe has the primary somatosensory cortex and is involved in spatial and proprioception functions as well as in attentional processes. The temporal lobe collects information from the primary visual cortex and from the primary auditory cortex and therefore is involved in object recognition and in language processing. The frontal lobe could be divided in the caudal part that contains the primary motor cortex and in the rostral one that is called the prefrontal cortex and has a pivotal role in higher cognitive performance. A particular mention goes to the limbic system, which is at the edge between the cortex and the deeper areas. It comprises the hippocampus fundamental to human memory processes.

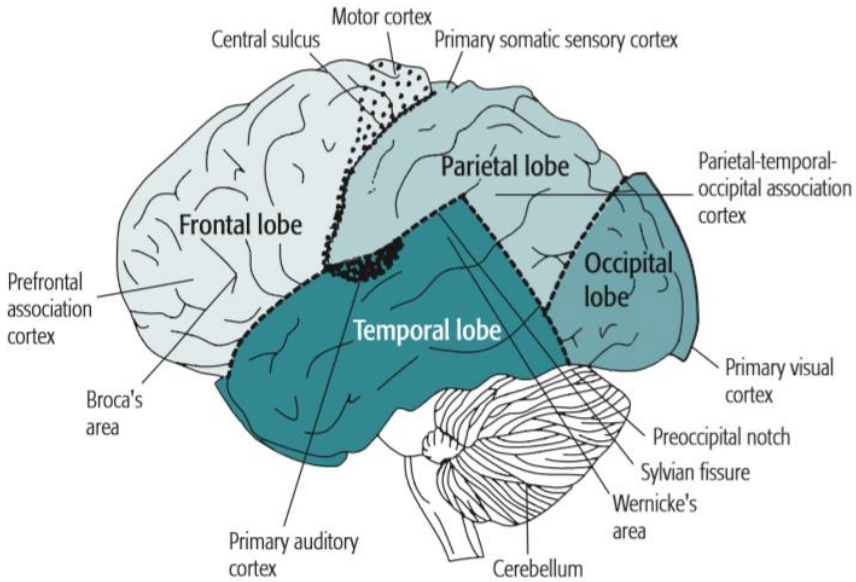


Figure 9. Frontal, Temporal, Parietal and Occipital lobes. The image shows the main anatomical features of the brain cortex.

From: Cognitive psychology and its implications (8th edition). New York. US: Worth Publishers. Author: Anderson J. R. (2015). Chapter: 1 The Science of Cognition p. 16.

1.2.2 Cognitive functions

Cognitive function could be defined as the mechanism that allows an integration process of elementary sensations to obtain perceptions. They allow world everyday knowledge and subjective experiences. Cognitive functions are executive functions, attention, visual perception, language, memory (**Abete et al. 2014**).

Executive functions are highest cognitive processes of human brain, they involve mainly three domains: cognitive flexibility, inhibitory control and working memory (WM). They are the basis for other fundamental mental processes as reasoning, problem solving and planning. In more details, cognitive flexibility is the process through which it is possible to change perspectives or approaches to a problem, adapt to new demands, rules or priorities (as in switching between tasks). Inhibitory control includes controlling one's attention, behavior, thoughts, and/or emotions irrespective of or on the basis of external stimuli and conditions. Working memory is a domain of memory related to executive functions, it consents simultaneously mental operations, allowing to manipulate information (**Diamond, 2013**).

Executive functions interest different areas of the frontal cortex, particularly they include two prefrontal cortices: the dorsolateral prefrontal cortex (dlPFC) and the anterior cingulate cortex (ACC) (**Anderson 2015**) (**Figure 10**).

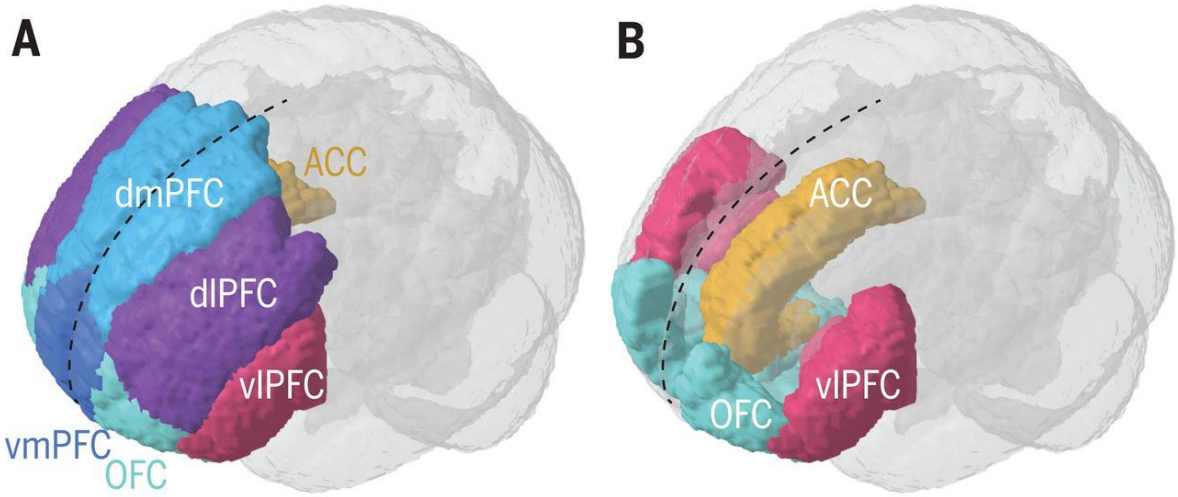


Figure 10. Subdivision of the brain prefrontal cortex. (**A and B**) Anterior Cingulate Cortex (ACC); dorsolateral Prefrontal Cortex (dlPFC); dorsomedial Prefrontal Cortex (dmPFC); ventromedial Prefrontal Cortex (vmPFC); ventrolateral Prefrontal Cortex (vlPFC); Orbital Frontal Cortex (OFC).
From: Science 27 Oct 2017: Vol. 358, Issue 6362, pp. 478-482. Author: Carlén M.

The dlPFC has importance especially in the regulation of intentions and in the control of behavior. For example, dlPFC is operating during dual tasks.
The ACC is operating especially when subjects must supervise conflict between challenging tendencies. An example of its activation derived from brain imaging studies using Stroop Test (**Anderson 2015**).

Memory is the procedure of coding, storing and retrieval of information. Human memory relies on frontal lobe for the formation and rescue of memories and on temporal lobe for the permanent storing of these traces (**Figure 11**).

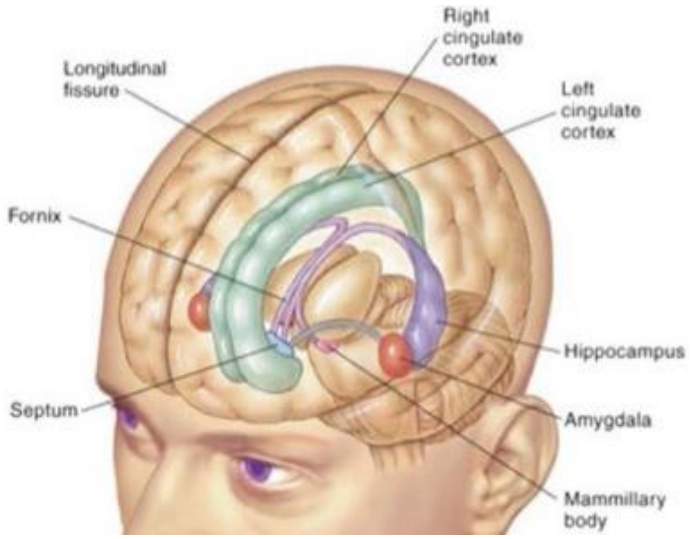


Figure 11. The limbic system. It regulates emotion, behavior, motivation, memory and olfaction. The limbic system comprises the cingulate cortex, the hippocampus and the amygdala.

From: Prospective Optimization. *Proceedings of the IEEE Institute of Electrical and Electronics Engineers*. 2014;102(5):10.1109/JPROC.2014.2314297. Authors: Sejnowski TJ, Poizner H, Lynch G, Gepshtein S, Greenspan RJ.

Information is not immediately available from the environment, but it must be processed (the coding phase). There are two perceptual systems designated to held briefly the sensory data to allow their analysis: the visual sensory memory and the auditory sensory memory. They could be defined as transient sensory store allowing to process the attended information and to send it to short term memory. The theory of short-term memory, by Atkinson and Shiffrin (1968), suggested that the attended information moves into a short-term memory store and through rehearsal it could go into a relatively permanent long-term memory store. Short-term memory has a limit in retaining new information, that is the memory span, which refers to the number of elements one can promptly repeat back. A crucial hypothesis in this theory was that the total of rehearsal controls the amount of information moved to long-term memory. Craik and Lockhart (1972) deduced that what was crucial was the deepness to which the track is processed and not for how long the information is rehearsed (**Anderson 2015**). Baddeley and Hitch (1974) and later Baddeley (2000), introduced the concept of working memory, a multicomponent system that manipulates data storage. Its components

are: phonological loop, visuospatial sketchpad, central executive and episodic buffer. Material obtained and elaborated by the working memory can be transferred to long-term storage (**Chai et al. 2018**). Long term memory according to Squire (1987) could be subdivided in two main components: explicit (or declarative) memory and implicit (or nondeclarative) memory. The first one includes memories that subjects could distinctly remember. The structure of the brain designated to this function is the hippocampus. Declarative (explicit) memory is also divided in episodic memory which includes information on where and when data were learned, and in semantic memory which includes general knowledge of the world. Nondeclarative (implicit) memory is defined as memory without conscious awareness, it is monitored mainly from the basal ganglia and the cerebellum (**Anderson 2015**).

Attention is the function underlying whole brain activity and it selects information to process. It is divided into different sub-processes dedicated for different features of attentional elaboration (**Glisky 2007**). This happens because in human information processing there is a limit due to the inability to elaborate more information at once. In Psychology this processing limit is compared to serial bottlenecks, points at which it is no longer possible to elaborate every information in parallel. Several theories have been articulated trying to explain at what time brain is no more able to integrate surrounding information. In general, theories are divided in early-selection theories and in late-selection theories. To explain these theories, some Authors have dealt with auditory attention, others with visual attention (for more details please refer to **Glisky 2007**).
Generally, attention could be divided on the basis of what kind of information brain has to process: selective attention denotes the capacity to deal with a specific task even if there are interfering stimuli. Divided attention concerns the faculty of managing two or more requests simultaneously. Sustained attention is the skill to retain concentration on a process for a long time (**Glisky 2007**). The parietal cortex influences information processing in visual cortex and in auditory cortex. Prefrontal regions (particularly the dlPFC and the ACC) control information processing in the motor area (**Figure 12**).

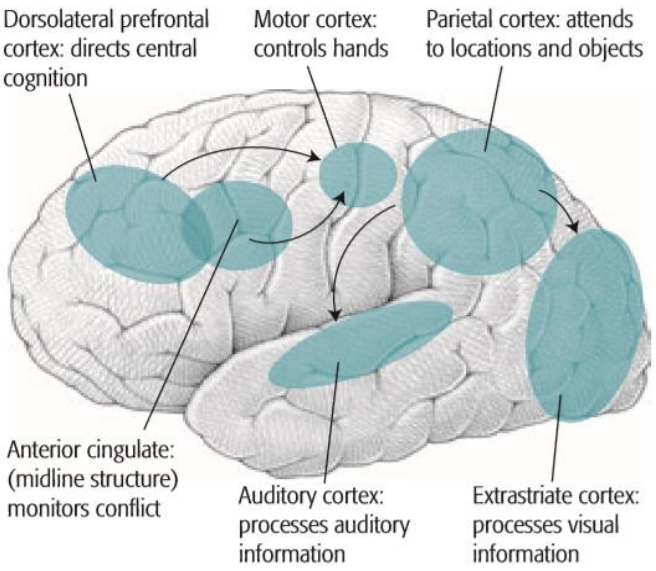


Figure 12. Some of the brain areas implicated in attention and some of the perceptual and motor regions they regulate. The parietal lobe is central in directing perceptual resources. The prefrontal regions (dorsolateral prefrontal cortex, anterior cingulate cortex) are particularly important in executive functions.

From: Cognitive psychology and its implications (8th edition). New York. US: Worth Publishers. Author: Anderson J. R. (2015). Chapter: 3 Attention and Performance p.54.

Visual perception is the process through which brain extracts information from the surrounding environment. Visual perception can be divided into an initial time, in which forms and things are obtained from the environment, and a later time, in which forms and objects are identified. A chemical reaction transforms light photons into neurons activity. Photoreceptors transmit the visual information, through optic nerve, to the visual cortex. From there, it is dislocated along "what" and "where" systems (**Figure 13**).

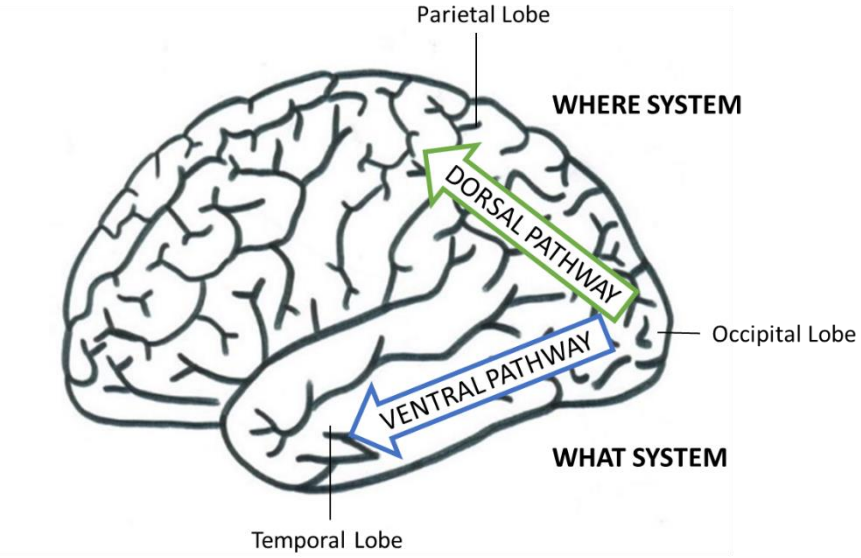


Figure 13. The dorsal pathway begins with V1 and arrives to the posterior parietal lobe. This pathway is also called the "Where System" or the "How system" and it is linked with motion, representation of object locations, and control of the eyes and arms, especially when visual information is used to guide saccades or reaching. The ventral pathway begins with V1and arrives to the inferior temporal lobe. This pathway is also called the "What System", and it is linked with form recognition and object representation. It is also associated with storage of long-term memory.

After sensory processing, the earliest sense of the information is called “primal sketch” according to Marr’s theory. These “sketches” are progressively provided with further information about the position in the environment: this is the Marr’s 2½-D sketch. The Gestalt laws of perceptual organization[1] are employed to assemble the elements into objects: this is the Marr’s 3-D model. Only after these procedures human brain can recognize the object and where it is located in the space (**Anderson 2015**).

Language is the faculty to associate sounds and meanings through grammatical rules based on one’s mother tongue. There are three pivotal functions of the language: 1. Communicative function; 2. Cognitive function; 3. Symbolic function (**Falabella 2013**). Language is mainly

[1] Gestalt laws of perceptual organization illustrate how the brain divides visual segments into objects. They are: the principle of proximity, the principle of similarity, principle of good continuation, the principles of closure and good form (for more details, please refer to Anderson 2015).

localized in the left hemisphere of human brain even in left-handed persons (**Figure 14**). The principal areas designated for language production and language comprehension are: prefrontal cortex (Broca's area), temporal cortex (Wernicke's area) and parietal cortex (supramarginal and angular gyri) (**Anderson 2015**). The distinction between production and comprehension involves several theories and mechanisms (for more details please refer to Anderson 2015).

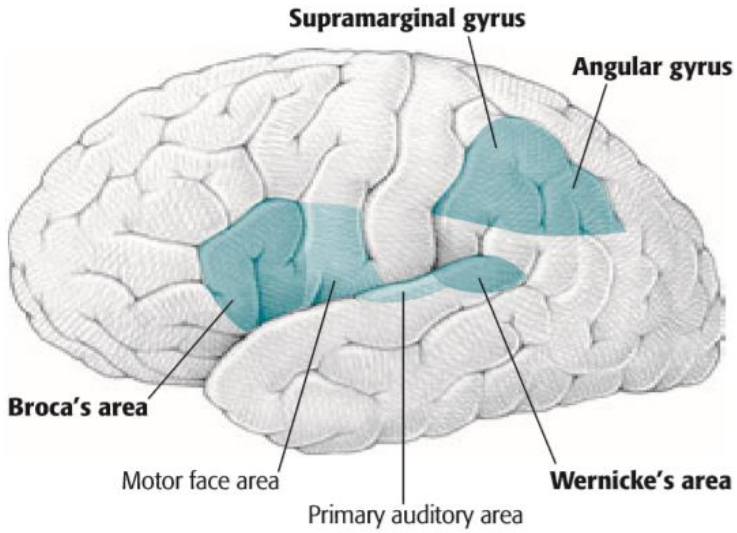


Figure 14. Main language areas in the left cerebral hemisphere.
From: Cognitive psychology and its implications (8th edition). New York. US: Worth Publishers. Author: Anderson J. R. (2015). Chapter: 12 Language Structure p. 282.

1.2.3 Cognitive Dysfunction in Hypertension

Several studies evidenced various cognitive domains affected by hypertension. Since the first studies about hypertension (1960) (**Iadecola et al. 2016**) it was quite clear that hypertension interferes with the ability of maintain attention and concentration. Later researchers have confirmed these first observations finding several cognitive domains damaged by hypertension, primarily executive functions, speed processing and memory (**Iadecola et al. 2016**).

Even if there are proofs of evidence of this strict relationship between hypertension and cognition, in routinely outpatient's facilities activity (in most cases) when the health problem is hypertension, physicians do not think to assess cognitive status and if it is performed, there is no a standard cognitive assessment for people suffering from hypertension. Increasing literature is showing this necessity, conceiving studies that compared cognitive test worldwide used to validate the best for cognitive impairment in hypertension (**Webb et al. 2014; Muela et al. 2017**). Several tests have been used to evaluate various cognitive domains in hypertension.

1.2.4 An overlook of the most used tests to assess cognitive impairment in hypertension

In literature, many Authors have dealt with the relationship between BP, neurodegeneration and cognitive decline (**Perrotta et al. 2016; Novak & Hajjar, 2010; Kilander et al. 1998**). Different reviews report several longitudinal and cross-sectional studies in which cognitive functions in patients with hypertension were monitored (**Walker et al 2017**). As said before, studies on hypertension observed major problems in executive functions, speed information processing and memory (**Iadecola et al. 2016**). This fact explains that the tests used in the assessment of hypertension are typically Trial Making Test A (TMT A) and TMT B, digit span, digit symbol test or Stroop test for executive functions and speed processing information and recall word list test for memory (**Iadecola et al. 2016**). Regarding the whole cognitive status, there is typically the aim to exclude any signs of overt dementia, and this justify the fact that usually studies present global battery to evaluate overall cognition. The main issue is that not all the general batteries fit for every cognitive problem. Some of these batteries are more precise to discriminate subtle deficits in neurodegeneration, others to quantify rougher or evident deficits. Reviews in the field of cognition and hypertension not always show what tests are used, they are limited to explain what cognitive functions they assess. Knowing details on how cognitive performance is measured, offers the possibility to develop a procedure to choose the best tests to assess cognition in hypertension. Hereunder, it will be taken into account neuropsychological tests used in literature examining Walker's et al review (**2017**) which have investigated how cognitive functions are evaluated in hypertension, subdividing hypertension in early and midlife hypertension, and late life hypertension.

Early and midlife hypertension:

Block design, Digit span, Mathematics, Reading. NHANES III;

Mini-Mental State Examination, verbal fluency task (animal naming), digit span backwards, constructional praxis, immediate recall of a ten-word list, delayed recall of a ten-word list, and pattern comparison task. NORMATIVE AGING STUDY;

Mini-Mental State Examination, Digit Symbol Substitution Test, Benton Visual Retention Test, and Verbal Fluency Test. NHLBI twin study USA;

100-point Cognitive Abilities Screening Instrument (CASI). The CASI includes tasks measuring attention, concentration, orientation, short- and long-term memory, language, visual construction, list-generating fluency, abstraction, and judgment. Honolulu-Asia Aging Study, USA;

Mini Mental State Examination (MMSE). Xi'an, China;

20-word free-recall test of short-term verbal memory, AH-4, Mill Hill vocabulary test and verbal fluency: phonemic and semantic. Whitehall II, UK;

Three-test battery, which includes Word List Learning, Word List Recall and Animal Fluency. REGARDS, USA;

Visual Reproductions Immediate and delayed, paired associate immediate and delayed, logical memory immediate and delayed, trial making test part B and Hooper Visual Organization Test. Framingham Heart Study, USA;

Simple Reaction Time Test, Symbol Digit Substitution Test, and Serial Digit Learning Test. NHANES III, USA;

The MMSE. EVA Study Group, France;

Delayed word recall test, 10-word delayed free recall task in which the learning phase included sentence generation with the study words, digit symbol subtest (DSS) of the Wechsler Adult Intelligence Scale-Revised and first-letter word fluency test using letters F, A, and S. ARIC, USA;

Iowa Screening Battery for Mental Decline, MMSE, Wechsler Adult Intelligence Scale – revised digit symbol substitution, color trail making test, color-word interference test, California Verbal Learning Test. Western Collaborative Group Study, USA;

Trial Making Test and MMSE. Male Cohort in Uppsala, Sweden;

Vocabulary (WAIS), digit span test, Claeson-Dahl test, block span test. Framingham Heart Study, USA;

Delayed Word Recall test, Digit Symbol Subtest of the Wechsler Adult Intelligence Scale-Revised, and the Controlled Oral Word Association (Word Fluency) test. ARIC, USA.

Late life hypertension

MMSE. COGNIPRES Spain;

MMSE. Kungsholmen Project, Sweden;

Six-item Screener (a test of global cognitive function derived from the widely used Mini-Mental State examination). REGARDS, USA;

Vocabulary (WAIS), digit span test, Claeson-Dahl test, block span test. Framingham Heart Study, USA;

Modified Mini-Mental State Examination, Boston Naming Test, Controlled Word Association Test, category naming, and complex ideational material and phrase repetition subtests from the Boston Diagnostic Aphasia Evaluation, Wechsler Adult Intelligence Scale–Revised similarities subtest and nonverbal identities and oddities subtest of the Mattis Dementia Rating Scale, Rosen Drawing Test and matching version of the Benton Visual Retention Test, multiple choice version of the Benton Visual Retention Test and the 7 subtests of the Selective Reminding Test: total recall, long-term recall, long-term storage, continuous long-term storage, words recall on last trial, delayed recall, and delayed recognition, 11 items from the Disability and Functional Limitations Scale and the Blessed Functional Activities Scale. Northern Manhattan Study, USA;

6-Item Screener. REGARDS, USA;

MMSE and hindo-MMSE Indo-US. Cross National Dementia Epidemiology Study, India/USA;

MMSE, Geriatric Depression Scale (GDS). Osservatorio Geriatrico Regione Campania, Italy;

Digits Forward and Backward portions of the Wechsler Adult Intelligence Scale–Revised, California Verbal Learning Test, Benton Visual Retention Test, Trail Making Test Parts A and B, Letter Fluency and Category Fluency, Boston Naming Test. Baltimore Longitudinal Study of Aging, USA;

East Boston Memory Test (EBMT), Symbol Digit Modalities Test and MMSE. Chicago Health and Aging, USA;

MMSE, sections B and H of the Cambridge Mental Disorders of the Elderly Examination (CAMDEX), Pfeffer Functional Activities Questionnaire, Hamilton Depression Scale. The Italian Longitudinal Study on Aging, Italy;

Synonyms test, Swedish version of Block Design test that is a part of WAIS, list of paired associates test, Digit Symbol Substitution Test. Men Born in1914, Sweden;

Modified MMSE and Digit Symbol Substitution Test. Cardiovascular Health Study, USA;
6-item memory test (the East Boston Memory Test [EBMT]) and 9-item version of the Pfeiffer Short Portable Mental Status Questionnaire (SPMSQ). East Boston cohort study, USA;
Short Portable Mental Status Questionnaire (SPMSQ). Duke Population Studies of the Elderly, USA;
Brief story, Orientation items were selected from the Pfeiffer Mental Status Questionnaire and digit span. East Boston Study, USA.

It is clear that there is still no standardized procedure for cognitive assessment in people with hypertension. All these studies provided evidences on the first assumptions about the involvement of hypertension in executive functions and its domains (**Iadecola et al.2016**) and secondarily in memory. In the last years, further studies tried to use as first step evaluation Montreal Cognitive Assessment (MoCA) (**Nasreddine et al. 2005**) in different cardiovascular fields **(Jurgens et al. 2012; Freitas et al.2012**). It comes from the need to better assess and to characterize those patients with a normal performance in the MMSE, but with cognitive complains (**Nasreddine et al. 2005**). MoCA is used as a global neuropsychological assessment and in the last decades several studies compared its efficacy to the MMSE or considering it even better (**Webb et al. 2014**; **Trzepacz et al.2015**; **Zamboni et al. 2017**). MoCA offers a first screening of the main cognitive functions that should be analyzed in cardiovascular problematics and it is more sensible to subtle deficits due to hypertension even at early stage (**Webb et al. 2014; Carnevale et al. 2018**). It could also discriminate more easily MCI from dementia. (**Julayanont et al.2014**). Along with MoCA, there are other neuropsychological batteries, the Mini-Cog test (**Patel et al. 2015**; **Walsh2015**) and the ADAS-Cog (Alzheimer's Disease Assessment Scale) (**Mohs et al. 1983**; **Dao et al**.**2017**) used as screening tool to discriminate MCI and dementia. Anyway, these batteries do not assess all the cognitive functions damaged by hypertension and they have been created to assess in particular AD not even at early stage (**Podhorna et al**. **2016**). Summarizing MoCA could be an efficient first step to individuate cognitive disfunctions at early stage in hypertension (**Carnevale et al. 2018**).
This battery is validated and translated in different languages worldwide: English, Dutch, Finnish, French, German, Italian, Polish, Portuguese, Spanish and Swedish and is composed by different subdomains which involve executive functions and visuospatial abilities, naming, memory, attention, language, abstraction, delayed recall and orientation. In more

details executive functions and visuospatial abilities were measured through a brief TMT B version test, copy of a cube and clock drawing; naming is tested through nomination of three animals; memory trial is divided in immediate and delayed recall and it consists in memorizing five words and in retaining them at the end of the other MoCA's trials. Attention tasks contain one string digit span forward (five numbers) and backward (three numbers), a brief double task test and a serial 7 subtraction. Language is tested by two sentences repetition and by phonemic verbal fluency task. Finally, abstraction is assessed asking object similarities and orientation is assessed through questions about date and patient location.
There is still lack of standardized procedure to deepening the functions that result impaired at the first screening with MoCA.

1.3 Forms of Dementia

As said before, neurodegeneration is a chronic detrimental process that affects the person in its entirety. Signs and symptoms hit brain areas and the related cognitive functions. According to WHO (2017) dementia could be defined as a chronic or progressive syndrome that interferes with normal ageing. The WHO distinguishes early, middle and late stage of the progression of dementia, based on what kind of symptoms the patient manifests.

The early stage is often underestimated because of its gradual onset. Prevalent symptoms are: lapses of memory, getting lost in everyday places, losing the sense of time.

The middle stage is characterized with clearer manifestations such as getting forgetful of latest happening and of people's name, getting lost at home, having problems in language and speaking and with taking of personal care. Moreover, subjects experienced mood and behavioral changes.

In the late stage patients gradually lost totally their autonomy in every activity, symptoms become obvious and invaliding. They include: being oblivious to time, places and relatives, mood changes, walking difficulties and becoming dependent (**WHO 2017**).

There are various forms of dementia. According to WHO (2017), the most common one is AD, followed by VaD, Lewy bodies dementia and fronto-temporal dementia. In daily clinical practice the difference between diagnoses is not so clear and it is common to have mixed forms.

Records of recent memory loss, language forgetfulness for specific words, difficulties in perceptual motor abilities as well as in executive functions, are evocative of AD. In this case the use of validated AD biomarkers (e.g. cerebrospinal fluid levels of beta-amyloid and phosphorylated tau, and amyloid imaging) could be helpful in differential diagnosis. In Lewy bodies dementia there are peculiar characteristics as visual hallucinations and parkinsonism while in fronto-temporal dementia the main symptoms involve behavioral traits and language deficits. DSM 5 (2013), in neurocognitive disorders (NCD) section, specifies other types of neurodegeneration due to: Traumatic brain injury, Substance/medication use, HIV infection, Prion disease, Parkinson's disease, Huntington's disease, Another medical condition, Multiple etiologies and Unspecified. Hereinafter it will be treated in more details VaD, for more information about the other NCDs please refer to DSM 5 (2013).

1.3.1 Vascular Dementia (VaD)

Increasing literature showed how hypertension and cognitive dysfunctions are strictly related (**Abete et al 2014**) being able to generate the so called VCI that can evolve in VaD (**Gorelick et al. 2011**). VaD is the prototype of disease that relates cardiovascular system and neurodegeneration. VCI could evolve in VaD since hypertension represents one of the major risk factors for etiopathology of major or mild vascular NCD (**DSM-5 2013**). VaD represents the second dementia immediately after AD. Its chief symptoms are of vascular etiology and its related cognitive impairment mainly involves attention and executive functions (**DSM-5 2013**). According to the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) (**version 2016**), beneath the label of VaD can undertake different forms of neurodegeneration of vascular etiology, each one with a diverse shade that could range from lesion of different cerebral area to clinical and physio-pathological criterion. These are: vascular dementia of acute onset, multi-infarct dementia, subcortical vascular dementia, mixed cortical and subcortical vascular dementia, other vascular dementia and vascular dementia unspecified. The form of VaD with multiple infarctions presents acute progressive or fluctuating cognitive dysfunctions interrupted by regression periods in which symptoms are stationary or improved (**DSM 5 2013**). A recent article by Emdin et al. (**2016**) examined the medical histories of 4.28 million people in the UK. The study found patients aged 30-50, who had high BP, had a 62 per cent higher risk of VaD, and a 26 per cent higher risk at age 51-70 showing how midlife hypertension is a major risk factor for cognitive dysfunctions (**Emdin et al 2016**). Furthermore, the study showed how the risk in developing cognitive dysfunctions was significant even if the risk of stroke was reduced.

According to DSM 5, when there is an NCD problem it is important to discriminate the real reasons for brain infarctions and white matter hyperintensities (WMHs), since they are rather recurring in patients over 65 years old.

1.3.2 VaD Neuroimaging Characteristics

1.3.2.1 Basics of Magnetic Resonance Imaging (MRI)

The MRI technique is based on physical properties of atomies. In particular, through magnetic field produced by an external device it is possible to direct and to align protons in

water nuclei of human body. Once aligned, protons are next perturbed by a Radio Frequency (RF) energy. The nuclei return to their original position, emitting in turn RF energy. Thanks to Fourier Transformation, frequency information is converted in shades of grey in a matrix arrangement of pixels. By changing the sequence of RF pulses applied and collected, different types of images are created. Repetition Time (TR) is the amount of time between succeeding pulse sequences applied to the same slice. Time to Echo (TE) is the time between the transfer of the RF pulse and the receipt of the echo signal. In more details, the most common MRI sequences are T1 and T2 weighted scans. They are produced taking advantage of different physical properties of protons in the atomies of tissues. As stated in Daroff et al. (**2016**) T1 (longitudinal relaxation time) is a measure of how long spinning protons take to realign with the magnetic field. T2 (transverse relaxation time) is a measure of how long spinning protons take to lose phase coherence among the nuclei spinning perpendicular to the main field. On the basis of the different properties of the tissues, there are different contrast and brightness, for example CSF is a visual marker that easily allow to distinguish between T1- and T2- weighted images. Indeed, CSF appears darker in T1- weighted images and brighter in T2- weighted images.

Another common sequence used in RMN acquisition is the FLAIR sequence (Fluid Attenuated Inversion Recovery) with a very long TR and TE, and the so called T2* with sufficiently long TE (>20 ms) and a low flip angle (to avoid T1-weighting) used to characterize microbleeds (**Barkhof et al. 2011**) (**Table 2**).

Sequence Name	Typical Duration (Minutes)
I. Coronal 3D T1-weighted gradient echo 1 mm isotropic voxels	8
II. Transverse T2-weighted TSE/FSE 3–5 mm slices	4
III. Transverse FLAIR TSE/FSE 3–5 mm slices	4
IV. Transverse T2* gradient echo 3–5 mm slices	4

Table 2. Sequences for standard RMN exam. From: Neuroimaging in Dementia. Springer Heidelberg Dordrecht London New York. 2011; DOI 10.1007/978-3-642-00818-4. Authors: Barkhof F., Fox N. C., Bastos-Leite A. J. and Scheltens P.

The imaging evaluation takes advantage of different criterions. One of the most used visual rating is the Fazekas Score (**Fazekas et al. 1988**). It is an easy scale that considers

periventricular and deep white matter and provides a practical and detailed description of the main lesions to search for scoring. Fazekas Score is set from 0 to 3. 0 = No WMHs; 1 = Focal or punctate lesions namely single lesions ≤9 mm or grouped lesions <20 mm; 2 = Beginning confluent lesions, namely single lesions 10–20 mm or grouped lesions >20 mm in any diameter. Another parameter is the presence of no more than connecting bridges between individual lesions; 3 = Confluent lesion, namely single lesions or confluent areas of hyperintensity ≥20 mm in any diameter. The image attached offers an overview of this rating (**Figure 15**).

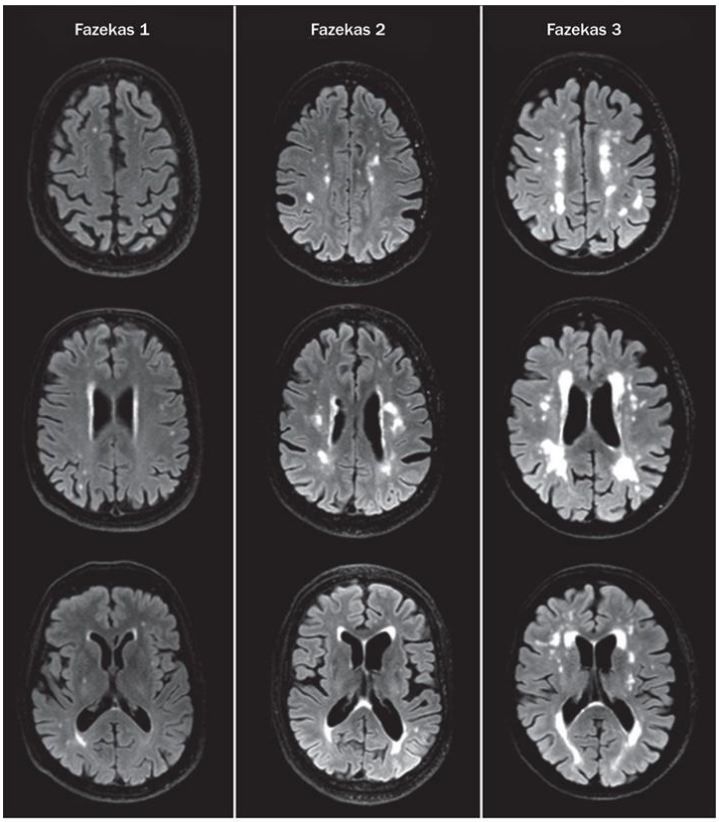


Figure 15. Visual rating of WMHs according to Fazekas. From: White matter hyperintensities, cognitive impairment and dementia: an update.2015. Nat. Rev. Neurol. doi:10.1038/nrneurol.2015.10. Authors: Prins, N. D. & Scheltens, P.

1.3.2.2 Neuroimaging and VaD

Literature has shown how BP affects brain vasculature, cortex and volume (**Iadecola et al**. **2016**). DSM 5 (**2013)** suggests the use of brain imaging as a routinely clinical practice for differential diagnoses between vascular and Alzheimer's type dementias and it hopes in specifically biomarkers that will be soon available and validated. Among biological markers of preclinical disease there are structural brain imaging, diffusion tensor imaging and iron imaging (**Tariq and Barber 2017**).

BP increases the risk for microbleeds or lacunar infarcts, WMHs and volume atrophy, involving different regions of the whole brain. The lesions may be focal, multifocal, or diffuse and they can occur in many combinations (**DSM 5 2013**) and generally they represent secondary hypertension-induced damage.

In general, microbleeds interest basal ganglia, lacunar infarcts are generally located in basal ganglia and in periventricular and in deep white matter, WMH interests periventricular regions and deep white matter, and finally volume atrophy involves all brain areas (**De Roos and Mitchell**, **2017**). When these lesions are visible, it is possible to made VaD diagnosis which is made thanks to neuroimaging techniques. In clinical practice, when a patient must be evaluated for suspected dementia, first examinations concern to assess the existence and the extent of brain atrophy and to determine the degree of eventually vasculature damage (**Barkhof et al. 2011**).

The most common neuroradiological findings in patients with a diagnosis of VaD are multiple subcortical microbleeds and periventricular leukoaraiosis ascribable to diffused ischemic sufferance at arteriolar level. There is less evidence of TC sensitivity than RM, both for focal lesions and for diffused parenchymal sufferance (**van Straaten et al 2004**), but conventional magnetic resonance (MR) is not sufficient to made VaD diagnosis (**Tedeschi et al. 2005**), hence the major use and development of several imaging technique that exploit RM in vascular neurodegeneration. MR has two main limits in the VaD diagnosis: inadequacy to attribute a clear vascular meaning to injury, inadequacy to detect the causal relationship lesion-cognitive decline (**Tedeschi et al. 2005**).

Conventional neuroimaging sequences usually applied in evaluating brain atrophy are: T1-weighted and T2- weighted. In particular, T1and 3D SPGR sequences offer information on brain volume and atrophy degree while T2 sequences evidence white matter lesions (**Tariq et al 2017**).

In addition to conventional MR, there are several "non-conventional" MR techniques, not always used in routinely clinical practice (**Tedeschi et al. 2005**). The so called non-conventional MR techniques are: spectroscopy, Diffusion Weighted Imaging (DWI), Diffusion Tensor Imaging (DTI), MR magnetization transfer, perfusion-weighted imaging. These methods offer a better characterization both for brain lesions, especially for WMHs, and for apparently normal cerebral tissue, identifying structural or functional damages more than that visible with conventional MR.

The DWI and the DTI techniques are based on free diffusion of water particles. Diffusion is physical property that describes particles movements. In biological tissue water molecules diffusion is limited by physical barriers, constituted essentially by cellular membranes, myelin sheaths surrounding axons and other structures (**Moseley et al. 2002; Alexander et al. 2007**). For these reasons brain diffusion is not isotropic that is uniformity in all directions, consequently, every pathological process that determines nervous tissue destruction, increases the diffusivity, producing a signal reduction that answer to a hypo-intensive images. For example, DWI is sensible to water spontaneous movements that become restricted in ischemic brain tissue. When this happens, the result is an extremely bright signal making the DWI technique recommended for detecting acute stroke.

Acquiring images in different directions and elaborating them through tensor matrix it makes possible to collect information about direction of diffusivity (anisotropy). DTI consents to study the quantity of anisotropy of water diffusion within axons which denotes the degree to which directionally order tissues are either maturing or losing their normal integrity (**Moseley et al. 2002**). As said in Tariq (**2017**) diffusion parameters offer a better sensitivity distinguishing underlying pathologies in progress than volumetric acquisition alone. Main DTI parameters are: Fractional Anisotropy (FA), Mean Diffusivity (MD), Axial Diffusivity (AD) and Radial Diffusivity (RD). In more details, FA is a measure that evaluates the microstructural integrity of white matter. The limitation of this measure is the less sensitivity to the specific type of change. MD is an "inverse" measure of the membrane density. MD is comparable for both grey matter and WM and higher in CSF. This parameter is sensible to cellular edema and necrosis. AD tends to be variable based on WM changes and pathology. Indeed, AD increases during brain maturation and decreases in axonal damages. RD is a value of de- or dys- myelination. Changes in axonal diameters or density may influence this measure (**Tromp 2016**).

FA is the measure that allows an overall assessment of WM and ranges between 0 (absence of water diffusion directionality) and 1 (is the case when the only direction is that of the fiber). The damage of nervous fibers of WM implies a reduction of FA values.

The advantage over conventional methods stands on the possibility to identify damage at microscopic level instead of macroscopic lesions, making it possible staging damage progression. A main limitation in the use of this approach came from the evidence that the initial region of interest (ROI)-based, or atlas-based methods of DTI analysis evidenced many technical flaws (**Carnevale et al. 2018**).

An evolution of MR DTI, that overcome these limitations, is fiber tracking, a post processing data analysis which allows by apposite algorithms to render information about diffusivity directions in a 3D axon connection through voxels (**Moseley et al. 2002**).

Thus far, however, no study exploited this strategy to examine microstructural WM damage in hypertensive patients, at a stage where they have normal brain imaging at conventional MRI and no diagnosis of dementia at the neurocognitive assessment (**Carnevale et al. 2018**).

All these evidences support the need of new strategies in approaching and preventing hypertension damages at cerebral and cognitive levels. There is still lack of literature on the parallel progression of cerebrovascular dysfunctions and related neurodegenerative pathologies as well as if it exists a time window in which it is possible a valid prevention also in term of delaying the onset of the pathology (**Carmichael, 2014; Tariq and Barber 2017**). Evaluating variations in cognitive performance may detect slight deterioration and hence might be a more sensitive outcome prior conversion to manifest dementia (**Tariq and Barber 2017**). The clinical approach to this category of patients usually starts when symptoms are clearly evident, and progression of dementia is perceived even by patient themselves. Indeed, patients generally report memory complains when they become aware of dysfunctions in normally activities. Growing researches focus on a compelling need for methods to determine as early as possible symptoms of neurodegeneration to identify at early stages those patients that would benefit of therapies aimed at limiting the transition to dementia.

The present research project, published on Cardiovascular Research (Volume 114, Issue 11, 1 September 2018, Pages 1536–1546), aims at identifying a specific signature of subtle and early alterations evidenced by cognitive assessment performed with specific batteries and tests, correlated with tractography data to discover brain signs of hypertension prior to become manifest at conventional imaging.

To the aim of this thesis, the published article shown below is improved with more details and specifications, highlighting the cognitive procedure performed.

CHAPTER 2. METHODS

2.1 Experimental Design

This study was conducted at IRCCS Neuromed, Department of Angiocardioneurology and Translational Medicine. The protocol was performed in compliance with the ethical standards established in the Declaration of Helsinki and approved by the Ethical Committee of our Institution, as registered in clinicaltrials.gov (NCT02310217). Informed consent was obtained from all individual participants included in the study. For the purpose of this publication, between November 2014 and September 2017, we screened subjects admitted at our outpatients' facility – Regional Excellence Hypertension Center of the Italian Society of Hypertension – affiliated to the Department of Neurological Complications of Cardiovascular Diseases of our Institute. We recruited individuals conforming to the following criteria: aged 40 to 65 years, compliant to give written informed consent, possibility to perform a dedicated 3 Tesla -MRI scan (**Figure 16**). The resulting 143 eligible patients were subjected to the assessment of strict inclusion/exclusion criteria by two independent raters. The exclusion criteria were the following: stroke, dementia, schizophrenia, seizures, Parkinson's disease, bipolar disorder and any other diagnosed neurologic or psychiatric disease, claustrophobia, secondary hypertension, end stage heart disease, renal failure, dialysis treatment, diabetes, atrial fibrillation and drugs that could affect cognitive functions. In the end, 70 patients met the criteria and entered the study. Then, in order to have a homogeneous sample of individuals, we further excluded subjects with already manifest signs of damage or alterations at conventional MRI and/or diagnosis of dementia, assessed as detailed in the specific sections below. After this latter screening, 28 patients reporting WM hyperintensities or lacunar damage (n=19), neurinoma (n=1), meningioma (n=1), angioma (n=1), brain calcifications (n=2) and artefacts due to movement (n=4) were further excluded, resulting in a final sample of 42 individuals. Each subject included in the study protocol underwent clinical examinations to assess covariates as office BP, smoking, body mass index (BMI) and education, and were categorized in hypertensive (n=23) and normotensive (n=19) individuals accordingly to their history of hypertension, current anti-hypertensive therapy and target organ damage, determined as detailed below in specific sessions. Since the protocol was designed as an observational study, there was no unified anti-hypertensive schedule for hypertensives and actual anti-hypertensive therapy was recorded for all patients and included the following main classes of drugs: angiotensin-

converting enzyme inhibitors and angiotensin II receptor blockers (ACE-I/ARBs), calcium channel blockers, diuretics and β-blockers.

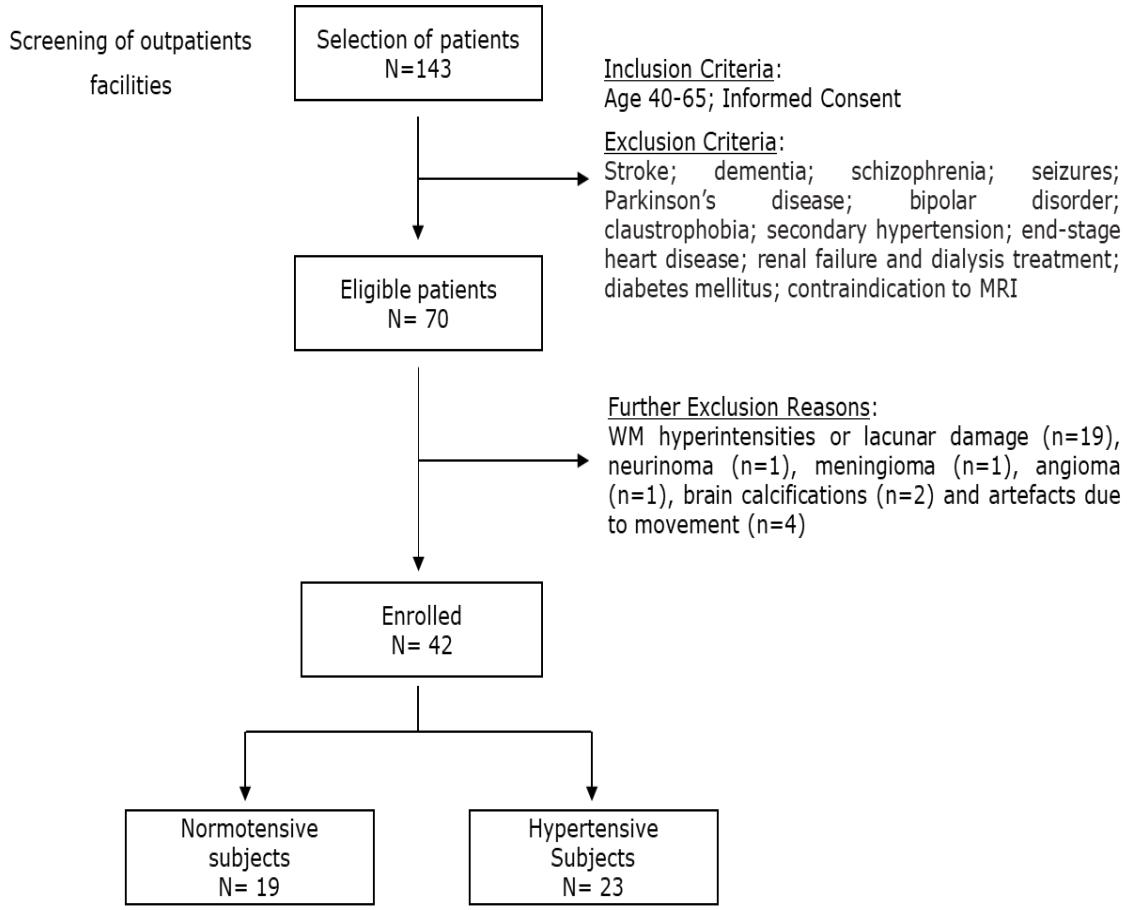


Figure 16. Flowchart of the population screened for the study. There are represented inclusion and exclusion criteria and the final sample examined for the research.

2.2 Clinical Procedures

2.2.1 Neuroimaging, DTI processing and Fiber-tracking

Brain MRI was performed on a 3T GE Signa Horizon scanner (General Electric Medical Systems, Milwaukee, WI, USA). After localization and calibration routines, the sequences used were as follows: T1 weighted images in axial orientation, acquired using a 3D SPGR

sequence (TR/TE/TI=5.6/1.7/400 ms, flip angle=11°, FOV=256 mm, slice thickness=1 mm, pixel spacing=1.0 mm×1.0 mm) with a 256×256 in-plane resolution; AX DP/T2 scan (TR/TE/TE2=3320/10/103.0 ms, FOV= 220mm, slice thickness=4mm); DTI was performed with a diffusion weighted spin echo-planar imaging sequence (TR/TE= 89.7/12200 ms, slice thickness 3 mm, 50 contiguous slices, maximum b-value=1000 s/mm2 in 30 optimized noncollinear directions; one volume was acquired without diffusion weighting). All images were anonymized; radiological assessment was performed by a blinded radiologist; subsequent DTI and structural image analysis was performed by a blinded operator through an automated analysis protocol. Conventional MRI on T1 and AX DP/T2 scans were used to assess the presence of macroscopic brain damage, presence of WM hyperintensity load, lacunae or other pathological alterations. A negative radiological result was considered an indispensable criterion to be eligible and the inclusion cut-off used for vascular-related damage was set at a Fazekas score ≤ 1. Diffusion images were processed using the standardized pipeline with the DTIfit tool. After pre-processing, analysis was performed using the PROBTRACK tool of probabilistic Bayesian Framework for tractography. PROBTRACK exploits a diffusion model estimated by BEDPOSTX, a tool for probabilistic diffusion parameters estimation capable of modelling crossing fibers. A standard tractography pipeline has been implemented using the plugin AutoPTX, following parameters and quality check procedures as described (**de Groot et al. 2016**). Tracts were categorized in associative, projection, limbic and callosal. Diffusion parameters were quantified with FA, MD, AD, and RD. The tract segmentation was achieved by thresholding the normalized tract density images. The threshold was selected according to the FA reproducibility criterion (**de Groot et al. 2015**), segmented tract volumes were computed to exclude WM tract atrophies. FA, MD, AD and RD were averaged for voxels inside tract segmentations. All tools were available from FSL Suite (version 5.0.8) (**Jenkinson et al. 2012; Woolrich et al. 2009; Smith 2002**). The analysis of brain cortex assessed cortical thinning or atrophy (exclusion criteria for the study) and was performed with FSL-VBM (Voxel based morphometry) (**Douaud et al. 2007**), an optimized VBM protocol (**Good et al. 2001**) carried out with FSL tools (**Smith 2002**). First, structural images were brain-extracted and grey matter-segmented before being registered to the MNI152 standard space using non-linear registration (**Andersonn et al. 2007**). Second, all native grey matter images were non-linearly registered to this study-specific template and "modulated" to correct for local expansion (or contraction) due to the non-linear component of the spatial transformation. The modulated grey matter images were then smoothed with an isotropic

Gaussian kernel with a sigma of 3 mm. Finally, voxelwise General Linear Model (GLM) was applied using permutation-based non-parametric testing, correcting for multiple comparisons across space with significance threshold of corrected p-value ($p < 0.05$).

2.2.2 Hypertension Diagnosis and Blood Pressure Measurement

Hypertension diagnosis was performed based on a systolic blood pressure higher than 140 mmHg, a diastolic blood pressure higher than 90 mmHg or they were previously diagnosed with hypertension and treated with anti-hypertensive drugs. BP was measured in sitting position after 5 minutes of rest, using an automated validated device (OMRON-705IT) with an appropriately sized cuff on the dominant arm. consecutive measurements were taken at 2 minutes apart and average values were recorded.

2.2.3 Ultrasonographic Analyses for Cardiac and Vascular Imaging

The overall 1-dimensional left ventricle (LV) measurements and the 2-dimensional views were obtained following the American Society of Echocardiography guidelines (**de Simone et al. 1992**). Left Ventricular Mass (LVM) was calculated using the Devereux's formula and normalized for the height elevated to 2.7 (indexed LVMI). Hypertrophic remodeling was diagnosed when LVMI>50 g/m2.7 (**de Simone et al. 1992; Landolfi et al. 2016**). Then we calculated Relative Wall Thickness (RWT) at end-diastole as 2 PWT/LVIDD. Diastolic function was assessed as the ratio of early to late ventricular filling and patients were categorized as diastolic dysfunction with an E/A ration < 1 (**Figure 17**).

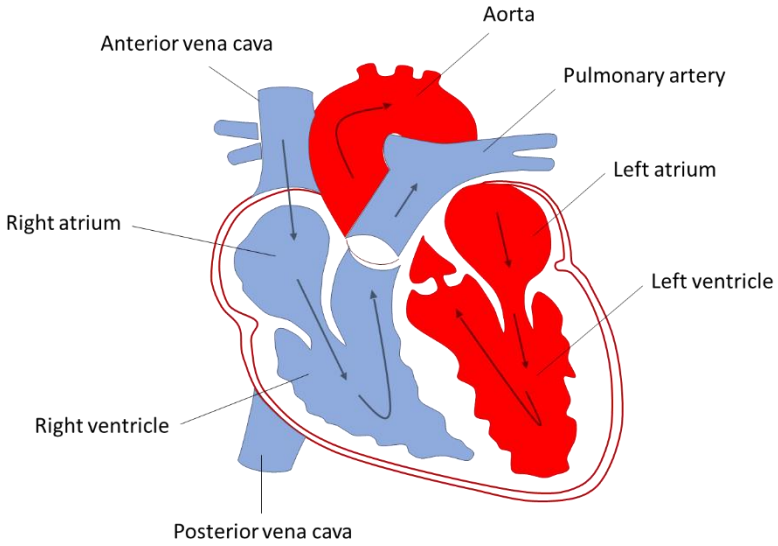


Figure 17. Representation of the heart, showing the main elements: atrium, ventricles, valves and arteries. The arrows show the normal direction of blood flow.

The measures of intima media thickness (IMT) (**Figure 18**) were obtained at the distal 1.0 cm below the carotid bifurcation in common carotid and at the proximal 1.0 cm of the internal carotid artery (**Howard et al. 1993; Espeland et al. 1999; Engelen et al. 2013**). Two blinded cardiologists reviewed the ultrasonographic analyses, to assess cardiac and vascular remodeling.

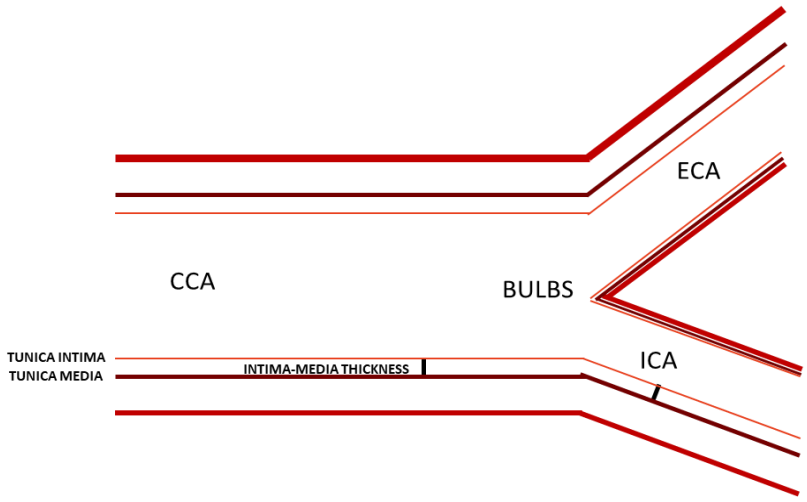


Figure 18. Image of carotid artery where the common carotid artery (CC), the bifurcation (Bulbs), the internal carotid artery (ICA) and the external carotid artery

(ECA) are easily identifiable. The measure of intima-media thickness (IMT) is reported.

2.2.4 Renal Function

Renal function was assessed by evaluating serum creatinine levels, albuminuria and estimated glomerular filtration rate (eGFR) through standardized procedures.

2.2.5 Cognitive Assessment

Patients underwent cognitive assessment administered by a certified psychologist, blinded to the clinical condition. The Instrumental Activities of Daily Living (IADL) test was used to assess potential impairment of daily life activities, by using a scale that measures emotional, cognitive, and physical functions (**Lawton and Brody, 1969**). Score ranges were from 0 (low function, dependent) to 8 (high function, independent), with a cut off value set at 8 for females and 5 for males. Normal performance on this test was considered an indispensable criterion to exclude severe dysfunction in personal autonomy. MoCA battery of tests was used as the gold standard neuropsychological evaluation in cerebrovascular diseases (**Nasreddine et al. 2005; Webb et al. 2014; Santangelo et al. 2015**). According to validation studies for patients with vascular dementia (**Nasreddine et al. 2005; Freitas et al. 2012**), patients who reported a MoCA inferior to 17 were considered demented and consequently excluded from the study protocol. Subdomains of MoCA were categorized according to normative data and were defined as following: visuospatial, executive functions, language, attention and memory. In more detail, as said before (see Par. 1.2.4), executive functions and visuospatial abilities were measured through a brief TMT B version test, copy of a cube and clock drawing; naming is tested through nomination of three animals; memory trial is divided in immediate and delayed recall and it consists in memorizing five words and in retaining them at the end of the other MoCA's trials. Attention tasks contain one string digit span forward (five numbers) and backward (three numbers), a brief double task test and a serial 7 subtraction. Language is tested by two sentences repetition and by phonemic verbal fluency task. Finally, they were assessed abstraction asking object similarities and orientation through questions about date and location. In this way MoCA test offers the possibility to perform further analyses (as discussed in Results Section).

Verbal paired-associate learning test was administered to evaluate subjects' anterograde learning, cut off range is ≥ 8.73 (**Squire and Shimamura 1986**). Semantic Verbal Fluency Test was used to assess the capability to access to semantic store through working memory. Correction grid considers a normal performance patient with a corrected score ≥37 (**Spinnler and Tognoni 1987**). Lastly Stroop Test was administered to patients to evaluate the ability of interfering stimuli interdiction. There's a cut-off for performance time and a cut-off for subject's errors (36.92 and 4.24 respectively) (**Caffarra et al. 2002**).
The execution of all the neuropsychological tests was completed always in the same order and they allowed to obtain a complete profiling of subjects' cognitive functions (**Table 3**).

TEST ADMINISTERED	**COGNITIVE FUNCTIONS**	**CUT-OFF/ C.S.**	**E.S.**
IADL (Lawton et al. 1989)	Emotional, Cognitive, and Physical Impairments' Functional Impact on Daily Activities.	8 F 5 M	-
Montreal Cognitive Assessment MoCA (Nasreddine et al., 2005)	General Cognitive Profile.	26	0-4
Semantic Verbal Fluency Test (Spinnler&Tognoni, 1987)	Anterograde Learning.	8.73	0-4
Verbal Paired-Associate Learning Test (Squire &Shimamura, 1986)	Access to Semantic Store Through Working Memory.	37	0-4
Stroop Test (Caffarra et al., 2002)	Evaluates the Ability of Interdiction of Interfering Stimuli. There is a Performance Time and a Performance for Subject's Errors.	4.24 36.92 S	0-4 0-4

Table 3. Overview of the cognitive procedure completed during the research protocol. C.S. = Corrected Score; E.S. = Equivalent Score.

2.3 Statistics

Based on preliminary data and previous studies assessing the impact of cardiovascular risk factors on cognitive functions (**Arntzen et al. 2001; Joosten et al. 2013**) and hypothesizing a predicted difference in MoCA score of 4 corrected points between mean values obtained for normotensive (NT) and hypertensive patients (HT), we calculated the power analysis for the study. Our analysis estimated to enroll at least 13 cases for each group to achieve an

alpha of 0.05 and a beta power of 0.90. Depending on the type of variables, characteristics of patients were presented as numbers (%) or means ± standard deviation (SD). Shapiro–Wilk Test was used to assess the normality of continuous variables. Comparisons between NT and HT were performed using t-test for independent samples for continuous variables or Mann-Whitney test for non-normally distributed variables. Categorical data were compared using Fisher exact Chi-Square test. Multivariate analysis was performed to assess the influence of relevant covariates in the significant differences emerged in the univariate analysis. Bivariate correlation analyses were conducted with Pearson correlation. When specified and accordingly to the datasets, partial correlation analyses or linear regressions were performed to assess the possibility that relevant covariates could influence the significant results observed. All the analyses were performed with SPSS (version 23; IBM). $P < .05$ was considered statistically significant.

CHAPTER 3. RESULTS

3.1 General characteristics of subjects and hypertension-induced target organ damage

In order to stage the level of hypertensive disease's progression, recruited patients with general characteristics showed in **Table 4** were subjected to the evaluation of typical signs of target organ damage due to chronic high BP. All the HT patients arriving at our observation were already on anti-hypertensive therapy, as listed in **Table 5**. Estimated duration of hypertensive condition was also recorded, accordingly to anamnestic history of patients (**Table 5**). At echocardiography, HT displayed signatures of hypertrophic remodeling, evidenced by increased thickness of LV walls, LVMI2.7 and RWT (**Table 4**). Hypertensive patients showed a significant diastolic dysfunction, even though the preserved cardiac ejection fraction (EF) observed in HT was indicative of an adaptive remodeling without loss of contractility, thus excluding organ failure (**Table 4**). In measuring carotid arteries' IMT, HT displayed a moderate wall thickening (**Table 4**), usually reflecting a status of chronic increased BP (**Eikendal et al. 2015**), reaching the significance only for the left internal carotid artery. On the other hand, the absence of atherosclerotic plaques excluded obstructive diseases that could compromise cerebrovascular functioning. Lastly, we evaluated renal function and observed that patients with hypertension had no alterations in microalbuminuria, serum creatinine, and eGFR (**Table 4**).

Sample Characteristics	NT n= 19	HT n=23	p value
Demographic			
Age. mean (SD)	52 (8)	55 (7)	0.272
Sex. number of females (percentage)	11 (57.86%)	11 (47.82 %)	0.527
Smokers. number (percentage)	3 (15.78%)	4 (17.39%)	0.893
BMI. mean (SD)	26 (4.9)	30.2 (4.5)	**<0.01
Blood Pressure			
Systolic Blood Pressure - mmHg. mean (SD)	122 (10.02)	138 (10.11)	***<0.001
Diastolic Blood Pressure - mmHg. Mean (SD)	77 (6.22)	87 (9.01)	***<0.001
Cardiac Remodeling			
LV end-diastolic diameter - mm. mean (SD)	4.86 (0.30)	5.04 (0.45)	0. 777
IV septal thickness- mm. mean (SD)	0.91 (0.12)	1.14 (0.14)	***<0.001
LV posterior wall thickness- mm. mean (SD)	0.90 (0.13)	1.10 (0.13)	***<0.001
LV mass index (LVMI2.7) - g/m2. mean (SD)	37.85 (8.88)	55.29 (9.65)	***<0.001
Relative wall thickness - RWT. mean (SD)	0.37 (0.04)	0.44 (0.05)	***<0.001
Dyastolic disfunction – E/A'<1 (percentage)	4 (21%)	17 (73%)	***<0.001
LV Ejection fraction - %. mean (SD)	65.47 (6.03)	67.43 (7.26)	0.276
Carotid Arterial Thickening			
Internal Carotid Artery (right) - IMT. mean (SD)	0.74 (0.17)	0.86 (0.21)	0.052
Common Carotid Artery (right) - IMT. mean (SD)	0.80 (0.13)	0.88 (0.12)	0.059
Internal Carotid Artery (left) - IMT. mean (SD)	0.76 (0.16)	0.87 (0.19)	*<0.05
Common Carotid Artery (left) - IMT. mean (SD)	0.79 (0.13)	0.87 (0.23)	0.088
Renal Damage			
Creatinine - mg/dL. mean (SD)	0.74 (0.17)	0.76 (0.15)	0.343
Albuminuria - mg/24hrs. mean (SD)	11.56 (15.67)	16.02 (16.63)	0.250
Estimated GFR – mL/min. mean (SD)	115.24 (34.00)	124.65 (39.39)	0.383
Cognitive Assessment			
IADL – score. mean (SD)	7.7 (0.73)	7.65 (0.71)	0.618
MoCA – score, mean (SD)	26.00 (2.35)	22.08 (2.60)	***<0.001
Semantic Verbal Fluency – score. mean (SD)	49.47 (11.6)	44.08 (11.5)	0.129
Paired-Associate Learning – score. mean (SD)	13.78 (3.9)	9.6 (4.9)	**<0.01
Stroop Color Word Test – score. mean (SD)	0.21 (0.6)	0.85 (1.4)	*<0.05
Stroop Interference Test – time. mean (SD)	16.45 (7.73)	25.5 (11.04)	***<0.001

Table 4. General characteristics of population under investigation and clinical assessment evidencing a mild peripheral organ damage in HT. General characteristics of patients' sample under investigation are listed in the Table, together with BP data. Evaluation of cardiac and vascular remodeling revealed a moderate

hypertrophy induced by hypertension. Assessment of renal function evidenced no sign of organ failure. Evaluation of general cognitive performance obtained by administration of the following tests: IADL (Instrumental Activities of Daily Living), MoCA battery, Semantic Verbal Fluency, Paired-Associate Learning, Stroop Color Test.

Hypertensive patients	**HT n=23**
HTN duration and therapies	
Age. mean (SD) Estimated HTN duration (years) mean (SD)	6.13 (3.73)
β-Blocker - number (percentage)	6 (26.0%)
ACE-I/ARBs - number (percentage)	19 (86.36%)
Calcium Blockers - number (percentage)	4 (18.18%)
Diuretics - number (percentage)	9 (40.90%)

Table 5. Characterization of hypertensive patients. List of antihypertensive therapies recorded for patient's admission and estimated duration of hypertensive conditions, on the basis of anamnestic history and drugs' assumption.

3.2 Fiber-tracking highlights a signature of WM microstructural alterations in hypertensive patients.

Accordingly to our inclusion/exclusion criteria, all individuals recruited had no damage at conventional T2-MRI scans, assessed by a blinded neuroradiologist. We further ascertained absence of topical cortical thinning of the grey matter, which could negatively impact on cognitive function, independently of WM damage. To unravel this issue, we performed VBM analysis on T1-scan that showed normal grey matter, with no significant cluster of differences between NT and HT individuals, with lowest p-value equal to 0.274 across the volume (**Figure 19**). Additionally, we checked whether the WM tracts segmented by fiber-tracking DTI-MRI had any difference in the size, by evaluating single tracts' volumes. No difference between HT and NT emerged, meaning that no tract evidenced malformation or atrophy (**Table 6**).

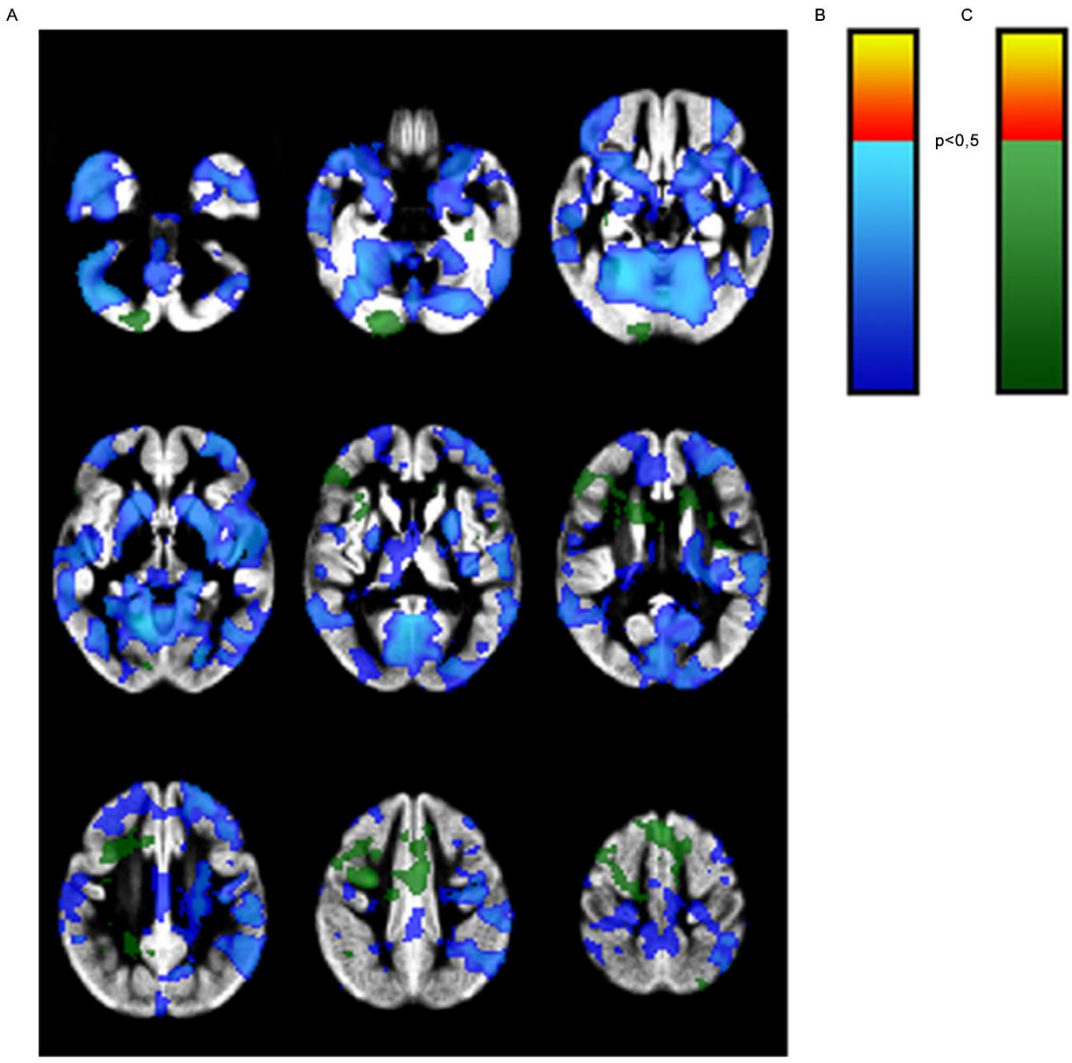


Figure 19. Voxel-based Morphometric Analysis of Grey Matter. (A) Voxel-Based Morphometric (VBM) Analysis of the gray matter in normotensive (NT) and hypertensive (HT) subjects showing in a colour-code the regions of brain cortex where (B) NT differed from HT (blue scale for non-significant differences – red scale for significant differences) and where (C) HT differed from HT (green scale for non-significant differences – red scale for significant differences). No cluster of voxels showed significant difference, being the lowest p-value = 0.274.

Tract Volume	NT	HT	p value
Associative			
SLF R	22631 (2692)	21725 (2666)	0.282
SLF L	21518 (3022)	20908 (2022)	0.439
ILF R	7864 (533)	7698 (726)	0.413
ILF L	7833 (919)	7797 (536)	0.879
IFO R	4389 (450)	4262 (463)	0.376
IFO L	4162 (379)	4131 (430)	0.805
UNC R	2653 (367)	2552 (295)	0.329
UNC L	2495 (307)	2546 (434)	0.667
Limbic			
CGC R	1449 (445)	1396 (413)	0.693
CGC L	1424 (323)	1408 (369)	0.878
CGH R	946 (119)	920 (111)	0.693
CGH L	944 (150)	903 (143)	0.750
Projection			
CST R	7690 (613)	7482 (1258)	0.950
CST L	7534 (723)	7568 (1164)	0.908
AR R	2626 (930)	3006 (1061)	0.230
AR L	2618 (672)	3012 (833)	0.193
ATR R	12037 (1242)	11579 (1372)	0.268
ATR L	12103 (1262)	11926 (1464)	0.681
STR R	6471 (380)	6293 (624)	0.264
STR L	6473 (546)	6479 (547)	0.971
PTR R	5341 (627)	5276 (601)	0.734
PTR L	5562 (731)	5299 (580)	0.201
Callosal			
FMI	2097 (503)	2235 (453)	0.359
FMA	9502 (977)	9019 (1027)	0.129

Table 6. Average tract-specific volume measurements obtained by the probabilistic tractography segmentation. Tract volumes are reported for fiber-tracts grouped as follows: Association Fibers (left and right SLF, ILF, IFO, UNC), Limbic System (left and right CGC, CGH), Projection fibers (left and right CST, AR, ATR, STR, PTR), and Callosal fibers (FMI and FMA) [see Abbreviations Section].

When we assessed the diffusion parameters of FA, MD, AD, and RD of the WM tracts segmented by fiber-tracking DTI-MRI, we revealed significant differences between HT and NT patients in specific tracts (**Figure 20 and Table 7**). Concomitant FA and MD variations are usually considered for extrapolation of specific tracts' alterations. In brief, lower FA indicates disorganized fascicles, affected by microstructural processes such as

demyelination, axonal degradation, or gliosis (**Alexander et al. 2007**). MD is a more sensitive measure even though less specific, and it results increased by pathological processes affecting neuronal membranes (**Alexander et al. 2007**). We observed a specific pattern of alterations in the WM fiber tracts of HT as compared to NT (**Figure 20 and Table 7**). In particular, HT showed a significant deterioration of WM connections in projection fibers of the Right Anterior Thalamic Radiation (r-ATR) (**Figure 20A**), association fibers of the Right Superior Longitudinal Fasciculus (r-SLF) (**Figure 20B**) and callosal fibers of the Forceps Minor (FMI) (**Figure 20C**).

	FA		MD		AD		RD	
Associative	**NT**	**HT**	**NT**	**HT**	**NT**	**HT**	**NT**	**HT**
SLF R	0.39 (0.03)	0.36 (0.02)*	0.77 (0.03)	0.81(0.04)***	1.10 (0.03)	1.13(0.04)**	0.61 (0.04)	0.65 (0.04)**
SLF L	0.39 (0.03)	0.39 (0.02)	0.76 (0.03)	0.80 (0.04)**	1.12 (0.03)	1.14 (0.04)*	0.61 (0.03)	0.63 (0.04)*
ILF R	0.39 (0.02)	0.38 (0.02)	0.86 (0.02)	0.87 (0.04)	1.24 (0.03)	1.25 (0.03)	0.66 (0.03)	0.68 (0.04)
ILF L	0.39 (0.02)	0.39 (0.02)	0.86 (0.02)	0.87 (0.03)	1.25 (0.03)	1.26 (0.03)	0.66 (0.02)	0.68 (0.04)
IFO R	0.40 (0.02)	0.39 (0.02)	0.85 (0.02)	0.88 (0.04)*	1.24 (0.05)	1.27 (0.04)	0.66 (0.02)	0.69 (0.04)
IFO L	0.40 (0.02)	0.39 (0.03)	0.86 (0.02)	0.88 (0.04)	1.25 (0.04)	1.28 (0.05)	0.67 (0.02)	0.69 (0.05)*
UNC R	0.33 (0.02)	0.32 (0.02)	0.89 (0.04)	0.93 (0.05)**	1.22 (0.05)	1.25 (0.05)*	0.73 (0.04)	0.77 (0.05)**
UNC L	0.33 (0.03)	0.33 (0.02)	0.88 (0.04)	0.91 (0.06)*	1.20 (0.05)	1.24 (0.06)*	0.73 (0.05)	0.75 (0.06)
Limbic								
CGC R	0.35 (0.03)	0.34 (0.02)	0.81 (0.03)	0.84 (0.04)*	1.12 (0.05)	1.15 (0.05)	0.65 (0.03)	0.68 (0.04)**
CGC L	0.39 (0.04)	0.37 (0.03)	0.81 (0.03)	0.83 (0.05)	1.17 (0.05)	1.18 (0.05)	0.63 (0.04)	0.66 (0.05)*
CGH R	0.23 (0.02)	0.22 (0.02)	0.99 (0.04)	1.03 (0.06)*	1.23 (0.05)	1.26 (0.07)	0.88 (0.04)	0.92 (0.06)*
CGH L	0.23 (0.02)	0.21 (0.03)	0.99 (0.06)	1.02 (0.10)	1.22 (0.07)	1.24 (0.10)	0.88 (0.06)	0.92 (0.10)
Projection								
CST R	0.45 (0.02)	0.44 (0.03)	0.89 (0.05)	0.90 (0.06)	1.33 (0.06)	1.33 (0.06)	0.67 (0.06)	0.68 (0.06)
CST L	0.44 (0.03)	0.44 (0.03)	0.90 (0.06)	0.89 (0.06)	1.33 (0.06)	1.32 (0.05)	0.68 (0.07)	0.68 (0.06)
AR R	0.30 (0.02)	0.30 (0.02)	0.93 (0.07)	0.98 (0.07)	1.22 (0.08)	1.27 (0.07)*	0.79 (0.07)	0.83 (0.07)
AR L	0.29 (0.02)	0.30 (0.02)	0.95 (0.06)	0.97 (0.06)	1.22 (0.06)	1.27 (0.07)	0.81 (0.06)	0.83 (0.06)
ATR R	0.36 (0.02)	0.34 (0.02)**	0.86 (0.03)	0.91 (0.07)**	1.19 (0.04)	1.24 (0.08)*	0.69 (0.04)	0.74 (0.06)**
ATR L	0.36 (0.02)	0.35 (0.02)	0.86 (0.04)	0.89 (0.05)	1.19 (0.05)	1.23 (0.05)*	0.69 (0.04)	0.72 (0.05)*
STR R	0.38 (0.02)	0.37 (0.02)	0.82 (0.05)	0.83 (0.06)	1.16 (0.04)	1.17 (0.07)	0.64 (0.05)	0.66 (0.06)
STR L	0.39 (0.02)	0.38 (0.02)	0.81 (0.04)	0.82 (0.05)	1.15 (0.03)	1.16 (0.05)	0.64 (0.04)	0.65 (0.05)
PTR R	0.37 (0.02)	0.36 (0.03)	0.88 (0.04)	0.88 (0.04)	1.24 (0.05)	1.24 (0.05)	0.70 (0.04)	0.70 (0.04)
PTR L	0.37 (0.02)	0.37 (0.02)	0.89 (0.04)	0.90 (0.04)	1.26 (0.06)	1.26 (0.04)	0.71(0.04)	0.71 (0.05)
Callosal								
FMI	0.47 (0.02)	0.45 (0.02)*	0.87 (0.04)	0.91 (0.04)**	1.37 (0.07)	1.41 (0.05)*	0.62 (0.04)	0.66 (0.04)**
FMA	0.45 (0.03)	0.45 (0.03)	1.04 (0.08)	0.99 (0.07)*	1.57 (0.09)	1.52 (0.08)	0.78 (0.08)	0.73 (0.07)

Table 7. Average tract-specific measurements of DTI parameters obtained by probabilistic tractography segmentation. FA (fractional anisotropy), MD (mean diffusivity), RD (radial diffusivity) and AD (axial diffusivity) are reported for the following fiber-tracts: Association Fibers (left and right SLF, ILF, IFO, UNC), Limbic System (left and right CGC, CGH), Projection Fibers (left and right CST, AR, ATR, STR, PTR), and Callosal Fibers (FMI and FMA).

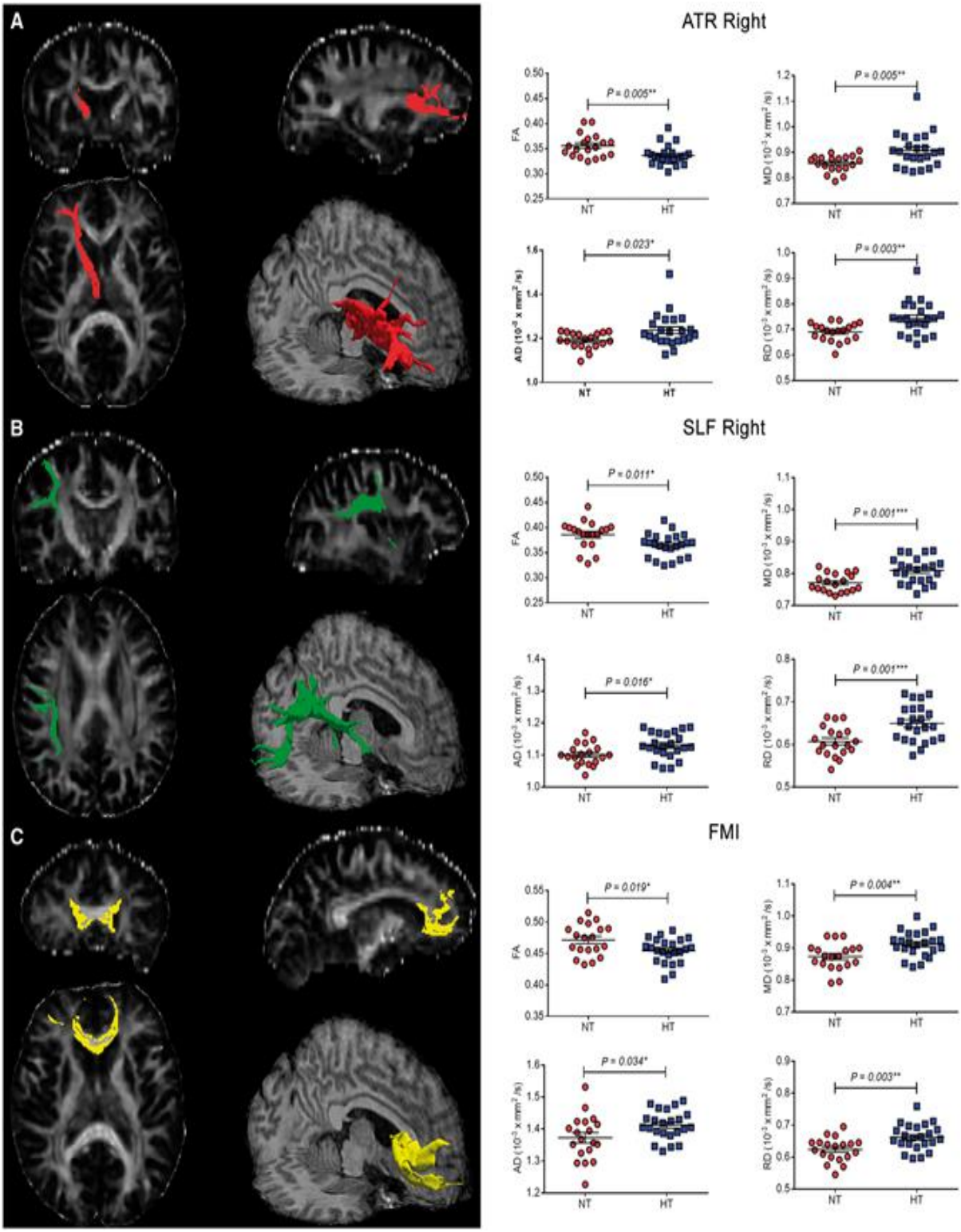

A
B
C
ATR Right
SLF Right
FMI
FA
MD (10⁻³ x mm²/s)
AD (10⁻³ x mm²/s)
RD (10⁻³ x mm²/s)
NT
HT
P = 0.005**
P = 0.005**
P = 0.023*
P = 0.003**
P = 0.011*
P = 0.001***
P = 0.016*
P = 0.001***
P = 0.019*
P = 0.004**
P = 0.034*
P = 0.003**

Figure 20. Tractographic reconstruction of relevant WM tracts altered in hypertensive patients. Coronal, Sagittal and Axial projections were obtained with a 3D rendering of reconstructed regions. (A-D) A representative tractographic reconstruction of the Right Anterior Thalamic Radiation (A), Right Superior Longitudinal Fasciculus (B) and Forceps Minor (C) are shown on the left. Graphs on the right report the individual mean values of FA, MD, AD, RD assessed for each specific WM tract.

Thus, over the whole WM tracts analyzed (**Figure 21A**), HT patients displayed a significant decrease in FA and concomitant increase in MD in the above described specific tracts (i.e. ATR, SLF and FMI), resulting in the signature represented in **Figure 21B**. Interestingly, while concurrent alterations of FA and MD were displayed only by right WM bundles of the specific tracts, MD alone revealed a damage progressing in the contralateral side (**Table 7**). In addition, AD and RD DTI parameters were considered, to take into account the potential impact of different neural mechanisms on WM abnormalities. Conventionally, incremental variations in RD are associated with myelin breakdown (**Alexander et al. 2007**), whereas in AD describe secondary processes of axon degeneration (**Alexander et al. 2007**). Our data also report a significant alteration of RD and AD in the tracts of interest (**Table 7**). Though showing no significant difference between the two categories of subjects, age is a known factor affecting diffusion parameters of WM (**Kennedy et al. 2009**). In order to test whether the significant differences between HT and NT patients emerged in the r-ATR, r-SLF, and FMI WM tracts were affected by aging, we performed a multivariate analysis with age as covariate. The difference in DTI parameters emerged in the univariate between HT and NT subjects was still significant in the ANCOVA, independently of age (**Table 8**). To further exclude that other potentially influencing covariates could interfere with the effects of hypertension on DTI parameters, we also tested gender, smoke and BMI by ANCOVA. None of these factors influenced the significant difference between HT and NT for the WM tracts reported in the univariate analysis (**Table 8**). More important, when we tested the potential influence of some anti-hypertensive drugs on the observed effects, we did not find any significant interference for none of the medications used (**Table 9**).

Parameter – Tract - Covariate	Type III Sum of Squares	Df	Mean Square	F	p value
FA – ATR R – age	0.003	1	0.003	6.899	<0.05*
FA – SLF R – age	0.003	1	0.003	5.689	<0.05*
FA – FMI – age	0.002	1	0.002	4.464	<0.05*
FA – ATR R – sex	0.004	1	0.004	8.574	<0.01**
FA – SLF R – sex	0.005	1	0.005	7.495	<0.01**
FA – FMI – sex	0.003	1	0.003	6.641	<0.05*
FA – ATR R – smoke	0.004	1	0.004	8.474	<0.01**
FA – SLF R – smoke	0.005	1	0.005	7.091	<0.05*
FA – FMI – smoke	0.003	1	0.003	5.811	<0.05*
FA – ATR R – BMI	0.004	1	0.004	7.805	<0.01**
FA – SLF R – BMI	0.006	1	0.006	10.563	<0.01**
FA – FMI – BMI	0.005	1	0.005	10.617	<0.01**

Table 8. R-ATR, R-SLF and FMI are significantly reduced in hypertensive patients, even when adjusting for age, sex, smoke and BMI. ANCOVA was performed to test covariates that could potentially influence as confounding factors the differences in WM tracts observed between hypertensive patients and normotensive subjects.

Antihypertensive Drugs	B	Std. Error	Beta	t	p value
FA – ATR R					
β-Blocker	0.05	0.010	0.075	0.491	0.327
Calcium blocker	-0.021	0.012	-0.266	-1.705	0.097
ACE-I/ARBs	-0.013	0.010	-0.274	-1.254	0.218
Diuretic	0.001	0.001	-0.132	0.006	0.995
FA – SLF R					
β-Blocker	-0.003	0.012	-0.042	-0.266	0.792
Calcium blocker	-0.029	0.015	-0.313	-1.952	0.059
ACE-I/ARBs	-0.011	0.012	-0.209	-0.933	0.357
Diuretic	-0.015	0.013	-0.224	-1.173	0.248
FA – FMI					
β-Blocker	0.015	0.010	0.228	1.488	0.145
Calcium blocker	-0.006	0.012	-0.070	-0.446	0.658
ACE-I/ARBs	-0.013	0.010	-0.280	-1.281	0.208
Diuretic	0.003	0.011	-0.061	-0.327	0.746

Table 9. None of the antihypertensive drugs assumed by hypertensive patients had any influence on the variations observed in Fractional Anisotropy of WM tracts identified in the signature. Table reports the linear regression analysis considering the different classes of antihypertensive drugs recorded in the anamnestic therapy of patients at the admission in the FA of r-ATR, r-SLF, r-CGC and FMI.

3.3 Hypertensive patients showed a specific pattern of cognitive alterations

Cognitive assessment began with the administration of IADL test for all individuals. Each patient showed normal performance on this test (**Table 4**) and, hence, was subjected to MoCA test (**Webb et al. 2014)**. HT displayed significantly impaired performance on MoCA, as compared to NT (**Table 4**). Further analysis of specific cognitive subdomains tested by the MoCA revealed significantly impaired memory, executive functions, attention and language domains (**Table 10**). The significantly worse score reported by HT patients in the verbal paired-associate learning revealed difficulties in retaining new information through working memory (**Table 4**). On the Stroop Test, HT did not perform as well as NT, thus indicating that hypertension negatively affects executive functions (**Table 4**). The Semantic

Verbal Fluency Test showed comparable performance in HT and NT, thereby indicating no alteration in language abilities that could affect performance on other tasks (**Table 4**).

MoCA Cognitive Subdomains	NT	HT	p value
Visuospatial			
Score, mean (SD)	3.3 (0.93)	3.1 (1.18)	0.922
Executive Functions			
Score, mean (SD)	3.6 (0.77)	2.6 (1.16)	**<0.01
Language			
Score, mean (SD)	5.4 (0.76)	4.8 (0.78)	*<0.05
Attention			
Score, mean (SD)	5.3 (1.15)	4.6 (1.34)	*<0.05
Memory			
Score, mean (SD)	2.8 (1.68)	1.5 (1.27)	**<0.01

Table 10. Hypertensive patients had a significantly reduced score in the general cognitive assessment obtained by MoCA test, and particularly in executive and memory functions. Table reporting the score values obtained in the subdomains assessed by MoCA test. Data are reported as mean values and SD.

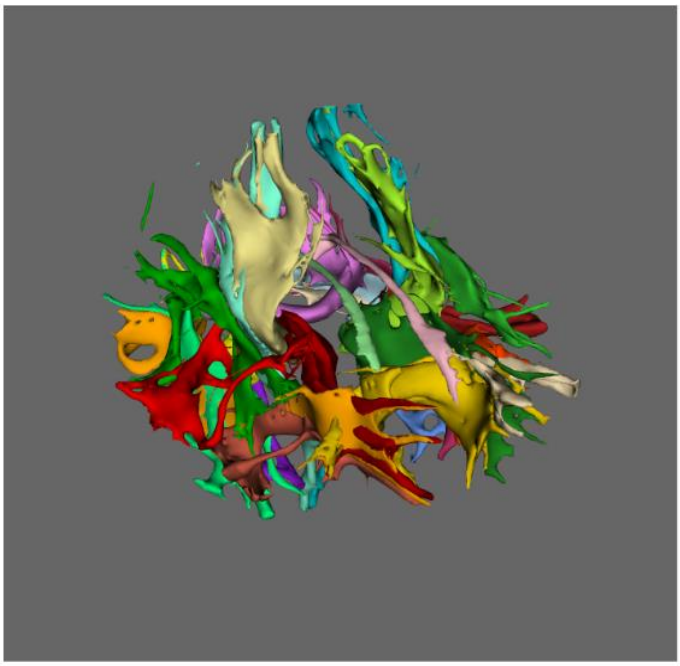
3.4 WM microstructural alterations scale with cognitive impairment and target organ damage.

In the end, we generated correlation models among microstructural WM alterations, cardiac remodeling, hypertensive condition and cognitive profile. We found a significant positive correlation between MoCA scores and FA of the projection and association fibers (ATR and SLF) (**Figure 21C and 21D**). FA values of the same projection and association fibers negatively correlated with the estimated duration of hypertension (**Figure 21E**) as MoCA scores did (**Figure 21F**). It is interesting to notice that the sample of hypertensive patients (n=18), excluded because of already manifest neurological damage, had a significantly longer estimated duration of hypertension when compared to the group of included patients (mean ± SD = 9.61 ± 5.57 vs 6.13 ± 3.73 years; *$p < 0.05$), thus suggesting that with disease advancement, brain damage progressively evolve toward an increasingly manifest injury. Further supporting this hypothesis, both the included and excluded patients had comparable blood pressure control (data not shown). Hence, in order to test whether the observed

correlations between DTI parameters and cognitive scores was modulated by hypertensive condition, we also performed a partial correlation analyses controlling for SBP and years of hypertensive conditions. There were no significant influences of SBP levels and overall duration of hypertension in the correlation observed neither between MoCA and FA r-ATR (SBP: df(39) = .239, n = 42, p = .132; years of hypertension: df(39) = .199, n = 42, p = .213) nor between MoCA and FA r-SLF (SBP: df(39) = .287, n = 39, p = .069; years of hypertension: df(39) = .276, n = 42, p = .081). Since altered FMI may be involved in impaired processing speed tasks (**Duering et al. 2011**), we tested the correlation between Stroop interference time, Stroop test errors and FMI-FA, finding a significant relationship suggestive of an impact of hypertension in inhibiting interfering stimuli, represented by the time performance (**Figure 21G and 21H**). Even the correlation observed between FA-FMI and Stroop Interference Time was controlled for the interaction with both SBP, duration of hypertension and age, given that this latter parameter emerged as influencing the deterioration of FA-FMI observed in HT. Interestingly, while there was a statistically significant negative partial correlation between Stroop Interference Time and FA-FMI whilst controlling for SBP (df(39) = -.321, n = 39, p = .040) no effect was observed for overall duration of hypertension (df(39) = -.282, n = 42, p = .074) or when controlling for age (df(39) = -.126, n = 42, p = 23.431). When controlling the correlation between Stroop test errors and FMI-FA for the same parameters it emerged a significant negative correlation when controlling for overall duration of hypertension (df(39) = -.312, n = 42, p = .047) while no effect were observed controlling for SBP (df(39) = -.300, n = 39, p = .057) or when controlling for age (df(39) = -.196, n = 42, p = .219). In the end, the significant negative correlation observed between indexed LVMI and MoCA (Figure 3I) revealed parallel progression of early cognitive alterations and initial peripheral organ damage. (Moazzami et al. 2018)

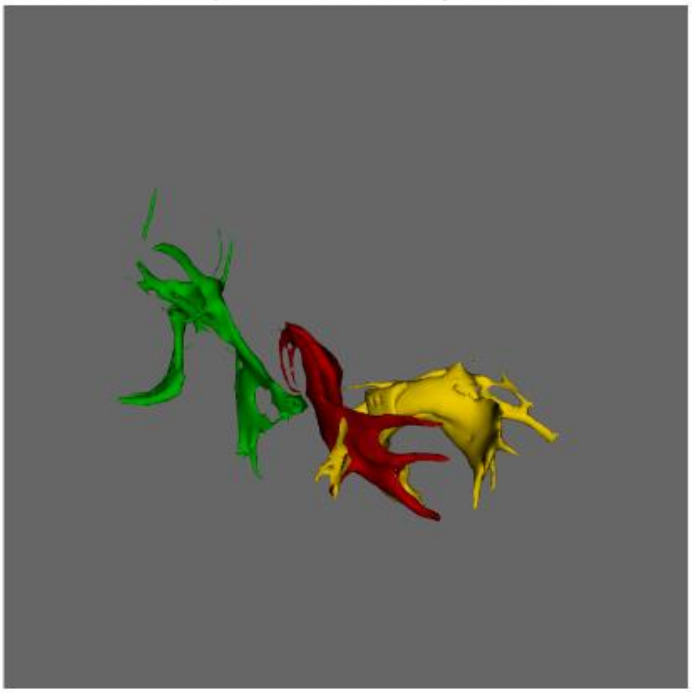
A

Reconstruction of main white matter tracts



B

Tracts with impaired diffusivity in HT

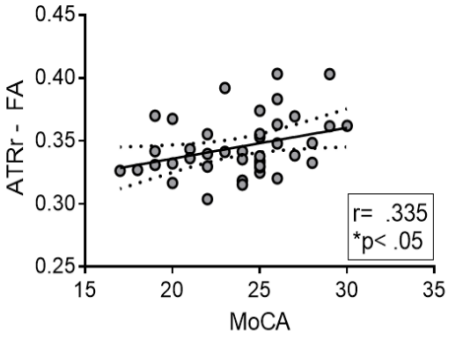


Anterior Thalamic Radiation

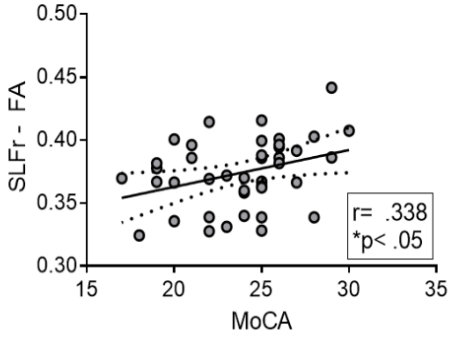
Superior Longitudinal Fasciculus

Forceps Minor

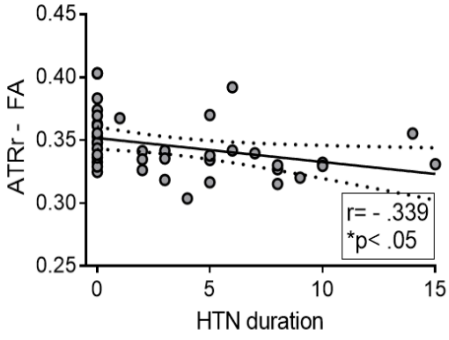
C


ATRr - FA
MoCA
r= .335
*p< .05

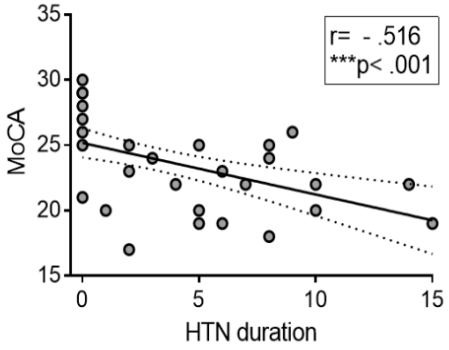
D


SLFr - FA
MoCA
r= .338
*p< .05

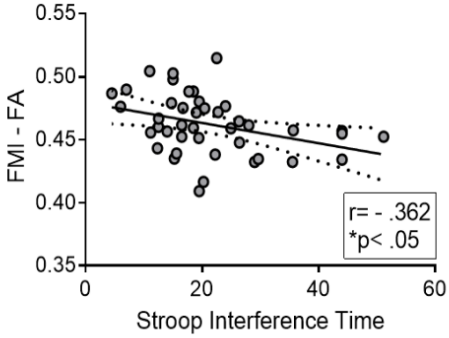
E


ATRr - FA
HTN duration
r= - .339
*p< .05

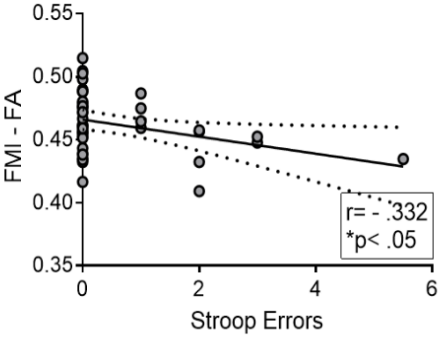
F


MoCA
HTN duration
r= - .516
***p< .001

G


FMI - FA
Stroop Interference Time
r= - .362
*p< .05

H


FMI - FA
Stroop Errors
r= - .332
*p< .05

I

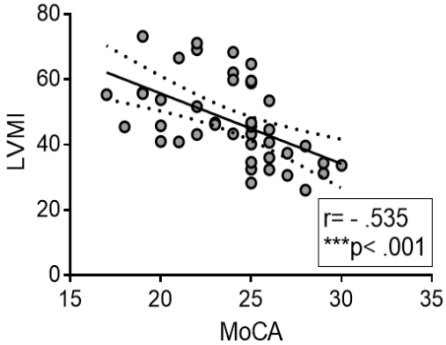

LVMI
MoCA
r= - .535
***p< .001

Figure 21. WM microstructural alterations scale with cognitive functions and target organ damage of hypertension. (A) Global 3D rendering of the overall tracts reconstructed by fiber tracking in all the subjects enrolled. (B) 3 D rendering of the sole tracts showing altered parameters of WM in hypertensive patients (in red ATR, in green SLF, in yellow FMI). (C, D) FA of the specific fiber tracts involved in memory and executive functions, namely the ATR (in C) and the SLF (in D) showed a positive correlation with scores reported at the MoCA test. (E, F) FA of the ATR and score at the MoCA test negatively correlated with the estimated duration of hypertension. (G, H) FA of the FMI, a fiber tract typically involved in processing speed, negatively correlated with Stroop interference time (G) and Stroop errors (H). (I) MoCA performance negatively correlated also with LVMI, index of hypertension peripheral organ damage. Dashed lines indicate 95% CI.

CHAPTER 4. DISCUSSION

This is the first study that highlights how hypertensive patients present microstructural alterations of specific WM tracts, at a stage where no sign of macroscopic structural brain damage was evidenced by conventional methods. The WM abnormalities, displayed in tracts segmented by probabilistic fiber tracking and associated to projection/association fibers and callosum system, were not visible on structural MRI and were associated with impairment of the related cognitive functions, typically prodromal of later dementia. Our results have been obtained in a hypertensive population characterized by the absence of comorbidities and an initial stage of peripheral organ damage, as shown by modest cardiac and vascular hypertrophic remodeling, but no overt sign of end-organ failure. The lack of structural brain injury induced by hypertension makes assessing prodromal signs quite challenging. DTI-MRI revealed itself as an important step toward using advanced neuroimaging as a diagnostic and prognostic biomarker (**Glasser et al 2013; Abhinav et al. 2014**). WM links different functional brain areas through bundles of fibers established between different cortical structures or between sub-cortical areas and the cortex. The fiber bundles could be thus defined, following anatomical projections. Based on this a-priori knowledge of the whole connections in the brain, we can track the WM bundles relating different functional areas and creating a unique tract identification for each subject (**de Groot et al. 2013, Li et al. 2015**). By applying this method, we performed DTI-MRI tractography, probabilistically estimating fiber orientation and number per each voxel, and extracting quantitative diffusion parameters (**Behrens et al. 2003; Behrens et al. 2007**). Despite HT subjects in our study were on anti-hypertensive therapy, the analysis evidenced a specific signature of their brains, characterized by a deterioration of projection fibers (ATR), association fibers (SLF) and callosum (FMI) systems. Interestingly, the injured tracts are related to specific cognitive functions that were concomitantly impaired, as envisaged by performance on cognitive assessment. The ATR is a projection fiber that connects the thalamus's anterior nucleus to the anterior cingulate gyrus and frontal cortex, and ATR damage has been linked to impaired memory (**Kiernan et al. 2013**), as revealed also in our HT patients by poor performance on MoCA test, and specifically in the memory subscale. The SLF, a tract composed of two long bi-directional bundles of neuronal axons, connects the cerebrum parietal, occipital, and temporal lobes with the ipsilateral frontal cortices (**Kiernan et al. 2013**). Characterized primarily as an association fiber, the SLF facilitates the formation of a bidirectional neural

network necessary for cognitive functions such as attention, memory, emotions, and language (**Kiernan et al. 2013**), functions as well impaired in HT under investigation in our study. Lastly, the FMI, also known as the anterior forceps, is a fiber bundle that connects the frontal lobes' lateral and medial surfaces, crosses the midline via the genu of the corpus callosum, and has been linked to processing speed tasks (**Duering et al. 2011**). This latter is some way measured by the results reported in the Stroop interference test, significantly worse in HT. Taken together, the altered WM tracts recapitulate a spatial signature of microstructural alterations that could be prodromal of the brain areas typically reported as affected by lacunar damage or hyperintensities in studies performed in patients at later stages of the disease and subjected to conventional MRI. Interestingly, this pattern of alteration has been extracted from a sample of patients with adequate range of blood pressure control, suggesting that an efficient anti-hypertensive therapy is not enough to exclude the onset of vascular dementia or protect the brain from WM microstructural damage. Further supporting this concept, the sample of patients that we excluded because of macroscopic WM damage evidenced by conventional MRI had a significantly longer estimated duration of hypertensive condition, despite showing ranges of blood pressure control similar to those of the samples of patients included in the study protocol. In addition, it should be further considered that several other risk factors, typically associated with hypertension as well, as aging, increased BMI and smoking, may influence per se WM integrity. The results obtained in the covariate analyses conducted in subjects under investigation in this work, would suggest a negligible role of potential interactions established between hypertension and confounding factors, thus strengthening the relevance of the signature identified. Likely, the strict inclusion/exclusion criteria chosen in this study allowed to study a population of HT as deprived as possible of confounding factors for the analysis of WM alterations. To fully understand which kind of treatment can protect WM and cognitive functions, studies aimed at addressing the mechanisms generating the damage need to be carried on. On this notice, two recent works added important knowledge in this field of research, showing how salt intake can affect cerebral blood flow (**Faraco et al. 2018**) and how pericyte loss can alter the vascular function in WM regions (**Montagne et al. 2018**), suggesting new targets or medical recommendations for adequate protection from hypertension-induced brain damage.

4.1 Conclusions and study limitations

Main strengths of this study consist in: 1) the comprehensive scan protocol executed on a 3T-MRI for the acquisition of high-resolution diffusion parameters of WM tracts obtained by fiber-tract probabilistic analysis, instead of less efficient atlas selections-based protocols; 2) restriction of the analysis to individuals with no evidence of brain damage at conventional MRI nor diagnosis of dementia. Overall these criteria allowed us to reveal early alterations of WM in patients where the conventional diagnostic methods failed to evidence an initial stage of brain injury. At same time, this study also presents some limitations that deserve discussion, in order to place our findings in the appropriate context and pave the way for future studies. First, we acknowledge that the strict inclusion criteria resulted in a quite limited sample size of patients under investigation. Secondly, we have only considered a baseline analysis of these patients, having no chance at this stage of predicting the evolution of the damage identified in HT patients. In conclusion, although larger studies are needed to better define the impact of such WM alterations on the evolution toward dementia, our findings support the possibility that non-invasive tool of advanced brain imaging coupled with cognitive assessment has the potential to provide invaluable information, which could possibly aid in the early prediction of long-term deficits in patients with hypertension. The current trend of personalized treatments calls for the acquisition of more data, to give a wider spectrum of information based not only on clinical assessments but depicting the lifestyle and habits of patients potentially linked to the etiology of hypertension. In future studies clinical, tractographical and cognitive data will be complemented by lifestyle and dietary habits information, in order to tailor an anti-hypertensive treatment on a per patient basis.

4.2 Perspectives

This research has given rise to a new aim, namely to understand how this impairment due to hypertension proceeds over time. As discussed before, thanks to fiber tracking, it is possible to detect early brain specific alterations in hypertensive patients before that these anomalies become visible in cortical structure (**Carnevale et al. 2018**). Several authors have dealt with the need to find prodromal signs or biomarkers for dementia (**Launer et al. 2015**; **Bateman et al. 2012**) but, as stated in Carmichael's work (2014), once found these biomarkers, the fundamental issue is when it should be effective starting prevention therapies. (i. e. an interval during which initial vascular damage processes must be monitored to hinder possible repercussions for the brain and cognition) (**Carmichael 2014**). Discovering at what time cortical brain damage starts under the effects of hypertension (and in which type of patients), could be an invaluable step forward in hindering the onset of later vascular dementia (**O'Brien et al. 2003**).

There are several longitudinal studies that treated the relationship between hypertension or cardiovascular risk factors and dementia (e.g. **Goldstein et al. 2013; Launer et al. 2015; Osone et a. 2015; Yaneva-Sirakova et al. 2018**). These researches were conducted taking advantage of different techniques (for example brain MRI, Cerebral brain perfusion (CBF), single-photon emission computed tomography (SPECT), 24-hour ambulatory blood pressure monitoring). They all highlighted the need to follow patients over time but to date, there have been no characterization of specific biomarkers able to early predict the passage from healthy condition to the impaired one. Moreover, there are no hypotheses on when there is this passage from normal to dysfunctional cognition. Moreover, there is no general consensus about time window among visits. In literature follow up are established at different intervals. There are studies in which assessments are established after few months and others in which follow up visits can occur even after 25 years from the first visit (**Nir et al. 2013; Launer et al. 2015**). In the middle there are several researches with different time range for evaluations. They generally consider from one to three years follow up with annually visits (**Cao et al. 2016; Williams et al. 2017; Yaneva-Sirakova et al. 2018**).

There are also several studies that exploit DTI in order to assess some cardiovascular pathologies such as cerebral small vessel disease. The study of Carnevale (2018) is an example of how this technique could be exploited in hypertension providing important biomarkers. Another issue highlighted in the article concerns lifestyle and dietary habits

information in order to tailor an anti-hypertensive treatment on a per patient basis (**Carnevale et al. 2018**).

Considering what was discussed, we are proceeding with follow up repeating the same procedure for each patient and implementing data to overcome previous study limitations.

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ABBREVIATIONS

Alzheimer's Disease **(AD)**

American Heart Association **(AHA)**

American Stroke Association **(ASA)**

Amyloid β **(Aβ)**

Angiotensin-Converting Enzyme **(ACE)**

Anterior Cingulate Cortex **(ACC)**

Anterior Thalamic Radiation **(ATR)**

Atrial Natriuretic Peptides **(ANP)**

Blood Brain Barrier **(BBB)**

Blood Pressure **(BP)**

Body Max Index **(BMI)**

Brain Natriuretic Peptides **(BNP)**

Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopath **(CADASIL)**

Cerebral Blood Flow **(CBF)**

Cingulate Gyrus part of Cingulum **(CGC)**

Common Carotid Artery **(CCA)**

Corticospinal Tract **(CST)**

C-type Natriuretic Peptides **(CNP)**

Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition **(DSM 5)**

Diastolic Blood Pressure **(DBP)**

Diffusion Tensor Imaging **(DTI)**

Diffusion Weighted Imaging **(DWI)**

dorsolateral Prefrontal Cortex **(dlPFC)**

Ejection Fraction **(EF)**

estimated Glomerular Filtration Rate **(eGFR)**

External Carotid Artery **(ECA)**

Fluid Attenuated Inversion Recovery **(FLAIR)**

Forceps Major **(FMA)**

Forceps Minor **(FMI)**

Fractional Anisotropy **(FA)**

General Linear Model **(GLM)**

Indexed LVM **(LVMI)**

Inferior Fronto-Occipital Fasciculus **(IFO)**

Inferior Longitudinal Fasciculus **(ILF)**

Instrumental Activities of Daily Living **(IADL)**

Internal Carotid Artery **(ICA)**

International Statistical Classification of Diseases and Related Health Problems 10th Revision **(ICD-10)**

Intima-Media Thickness **(IMT)**

Kinin Kallikrein System **(KKS)**

Left Ventricle **(LV)**

Left Ventricular Mass **(LVM)**

Leukocyte Adhesion Molecules **(LAMs)**

Longitudinal relaxation time **(T1)**

Magnetic Resonance **(MR)**

Magnetic Resonance Imaging **(MRI)**

Mean Diffusivity **(MD)**

Mild Cognitive Impairment **(MCI)**

Mini Mental State Examination **(MMSE)**

Montreal Cognitive Assessment **(MoCA)**

NeuroCognitive Disorder **(NCD)**

Nitric Oxide **(NO)**

Parahippocampal Part of Cingulum **(CGH)**

Posterior Thalamic Radiation **(PTR)**

Radial Diffusivity **(RD)**

Radio Frequency **(RF)**

Receptor for Advanced Glycation End product **(RAGE)**

Region Of Interest **(ROI)**

Relative Wall Thickness **(RWT)**

Renin-Angiotensin-Aldosterone System **(RAAS)**

Repetition Time **(TR)**

Standard Deviation **(SD)**

Superior Longitudinal Fasciculus **(SLF)**

Superior Thalamic Radiation **(STR)**

Systolic Blood Pressure **(SBP)**

Target Organ Damage **(TOD)**

Time to Echo **(TE)**

Transverse relaxation time **(T2)**

Trial Making Test **(TMT)**

Uncinate Fasciculus **(UNC)**

Vascular Cognitive Impairment **(VCI)**

Vascular Dementia **(VaD)**

Voxel Based Morphometry **(VBM)**

White Matter Hyperintensities **(WMHs)**

Working Memory **(WM)**

World Health Organization **(WHO)**

ANNEX:

This PhD thesis work gave rise to an article published on Cardiovascular Research (2018) doi:10.1093/cvr/cvy104.

TITLE: **BRAIN MRI FIBER-TRACKING REVEALS WHITE MATTER ALTERATIONS IN HYPERTENSIVE PATIENTS WITHOUT DAMAGE AT CONVENTIONAL NEUROIMAGING**

AUTHORS: Lorenzo Carnevale, **Valentina D'Angelosante**, Alessandro Landolfi, Giovanni Grillea, Giulio Selvetella, Marianna Storto, Giuseppe Lembo and Daniela Carnevale.

ABSTRACT

Aims: Hypertension is one of the main risk factors for dementia. The subtle damage provoked by chronic high blood pressure in the brain is usually evidenced by conventional magnetic resonance imaging (MRI), in terms of white matter (WM) hyperintensities or cerebral atrophy. However, it is clear that by the time brain damage is visible, it may be too late hampering neurodegeneration. Aim of this study was to characterize a signature of early brain damage induced by hypertension, before the neurodegenerative injury manifests.

Methods and Results: This work was conducted on hypertensive and normotensive subjects with no sign of structural damage at conventional neuroimaging and no diagnosis of dementia revealed by neuropsychological assessment. All individuals underwent cardiological clinical examination in order to define the hypertensive status and the related target organ damage. Additionally, patients were subjected to DTI-MRI scan to identify microstructural damage of WM by probabilistic fiber-tracking. To gain insights in the neurocognitive profile of patients a specific battery of tests was administered. As primary outcome of the study we aimed at finding any specific signature of fiber-tracts alterations in hypertensive patients, associated with an impairment of the related cognitive functions. Hypertensive patients showed significant alterations in three specific WM fiber-tracts: the anterior thalamic radiation, the superior longitudinal fasciculus and the forceps minor. Hypertensive patients also scored significantly worse in the cognitive domains ascribable to brain regions connected through those WM fiber-tracts, showing decreased performances in executive functions, processing speed, memory, and paired associative learning tasks.

Conclusions: Overall, WM fiber-tracking on MRI evidenced an early signature of damage in hypertensive patients when otherwise undetectable by conventional neuroimaging. In perspective, this approach could allow identifying those patients that are in initial stages of brain damage and could benefit of therapies aimed at limiting the transition to dementia and neurodegeneration.

Keywords: White matter tractography • Hypertension • Cognitive impairment • Presymptomatic diagnosis

In addition, during my PhD fellowship I had the opportunity to take part in a project focused on preventing cognitive dysfunction in hypertension through a new nutraceutical compound that gave rise to an article published on: **Immunity & Ageing (2018) 15:7 DOI 10.1186/s12979-017-0113-4.**

TITLE: EFFECTS OF A NEW NUTRACEUTICAL COMBINATION ON COGNITIVE FUNCTION IN HYPERTENSIVE PATIENTS.

AUTHORS: Giuseppe Giugliano, Alessia Salemme, Sara De Longis, Marialuisa Perrotta, **Valentina D'Angelosante**, Alessandro Landolfi, Raffaele Izzo and Valentina Trimarco.

ABSTRACT

Background: Chronic increased arterial blood pressure has been associated with executive dysfunction, slowing of attention and mental processing speed, and later with memory deficits. Due to the absence of a concrete therapeutic approach to this pathophysiological process, in the last decades there has been an increasing interest in the use of nutraceuticals, especially those with antioxidant properties, which own strong neuroprotective potential, that may help to improve cognitive function and to delay the onset of dementia.

Results: We evaluated the effects of the treatment with a new nutraceutical preparation containing different molecules with potent antioxidant properties (AkP05, IzzeK®) and placebo on a cohort of thirty-six hypertensive patients. At baseline, neuropsychological evaluation, arterial stiffness and biochemical parameters of the subjects were comparable. After 6 months of treatment, there was a significant reduction of the augmentation index in the AkP05-treated group. Moreover, the measurement of cognitive function, evaluated with MoCA test and Word Match Testing, showed a significant improvement in patients receiving the active treatment. In addition, the group treated with nutraceutical reached a better Stroop test score, while subjects that received placebo did not showed any improvement. Finally, a positive relationship between SBP variation and the psychometric assessment with the EQ-VAS scale was observed only in the active treatment group.

Conclusions: In this study, we demonstrated that the therapy with a new nutraceutical preparation is able to significantly increase the scores of important neuropsychological tests in hypertensive patients already on satisfactory blood pressure control. Although future studies are needed to better characterize the molecular mechanisms involved, these results

candidate the new nutraceutical combination as a possible therapeutic strategy to support the cerebrovascular functions and delay the onset of dementia in hypertensive patients.

Keywords: Nutraceutical, Neuropsychological evaluation, Arterial stiffness.

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